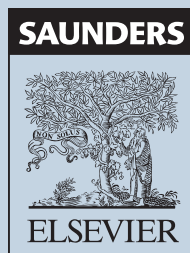# Equine Respiratory Medicine and Surgery

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Preface

The horse has an impressive capacity for strenuous exercise, being capable of running at speeds up to 20 meters per second for long distances, even while carrying a rider. Not unexpectedly, the equine respiratory system is extremely well adapted to provide the gas exchange requirements of such exercise that can include an oxygen demand of 50–80 liters per minute, which is approximately twice that of an elite human athlete on a weight-for-weight basis. To meet this oxygen demand, the racing horse must inhale and exhale in the region of 15 liters of air, twice per second, which equates to an impressive minute volume of 1,800 liters. Clearly, any one of the numerous disorders that impair the horse's ability to ventilate its lungs and exchange oxygen compromises exercise performance, prompting veterinary attention.

Equine Respiratory Medicine and Surgery provides clinicians, undergraduate and postgraduate students, and research scientists with a fully comprehensive, state-of-the-art reference source, written by world leaders in the field of equine respiratory tract disease. The book begins with a review of the relevant applied basic science. This is followed by sections that cover current diagnostic techniques, infectious diseases, disorders of the upper and lower respiratory tract, neonatal respiratory tract disorders, and, finally, disorders of the chest wall, diaphragm, and pleurae.

The book highlights the remarkable progress that has been made in our understanding of diseases of the equine respiratory tract over the last 10–15 years, and the resultant improvements in diagnosis of these diseases and management of affected horses. We have clearly moved on considerably from the era of Vegetius Renatus (4th century Roman author), who advocated the following treatment for "horses with a grievous cough:"

> *The shank of a fat boar is boiled till the flesh be loosened. Add 3 ounces of deer marrow, suet of a he goat, half ounce of bull glue, 3 sextari of raisin wine, a sextarius of psafin, half a hemina of sharpest vinegar, and 3 ounces of gumdragant, linseed and fenugreek*

Vegetius Renatus
*Of the Distempers of Horses and
of the Art of Curing Them.*
English Translation. Printed for A. Millar,
London, MDCCXLVII

We hope that future readers of our book will not use it as a source of entertainment when they write editorials 2000 years from now, but will recognize that progress in the diagnosis and treatment of equine respiratory diseases has been a consequence of modern technology. Improvements in equipment for endoscopy, improved diagnostic imaging techniques, use of immunohistochemistry and polymerase chain reaction for diagnoses, and the advent of safe techniques for lung biopsy have all advanced the practice of equine respiratory medicine. We know that the next 10 years will be just as exciting as the previous decade, and we look forward with excitement to future advances.

**Bruce McGorum, Paddy Dixon,
Ed Robinson, and Jim Schumacher**

Acknowledgments

1

Anatomy of the Respiratory System

N Edward Robinson and Paul W Furlow

The major function of the respiratory system is to deliver oxygen to and remove carbon dioxide from the blood. Air is delivered into the lungs through a series of conducting airways that connect the ambient air to the alveoli where gas exchange with blood takes place. These conducting airways include the nares, nasal cavity, pharynx, larynx, trachea, bronchi, and bronchioles. Gas exchange occurs in the alveolar ducts and alveoli, both of which are lined by an extensive pulmonary capillary network so that there is a huge vascular surface area for oxygen and carbon dioxide diffusion. Blood reaches the pulmonary capillaries from the right ventricle through the pulmonary arteries and returns to the left atrium via the pulmonary veins. In addition, the bronchial circulation, which is a branch of the systemic circulation, provides nutrients to the bronchi, large vessels, and pleura. This chapter describes the gross and microscopic structure of the respiratory system and forms a basis for future chapters. The structures are described in the sequence that they might be approached during a clinical examination.

The Nose

The external nares and false nostril

The respiratory system begins at the external nares, which provide very mobile valves that can be closed to prevent water entry during swimming or can be opened maximally during exercise to facilitate high airflow rates. The alar cartilages provide rigidity to the external wall of the nostril. Movements of the external nares are a result of the action of the muscles (levator nasolabialis, dilator naris lateralis, and transversus nasi) that attach to these cartilages. The lateralis nasi inserts into the cartilaginous extension of the ventral turbinate so that it draws the lateral wall of the nasal vestibule outwards and compresses the false nostril, thereby enlarging the nasal opening.

The junction between the skin and the nasal mucosa is visible just within the external nares. On the ventral aspect of this mucocutaneous junction is the opening of the nasolacrimal duct. This is one source of the small amount of moisture frequently seen on the horse's external nares.

If one extends an index finger into the nose and up the lateral surface of the external nares, one enters the blind, hair-lined cavity known as the false nostril. It is approximately 10 cm in depth and its function is unknown. The sebaceous glands in the walls of the false nostril can give rise to epidermal cysts.

The nasal cavity

Extending a finger up the medial and ventral aspect of the external nares, one enters the nasal cavity through its narrowest part known as the nasal valve. On the medial side of the nasal valve is the nasal septum while on the lateral side is the alar fold, which is a mucosal extension of the ventral turbinate. The nasal septum separates the two sides of the nasal cavity. In its caudal portion it is bony but rostrally it is cartilaginous. It is covered with a highly vascular mucosa. The rostral end of the mucosa to the level of the second or third cheek tooth is covered by a non-ciliated stratified cuboidal epithelium with a low density of mucous cells. Caudally, the epithelium becomes ciliated pseudostratified columnar and mucous cell density increases progressively.

Nasal turbinates

Each side of the horse's nasal cavity has two turbinates that divide the cavity into three air passages, the ventral, middle and dorsal meatuses. The ventral meatus, which has the largest cross-sectional area, provides the direct pathway for airflow between the external nares and the nasopharynx and is the primary path for an endoscope or stomach tube. The dorsal meatus extends into the ethmoid region. The turbinates enlarge the mucosal surface of the nasal cavity, which facilitates its air-conditioning and defense functions.

It is easiest to understand the gross structure of the turbinates if one imagines them initially as a mucosa-covered bony plate extending into each nasal cavity from its lateral wall. To be accommodated within the nasal cavity, each of these structures must scroll. The ventral turbinate scrolls upwards while the dorsal turbinate scrolls downwards (Fig. 1.1). This scrolling results in the shell-like structures known as the nasal conchae. The conchae of both the dorsal and ventral turbinates form dorsal and ventral conchal sinuses. The dorsal conchal sinus is contiguous with the frontal sinus and the ventral conchal sinus with the maxillary sinus (Fig. 1.2).
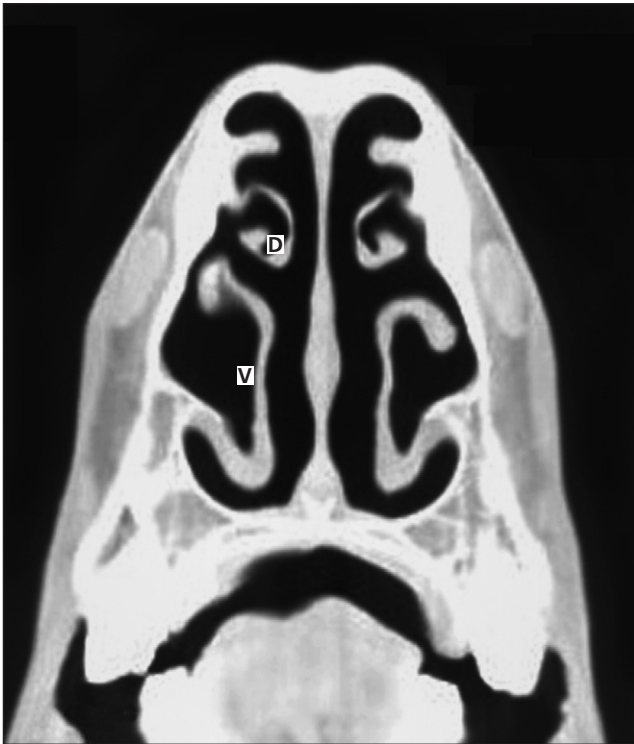
Fig. 1.1. Cross-sectional image of the nose at the level of the first cheek tooth. The scrolling of the dorsal (D) and ventral (V) turbinates is clearly visible.

Fig. 1.2. Cross-sectional image of the nose at the level of the junction of the fourth and fifth cheek teeth. The dorsal (d), middle (m), ventral (v), and common (c) meatuses are visible. The ventral conchal sinus (VCS) communicates with the rostral maxillary sinus (RMS) over the nasolacrimal canal (n). The dorsal conchal sinus (DCS) communicates with the frontal sinus (F).

The ventral turbinate is the shorter of the two turbinates extending between the first and sixth cheek teeth. Rostrally, the ventral turbinate continues to the external nares as the alar fold. The longer dorsal turbinate terminates rostrally at the first cheek tooth and caudally extends to the ethmoid region. The epithelial lining of the rostral portion of the alar fold is stratified squamous epithelium with numerous duct openings from the large number of submucosal glands. Caudally, there is a progressive transition of epithelium through stratified non-ciliated cuboidal, and ciliated pseudostratified columnar, to typical respiratory epithelium with numerous mucus cells (Kumar et al 2000) (Fig. 1.3).

The submucosa of the nasal cavity is highly vascular allowing it to warm inspired air and regulate mucus production. Postganglionic sympathetic nerves (supplied via cervical sympathetic preganglionic fibers synapsing in the superior cervical ganglion) innervate the nasal blood vessels. Upon release of norepinephrine these nerves cause vasoconstriction. Congestion of the nasal mucosa is a feature of Horner syndrome and is a consequence of loss of the sympathetic nerve supply. Parasympathetic innervation of the nose is from the facial nerve (CN VII) and has little effect on the diameter of blood vessels, suggesting that vasodilatation is a la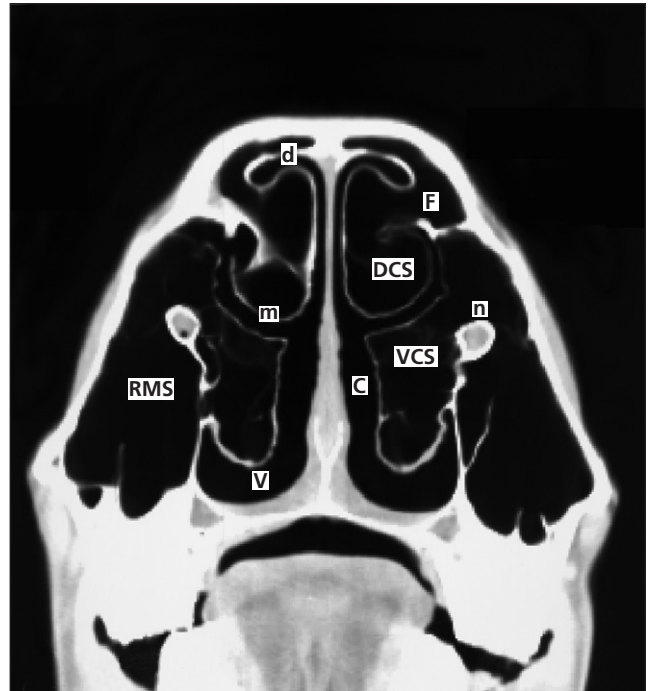rgely passive process. When activated, the parasympathetic nerves in the nose chiefly regulate glandular blood flow and secretion.

The ethmoids

The ethmoid region of the nasal cavity is clearly visible when an endoscope is directed dorsally from within the ventral meatus of nasal cavity (Fig. 1.4). One sees the rostral surface of the ethmoturbinates, a mass of highly vascularized, scroll-like plates of bone that ramify toward the olfactory region of the brain (Fig. 1.5). The ethmoid region receives most of its blood supply from intracranial sources (Bell et al 1995). The olfactory epithelium that lines the ethmoturbinates contains three types of cells: sensory neurons, exhibiting odor receptors that transmit electrical signals to the brain; sustentacular cells, which provide protection to the neurons and secrete mucus; and basal cells, which differentiate to replace dead sensory neurons. The axons of nerves in the olfactory epithelium converge to form the olfactory nerve (CN I) giving rise to the sense of smell. Progressive ethmoid hematoma is a slowly growing, uncommon, hemangiomatous mass that originates in the ethmoidal region and can cause chronic epistaxis.

The maxillary sinus drains into the caudal part of the middle meatus of the nasal cavity via the nasomaxillary
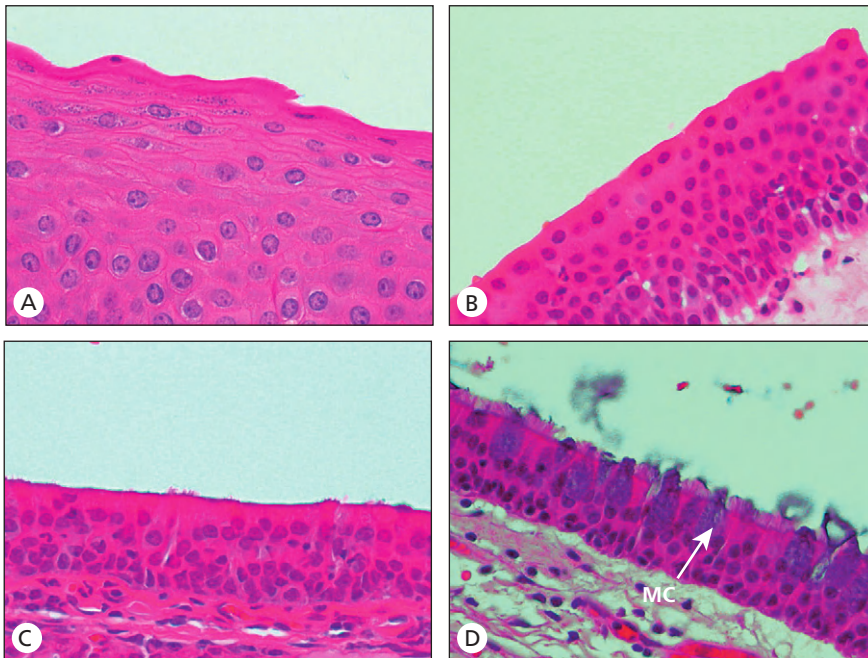
Fig. 1.3A–D. Types of epithelium found in the equine nose. (A) Stratified squamous epithelium typical of the most rostral part of the ventral turbinate. (B) Non-ciliated stratified cuboidal epithelium with low mucus cell count found on the rostral third of the nasal septum and the alar fold. (C) Transitional epithelium that occurs on the rostral third of the turbinate bones and the middle third of the nasal septum. (D) Ciliated pseudostratified respiratory epithelium with many mucus cells (MC) typical of caudal parts of the turbinates and nasal septum.
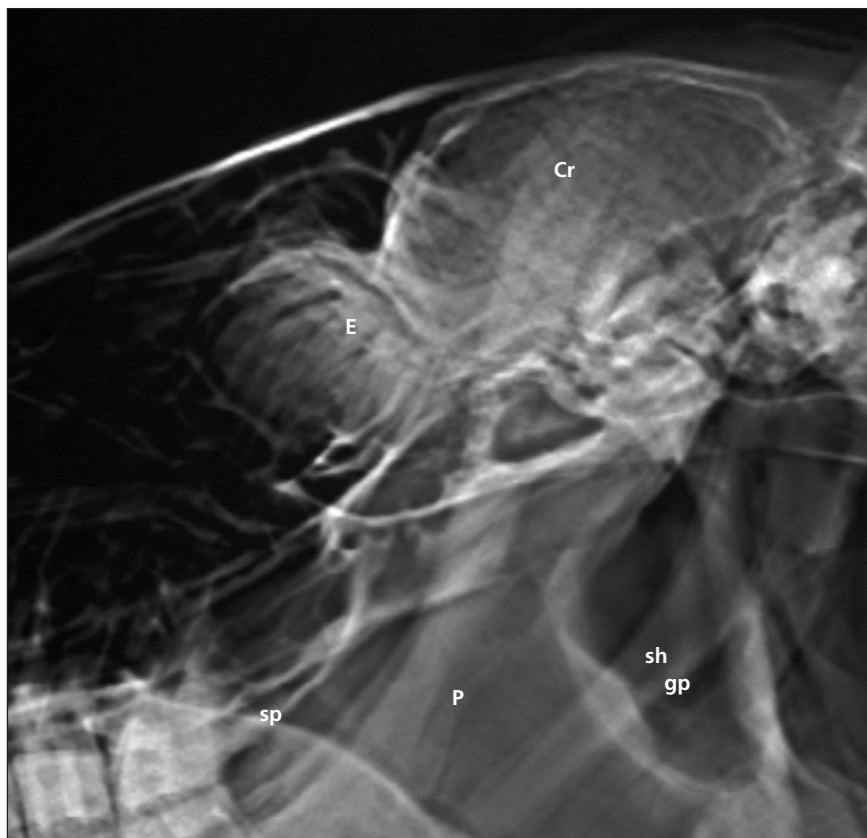
Fig. 1.4. Lateral radiograph of the caudal nasal cavity and pharynx (P) showing the cranium (Cr), ethmoturbinates (E), guttural pouch (gp), stylohyoid bone (sh) and soft palate (sp).

opening. Although this opening cannot be seen directly with an endoscope, purulent drainage from the sinus appears lateral to the caudal part of the dorsal turbinate where it joins the ethmoid region (Fig. 1.5).

The paranasal sinuses

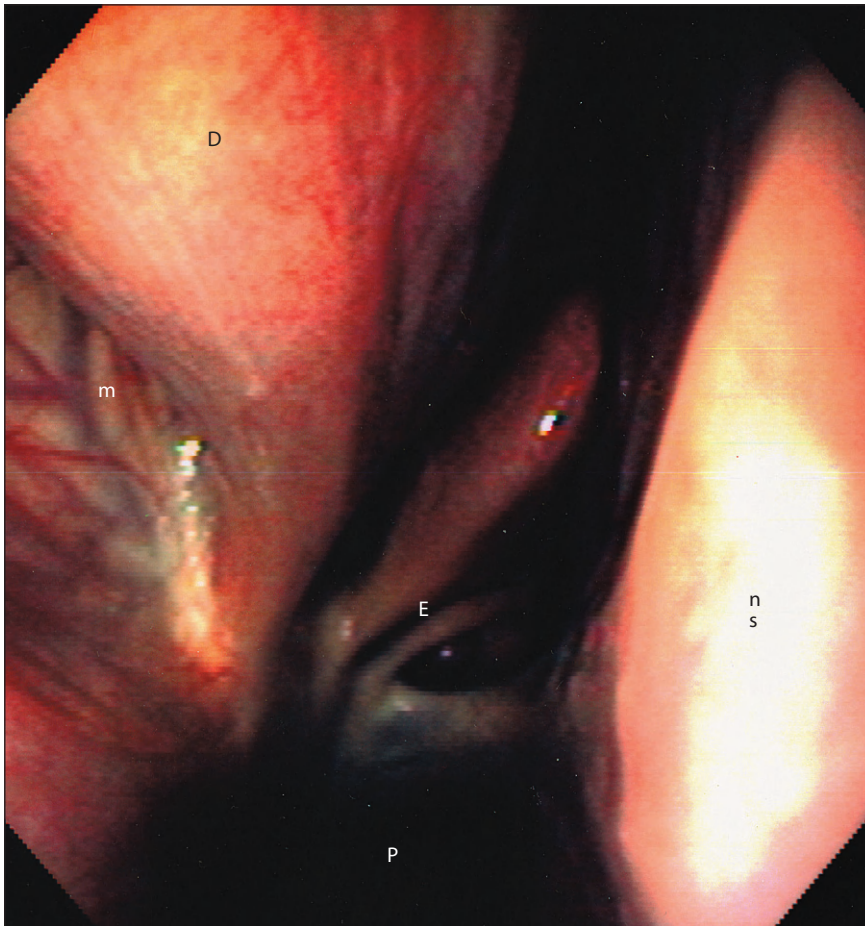There are seven pairs of paranasal sinuses in the horse (Fig. 1.6). These are the rostral and caudal maxillary, ventral and dorsal conchal, frontal, sphenopalatine, and ethmoid sinuses. The rostral maxillary sinus (RMS) is located dorsal to the third and fourth maxillary cheek teeth. The lateral portion of the RMS communicates with the medial portion, known as the ventral conchal sinus (VCS), over the infraorbital canal. The RMS is separated from the caudal maxillary sinus (CMS) by a bony septum usually positioned between the fourth and fifth cheek teeth. The CMS is located dorsal to the fifth and sixth cheek teeth. The frontal sinus is triangular and located dorsal to the ethmoturbinates and rostral to the cranium. The frontal sinus and the dorsal conchal sinus functionally form a single compartment known as the conchofrontal sinus. Within the ethmoturbinates lie many small sinuses that comprise the ethmoidal sinus that drains laterally into the CMS by way of the sphenopalatine sinus that lies adjacent to CN II–VI and blood vessels (Bell et al 1995, McCann et al 2004).

Drainage of the paranasal sinuses does not strictly rely on gravity alone but also on ciliary transport directed toward the middle meatus of the nasal cavity (Barakzai 2004). The RMS and VCS drain into the middle meatus via the slit-like nasomaxillary aperture (NMA), which is located at the highest point in the VCS. The remaining paranasal sinuses drain into the CMS and thence into the posterior part of the nasal cavity via the NMA of the CMS. The drainage from these two separate NMAs merges. When the drainage from the sinuses is purulent it can be observed entering the nose lateral to the caudal limit of the dorsal turbinate (Fig. 1.5). The narrow drainage pathways are easily obstructed as a result of mild inflammation.

The Pharynx

The pharynx delivers air from the posterior nasal cavity to the larynx and is also the pathway that delivers food from the oral cavity to the esophagus. In horses, the oral cavity
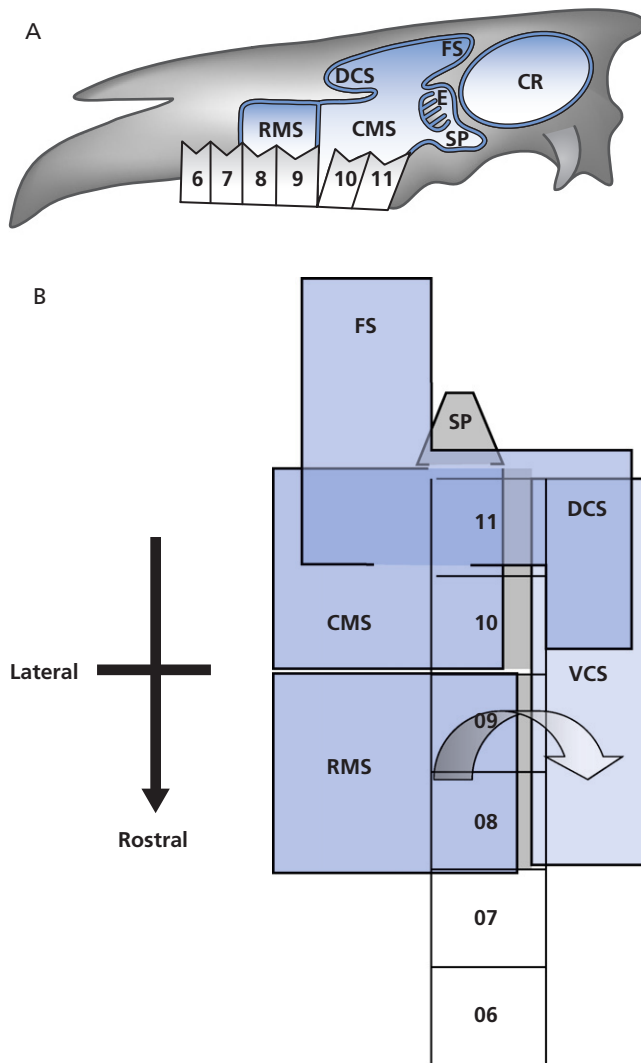
A

B

Lateral

Rostral

Fig. 1.6. The paranasal sinuses of the horse. (A) Lateral view. (B) Diagrammatic representation to show the relationship to the cheek teeth and the intercommunications. CMS = caudal maxillary sinus, CR = cranium, DCS = dorsal conchal sinus, E = ethmoidal sinuses, FS = frontal sinus, RMS = rostral maxillary sinus, SP = sphenopalatine sinus, VCS = ventral conchal sinus. Arrow shows communication between RMS and VCS over the infraorbital canal. Teeth are labeled in the Triadian system: 06–08 = premolars, 09–11 = molars.

and pharynx are normally separated by the soft palate except during swallowing. For this reason, horses are obligate nasal breathers.

The nasopharynx is lined with pseudostratified columnar ciliated epithelium containing goblet cells and the oropharynx is lined by stratified squamous epithelium. Lymphoid tissue that is organized into follicles is visible in rows along the dorsal wall and represents part of the equine tonsil (Kumar & Timoney 2001). In young horses, these follicles can become large and edematous. This condition, known as follicular lymphoid hyperplasia, is generally self-limiting as the horse matures. In addition, a lingual tonsil has been described at the base of the tongue (Kumar & Timoney 2005).

Unlike the nasal cavity, larynx and trachea, the pharynx lacks rigid support from bone or cartilage. The patency of the pharynx depends on the activity of muscles associated with the hyoid bone, soft palate and tongue (Holcombe et al 1997, Holcombe et al 1998, Tessier et al 2004). These are discussed extensively in Chapter 29. Dysfunction of these muscles or their nerves has been associated with pharyngeal collapse or dorsal displacement of the soft palate, conditions that are exaggerated during exercise.

When the pharynx is viewed endoscopically from the posterior nares, the following structures are generally visible: the soft palate forms the ventral floor, the slit-like guttural pouch openings are visible on the lateral wall, and the larynx and glottis are visible ventrally (Fig. 1.7). If the soft palate is located in its normal position, the pointed epiglottis is visible. The epiglottis is held down against the soft palate by the action of the hyoepiglotticus muscle (Holcombe et al 2002). If the soft palate is displaced dorsally, the epiglottis is hidden.

When the endoscope tip is directed dorsally within the pharynx, the dorsal pharyngeal recess becomes visible. In cases of follicular lymphoid hyperplasia, the dorsal pharyngeal recess can become severely affected and edematous. In mules, a muscular sphincter surrounds the entrance into the dorsal pharyngeal recess.

The anatomy of the guttural pouches is described in Chapter 28. They are lined by ciliated epithelium with mucus-secreting cells and have a close association with the vagus (CN X), glossopharyngeal (CN IX) and hypoglossal (CN XII) nerves (Manglai et al 2000a,b). The internal carotid artery runs in the wall of the guttural pouch and it is thought that this association allows for cooling of arterial blood on its way to the brain (Baptiste et al 2000). The 5-cm long slit-like openings into the guttural pouches extend downward and backward in the lateral wall of the pharynx, beginning just caudal to the level of the posterior nares. A fold of mucus membrane that needs to be lifted to pass an endoscope into the guttural pouch covers the medial wall of the guttural pouch opening. During swallowing, the guttural pouch openings dilate widely and the mucus membrane folds almost make contact with each other in the pharyngeal midline (Fig. 1.7B).

The Larynx

The main function of the larynx is to prevent inhalation of food into the lower airway during swallowing. The larynx also has evolved a second function, which is phonation. The cartilaginous support of the larynx is provided by the ring-shaped cricoid cartilage adjacent to the first tracheal ring, the large thyroid cartilage, a pair of arytenoid cartilages that support the vocal folds and the epiglottis that provides a protective flap to cover the glottis during

Fig. 1.7. (A) Endoscopic view of the pharynx showing 1, the tissue flaps that cover the openings to the Eustachian tubes and guttural pouches; 2, the dorsal pharyngeal recess; and 3, the larynx. (B) The guttural pouch openings during swallowing. Note how the tissue mucosal flaps over the openings converge in the midline of the pharynx.

deglutition (Fig. 1.8A). Corniculate and cuneiform cartilages are attached to the arytenoid and epiglottic cartilages respectively. The cricoid cartilage, which is the most caudal, has a broad dorsal surface that provides the origin of the cricoarytenoideus dorsalis muscle. It also has articular surfaces for the arytenoids and for the caudal cornu of the

thyroid cartilage. The thyroid cartilage has a narrow ventral body from which arise two plate-like laminae that form the lateral walls of the larynx and articulate with the cricoid in diarthrodial joints at their caudal cornua. The ventral borders of the laminae unite rostrally at the body of the thyroid and thereby form a ventral triangular space – the thyroid notch – that is bounded caudally by the ventral part of the cricoid cartilage. The cricothyroid membrane fills the thyroid notch, and is easily palpable. The rostral border of each thyroid lamina is attached to the hyoid bone by the thyrohyoid membrane. The paired arytenoids are mobile and under the control of the intrinsic laryngeal muscles. They lie medial to the thyroid laminae and rostral to the cricoid cartilage. At the caudal edge of the medial surface of each arytenoid is the diarthrodial cricoarytenoid articulation. On the lateral surface dorsal to the articulation is the muscular process for the insertion of the cricoarytenoideus dorsalis muscle. A notch in the arytenoid cartilage separates the caudal muscular process from the rostral corniculate cartilages that are attached to the arytenoids by cartilaginous joints. The bilateral corniculate cartilages are visible endoscopically dorsal and lateral to the glottis. In the normal horse, these abduct almost symmetrically during inhalation but, in the horse with recurrent laryngeal neuropathy, the left corniculate fails to abduct or lags considerably. The most ventral point of the arytenoid cartilage is the vocal process. This forms the attachment for the vocal ligament, a band of elastic fibers that originates on the caudal border of the thyroid body and underlies the membranous vocal fold. Movements of the arytenoids and corniculate cartilages abduct and adduct the vocal folds. The single almost triangular epiglottic cartilage is pointed rostrally and normally visible endoscopically above the soft palate. Attached to either side of its base are the bar-like cuneiform cartilages that project caudodorsally.

Three extrinsic muscles regulate the position of the larynx in relation to the head and neck. A small muscle, the hyoepiglotticus, connects the ventral surface of the epiglottis to the basihyoid bone and thereby pulls the epiglottis ventrally to enlarge the entrance to the glottis. Dysfunction of this muscle results in inspiratory epiglottic retroversion during exercise. The thyrohyoideus connects the lateral surface of the thyroid laminae to the caudal border of the stylohyoid bone. Contraction of the thyrohyoideus pulls the larynx rostrad. The sternothyrohyoideus originates at the manubrium of the sternum and inserts onto both the caudal border of the thyroid laminae and the basihyoid bone and the lingual process of the hyoid bone. Contraction pulls the larynx backward and downward.

Intrinsic muscles of the larynx (Fig. 1.8B) regulate the position of the vocal folds and the size of the glottic opening (rima glottidis). Contraction of the cricoarytenoideus dorsalis abducts the vocal folds and enlarges the rima glottidis. Paresis of this muscle results in left laryngeal
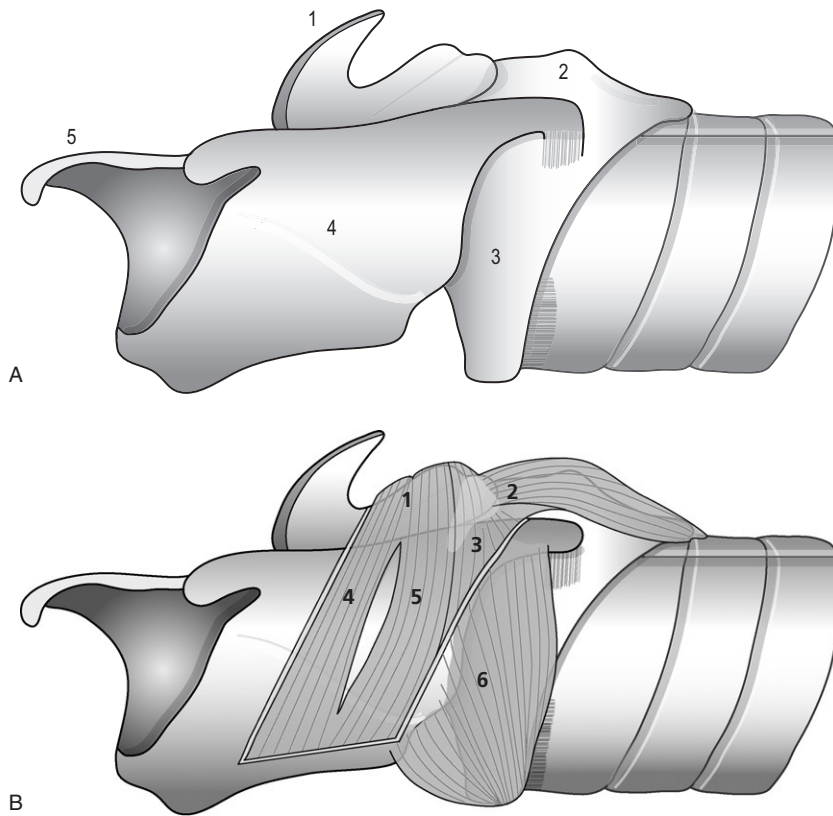
Fig. 1.8. (A) The cartilages of the larynx. 1 = cuneiform cartilage, 2 = muscular processes of the arytenoid cartilages, 3 = cricoid cartilage, 4 = lamina of thyroid cartilage, 5 = epiglottic cartilage. (B) The intrinsic muscles of the larynx. 1 = arytenoideus transversus, 2 = cricoarytenoideus dorsalis, 3 = cricoarytenoideus lateralis, 4 = ventricularis, 5 = vocalis, 6 = cricothyroideus. After Budras, Sack & Röck: The Anatomy of the Horse (page 44). Schlütersche GmbH & Co. KG, Hannover, Germany, 2003.

hemiplegia. Contraction of the arytenoideus transversus also abducts the vocal folds. The adductor muscles of the vocal folds are cricoarytenoideus lateralis, arytenoideus transversus, vocalis and ventralis. Contraction of the latter muscles during swallowing forms a sphincter that closes the glottis and prevents food inhalation. Innervation of the intrinsic muscles of the larynx is via the recurrent laryngeal nerves except for the cricothyroideus, which receives innervation via the cranial laryngeal nerves.

The laryngeal mucosa is contiguous with that of the pharynx and trachea. It is tightly adherent to the dorsal surface of the epiglottis, over the vocal ligaments and to the cricoid cartilage. Between the lateral border of the epiglottis and the cuneiform/arytenoid cartilages, the mucosa forms the aryepiglottic fold that can sometimes cause an inspiratory obstruction during exercise. The loose mucosal folds on the ventral side of the epiglottis can sometimes displace dorsally to entrap the epiglottis. Most of the larynx is lined by respiratory, pseudostratified, columnar, ciliated epithelium with goblet cells but the epiglottis and vocal folds are covered by a stratified squamous epithelium.

There are numerous mucus glands beneath the laryngeal mucosa, especially in the epiglottis.

The laryngeal saccules are bilaterally paired, 2.5–5.0 cm deep, mucosa-lined cavities that extend upwards and backwards on the medial surface of the thyroid cartilage. The entrance to the saccules is from the lateral ventricle of the larynx, a pocket-like depression lateral to the vocal folds. The function of the laryngeal saccules is unknown but they are thought to participate in generation of the noise characteristic of horses with recurrent laryngeal neuropathy.

The Trachea

The trachea provides a flexible connection between the larynx and bronchial bifurcation (carina). The trachea can be palpated immediately beneath the skin on the ventral midline of the neck. Its lowest point is where it enters the thoracic inlet immediately above the manubrium of the sternum and it is here that mucoid secretions tend to accumulate. The trachea then ascends and terminates at the bronchial bifurcation, which is just dorsal to the left atrium.
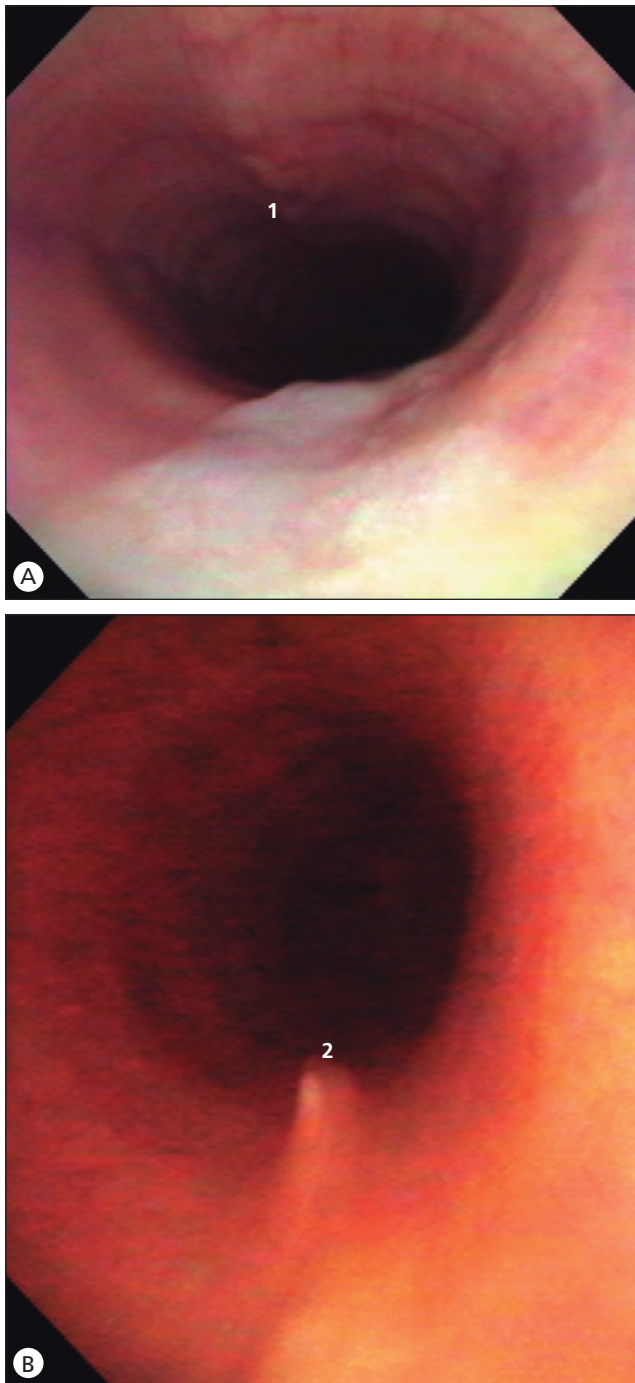
Fig. 1.9. Endoscopic views of the trachea. (A) A ventral stream of mucus in a horse with recurrent airway obstruction. The larger bronchial vessels are clearly visible as is the junction of the ends of the tracheal cartilages in the dorsal wall (1). (B) Cartilage spike on the ventral floor of the trachea (2).

The trachea is supported throughout its length by C-shaped sections of cartilage that are tightly apposed to one another so as to give rigidity to the airway (Art & Lekeux 1991a,b). A band of smooth muscle, known as the trachealis muscle, connects the free ends of the cartilages dorsally. When this muscle relaxes, the ends of the cartilage do not meet. Contraction of the trachealis pulls the cartilage tips together so that the trachea becomes more rigid. During endoscopic examination, one can see the outline of the cartilages ventrally and laterally and often appreciate the flatter dorsal surface that overlies the trachealis muscle (Fig. 1.9). In older horses, it is not uncommon to see "spikes" of epithelium-covered cartilage protruding into the tracheal lumen on its ventral floor (Fig. 1.9B). In some horses, these can be extensive and form ridges. Their cause is unknown and they appear to be benign.

In older Shetland ponies and in Miniature Horses, the trachea often becomes flattened dorsoventrally and the region between the tips of the tracheal cartilages that includes the trachealis muscle becomes wider. In these same animals, the trachea sometimes rotates in the middle third of the neck so that the trachealis is on the right side of the neck rather than dorsal. This can cause problems if it is necessary to perform a tracheotomy. A midline incision in the neck of these animals enters the trachea near the margin of the cartilage leaving no rigid support on the right side of the tracheotomy.

The pseudostratified columnar tracheal epithelium consists primarily of mucus-producing goblet cells and ciliated cells above a layer of basal cells. The horse has few submucosal bronchial glands. The lamina propria contains many branches of the bronchial circulation that serve to warm and humidify the air and participate in the inflammatory response of the trachea. The larger of these vessels are visible endoscopically beneath the mucosa. The lamina propria is richly supplied with both sensory and autonomic nerves. Non-myelinated sensory nerves containing neuropeptides ramify into the epithelium. Parasympathetic nerves reach the cranial and caudal trachea via the cranial laryngeal nerve and vagus, respectively. Parasympathetic ganglia located along the dorsal wall of the trachea send postganglionic fibers to a submucosal and a muscular plexus. Postganglionic sympathetic fibers enter the wall of the trachea in association with the vagus nerve and terminate in the lamina propria around the bronchial blood vessels. Sympathetic nerves innervate the trachealis muscle only in its cranial third (Yu et al 1994).

The Bronchi

As an endoscope is advanced towards the carina, the openings of the main bronchi become visible. The right main-stem bronchus forms an almost straight line with the trachea, while the left bronchus deviates slightly. On each side, the first bronchus arising from the lateral wall of the main-stem bronchus is to the cranial part of the lung. There is also a large ventral branch to the region of lung

corresponding to a middle lobe. The bronchus to the accessory or intermediate lobe of the lung arises medio-ventrally from the right bronchus (Fig. 1.10). After giving off these large branches, the main bronchi then run parallel to the dorsal border of each lung toward its caudal extremity (Fig. 1.11). Branches are given off both dorsally and ventrally to various parts of the lung. The branching pattern is consistent and has been mapped and labeled (Smith et al 1994).

The bronchial branching pattern is monopodial. Each main bronchus continues almost directly toward the lung periphery and gives off a series of smaller branches (Fig. 1.11B). The exact number of branches of the horse's tracheobronchial tree has not been determined but varies with lung region. There are more branches between the carina and caudal extremity of the lung (we have counted more than 40) than between the carina and the tip of the cranial lobe. Bronchi are identified by the presence of cartilage in their walls and include all airways greater than approximately 2-mm diameter.

The trachea and bronchi are lined by a pseudostratified columnar epithelium that overlies the basement membrane and consists of ciliated and non-ciliated cells that differentiate from basal cells (Fig. 1.12). The non-ciliated cells are primarily mucus-secreting cells (also known as goblet cells). The mucus-secreting goblet cells produce the mucins, which form a large percentage of the mucoid layer that lines the airways. The horse has very few submucosal glands in its tracheobronchial tree (Widdicombe & Pecson 2002). The mucoid layer is propelled craniad by the ciliary cells (see Chapter 5). The lamina propria is immediately beneath the basement membrane and contains a rich supply of bronchial blood vessels and nerves. The former are involved in warming and humidifying the air. The nerves include non-myelinated neuropeptide-containing sensory nerves and branches of the sympathetic nervous system that supply the bronchial blood vessels (Sonea et al 1993a,b, 1994a,b, 1999). Smooth muscle encircles the bronchi and bronchioles and receives parasympathetic and inhibitory non-adrenergic non-cholinergic innervation (Broadstone et al 1991, Yu et al 1992, 1994).

The Lungs

Apart from their first few centimeters, the bronchi are totally surrounded by the lung parenchyma (Fig. 1.11). Unlike the lungs of most other mammals, the horse lung is not divided into distinct lobes. Viewed from the costal surface, both the right and left lung have a similar shape with a ventral notch that accommodates the heart separating the smaller cranial portion from the larger caudal portion. The right lung is larger than the left in part because it includes the intermediate or accessory lobe, which fills the space caudal to the heart and cranial to the
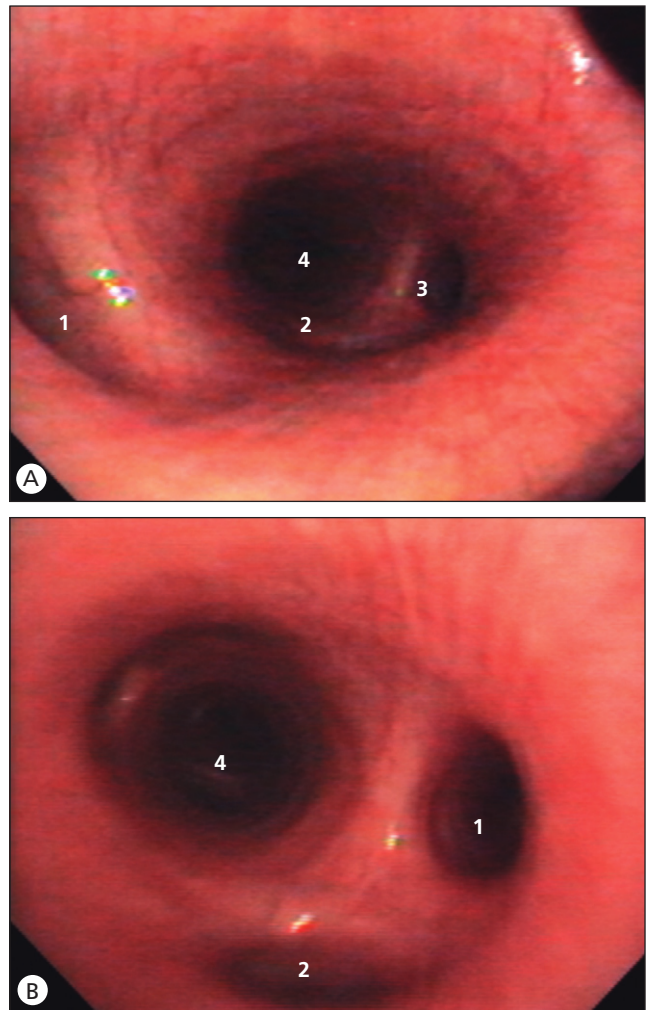

Fig. 1.10. Main branches of (A) the right and (B) the left bronchi. 1: branch to cranial region of lung; 2: branch to cardiac region of lung; 3: branch to accessory lobe; 4: branch to caudal lobe.

diaphragm. The surface of the horse lung shows the fibrous connective tissue septa that divide the lung into lobules. However, these are less distinct than in the pig or cow. The separation of the lung into lobules limits the collateral movement of air between different lung regions (Robinson & Sorenson 1978, Robinson 1982). The greatest bulk of the horse lung is in its caudodorsal region. This means that in the standing animal, much of the lung is almost dorsal to the diaphragm (Figs 1.11 and 1.13).

The bronchovascular bundle

Anyone who views a radiograph of the lung notices that the bronchi and large blood vessels run together (Fig. 1.11B). The distribution of bronchi and arteries is almost identical down to the most peripheral airways. While most veins follow the larger bronchi, some also track through the lung away from the airways and arteries.
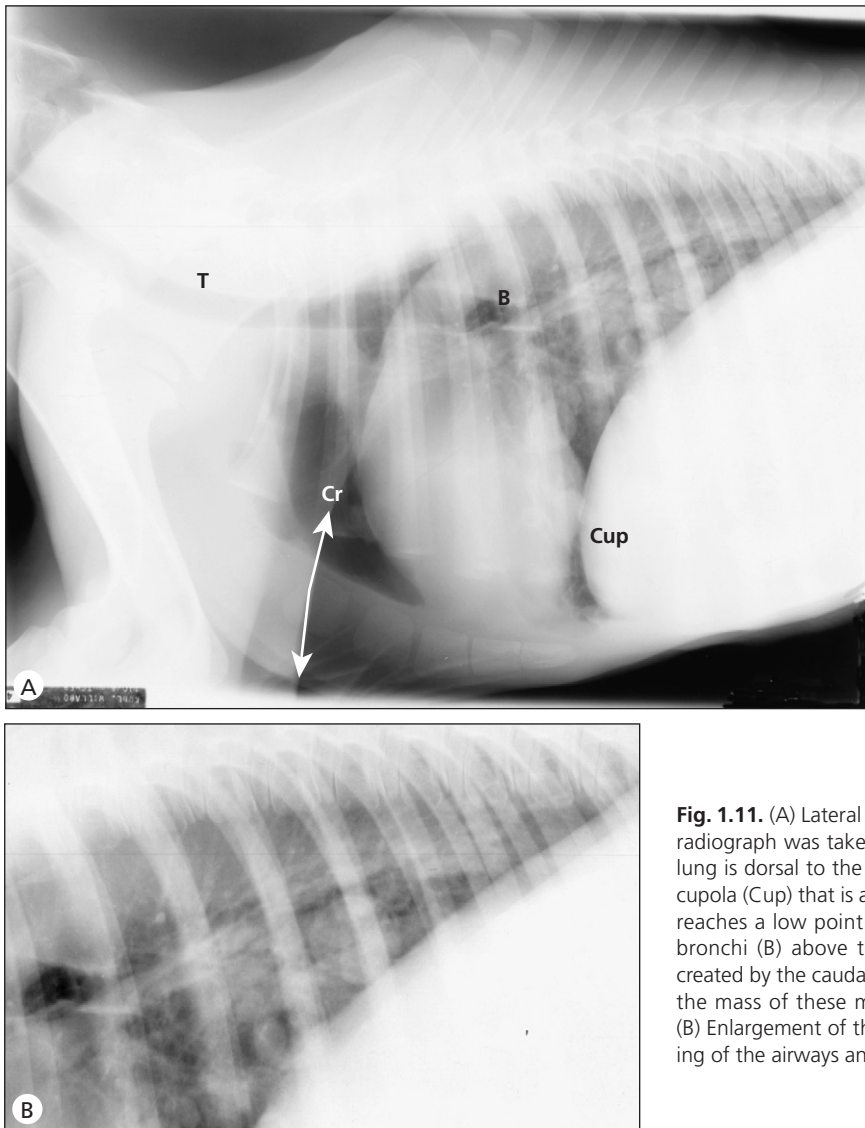
Fig. 1.11. (A) Lateral view of the thorax of a foal with pneumonia. The radiograph was taken with the foal standing. Note that much of the lung is dorsal to the diaphragm, which slopes steeply forward to the cupola (Cup) that is at the level of the sixth to seventh rib. The trachea reaches a low point at the thoracic inlet (T) and bifurcates into the bronchi (B) above the heart. The white arrow marks the shadow created by the caudal margin of the shoulder muscles. In older horses, the mass of these muscles obscures much of the cranial lung (Cr). (B) Enlargement of the caudal region of the lung to show the branching of the airways and vasculature in this abnormal lung.

The associated bronchi and blood vessels are contained in the bronchovascular bundle, which is a loose connective-tissue sheath that also contains lymphatics and nerves. The loose connective tissue is a sink in which edema accumulates whenever fluid filtration rate in the lung is increased, for example in pulmonary edema. When this occurs, the fluid increases the radiographic density in the peribronchial region.

Bronchioles

Several generations of bronchioles, which can be differentiated from bronchi by the absence of cartilage in their walls, connect the small bronchi to the alveolar ducts and alveoli. Because of their small size and the absence of cartilage, bronchioles cannot be distinguished radiographically from the surrounding alveoli. For this reason, radiographs are not useful for the identification of bronchiolar inflammation such as occurs in inflammatory airway disease. In some mammals, there are respiratory bronchioles, the latter being identified by alveoli that open directly from the bronchiolar wall. Horses lack these respiratory bronchioles and the terminal non-respiratory bronchiole connects directly to the alveolar duct (McLaughlin et al 1961, Tyler et al 1971). In the bronchioles, the epithelium is a single layer of cuboidal cells (Fig. 1.14). The primary secretory cell is the Clara cell that has an extensive network of smooth endoplasmic reticulum (Plopper et al 1980). Ciliated epithelial cells are also present in the bronchioles but are less dense in number than in the larger airways. Mucus-secreting goblet cells do not occur in the bronchioles of young healthy horses but are found in horses that have airway inflammation (Kaup et al 1990). As in the bronchi, a layer of smooth muscle encircles the bronchioles.
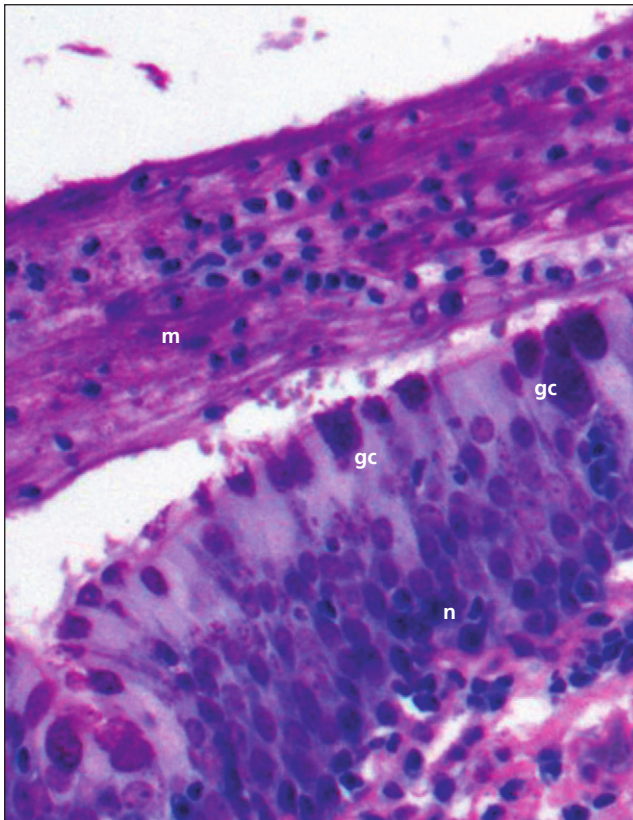
Fig. 1.12. Bronchial epithelium of a horse with recurrent airway obstruction (heaves). Note the thick layer of mucus (m) containing many neutrophils, the multiple layers of epithelial nuclei (n) in the pseudostratified columnar epithelium, and the mucus-producing goblet cells (gc) interspersed among the ciliated epithelium. (Giemsa stain.)

Fig. 1.13. Ventrodorsal radiograph of the lung of a foal with pneumonia. The shadow of the diaphragm is outlined. Note that the lung (L) extends far caudally and dorsally on either side of the abdomen. The bronchi and vessels (V) in the caudal part of the lung are difficult to see because the abdomen overlies that part of the lung. Without careful scrutiny, it would be easy to miss the abscess (A) because it is obscured by the abdominal contents.

Alveolar ducts and alveoli

Gas exchange occurs in the alveolar ducts and alveoli. The former are extensions of the bronchioles and can form several generations, each of which has numerous alveoli in its walls. The alveolar structure of the lung results in a large surface area for gas exchange. In a 510-kg horse, the total alveolar surface area is reported to be 2,456 m^2 (Gehr & Erni 1980) (one-quarter of a hectare) of which about 1,500 m^2 is in contact with pulmonary capillaries and available for gas exchange (Gehr & Erni 1980, Stone et al 1992). Two types of epithelial cells line the alveoli. The terminally differentiated squamous type I cell covers most of the surface but the cuboidal type II cell is more numerous (Stone et al 1992). The type I cell is characterized by very thin cytoplasmic extensions that extend away from the nucleus over the alveolar surface in the same way that egg white extends away from the egg yolk in a frying pan. Its cytoplasm has few organelles other than pinocytotic vesicles and a few mitochondria. By contrast with the type I cell, the type II cell shows evidence of being metabol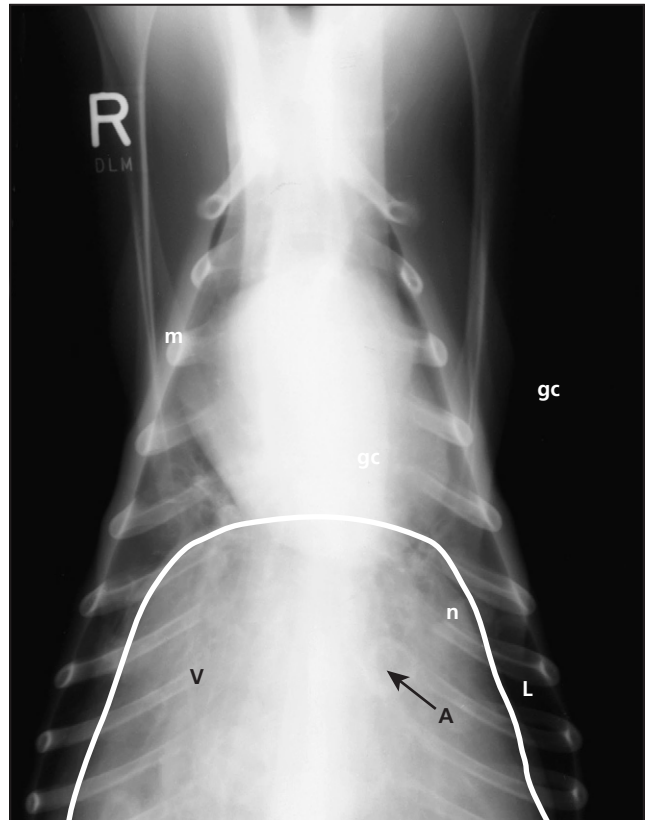ically very active. Its cytoplasm is rich in endoplasmic reticulum and the Golgi apparatus is large. The characteristic feature of the type II cell is the presence of large vesicles that contain the precursors of pulmonary surfactant, the phospholipid that is essential for lung stability. Surfactant is released from the type II cells in the form of myelin coils that unfurl when they reach the alveolar surface. Type II cells also re-uptake components of surfactant to be resynthesized. In addition to production of surfactant, type II cells also reabsorb edema fluid from the alveoli, and, when the alveolar surface is injured and type I cells are lost, the type II cells differentiate into type I cells to recover the surface.

The alveolar septum

Neighboring alveoli are separated by the alveolar septum that contains the pulmonary capillaries (Fig. 1.15). The air is separated from the capillary blood by the type I epithelial cell, a basement membrane, a variable amount of interstitium and the endothelial cell. The quantitative
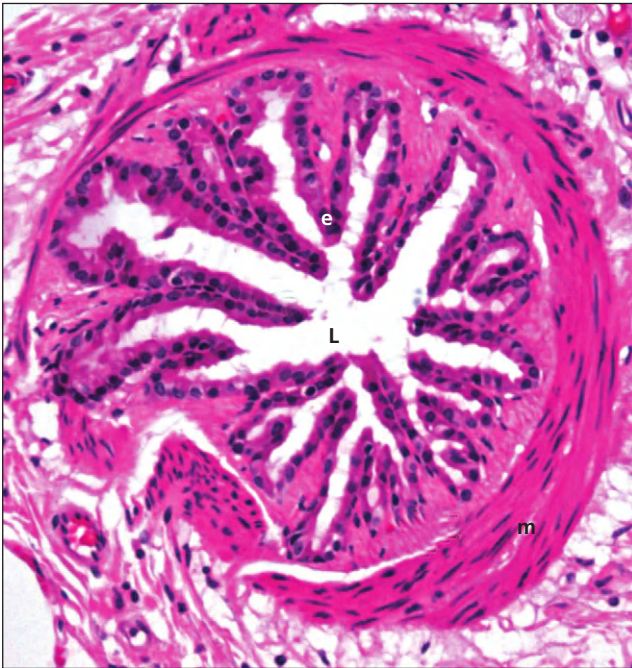
Fig. 1.14. Bronchiole from a healthy horse. The epithelial layer (e) consists of a single layer of cuboidal cells that encircles the lumen (L). The epithelium is folded because the lung was collapsed when the section was taken. In life the perimeter of the airway would form a circle. A layer of smooth muscle (m) encircles the airway. There are no mucus-producing goblet cells in this healthy bronchiole. (Hematoxylin and eosin)

characteristics of this barrier have been described in the horse and other species (Stone et al 1992). On one side of the septum, the separation of air and blood is less than 0.1 µm because there is no interstitium and few organelles in the epithelial or endothelial cells. On the opposite side, the membrane is somewhat thicker because an interstitial space separates the two cell types and they contain more organelles. It is thought that gas exchange occurs on the thin side of the septum and fluid exchange between the capillary and interstitium occurs on the thicker side

(Fig. 1.16). The capillary network in the septum is so extensive that it has been compared to a sheet of blood. Because there are no lymphatics in the alveolar septum, fluid that filters from the pulmonary capillaries must track through the interstitium to the lymphatics in the peribronchial tissue.

Lymphatic networks

The lung possesses two networks of lymphatics. One surrounds the bronchi while the other is subpleural. Both these networks connect to the hilar and mediastinal lymph nodes and drain into the thoracic duct.

The Pulmonary Circulation

The pulmonary circulation receives the whole output of the right ventricle and delivers it through the pulmonary capillaries and back to the left atrium. It is the branch of the circulation involved in the uptake of oxygen and removal of carbon dioxide. Its anatomy and function are described in Chapter 3.

The Bronchial Circulation

The bronchial circulation is a branch of the systemic circulation from which it receives about 2% of the cardiac output (Magno 1990). It provides the nutritional blood flow to the walls of the bronchi and large blood vessels and to the pleura. In the bronchi, the submucosal plexus of bronchial vessels is important for warming and humidifying air and in the immune response. As the intensity of exercise increases, so does the magnitude of bronchial circulatory blood flow (Manohar et al 1992). The venous drainage of the bronchial circulation is complex. Some returns to the azygos vein but some also enters the pulmonary veins thereby adding venous blood to the oxygenated blood that is leaving the capillaries. If the blood supplied by the pulmonary circulation becomes reduced in
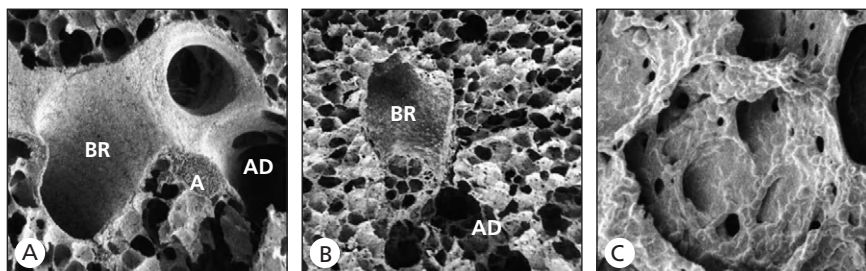
Fig. 1.15. Scanning electron micrographs of the alveoli of a horse. (A) An artery (A) adjacent to several bronchioles (BR). An alveolar duct (AD) and alveoli are also visible. (B) A bronchiole (BR) terminating in an alveolar duct (AD) and surrounded by alveoli. (C) Several alveoli that have been overdried during preparation so that the outlines of the numerous erythrocytes in the alveolar capillaries are visible. Reproduced with the permission of W.S. Tyler, University of California, Davis, CA.

Fig. 1.16. Transmission electron micrograph of a pulmonary capillary in the alveolar septum. The septum separates two alveoli. On the lower side of this capillary, the endothelium and epithelium are separated by a thick layer of interstitium that allows movement of interstitial fluid within the alveolar septum. On the upper si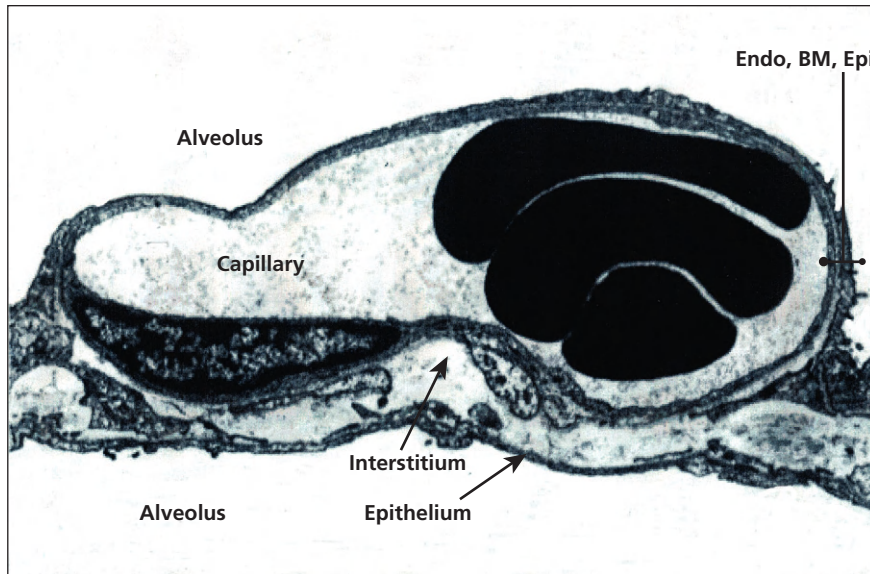de, the endothelium (Endo) and epithelium (Epi) are separated only by a thin common basement membrane (BM). This thin side, which is less than 1 μm in thickness, is probably where most gas exchange occurs.

a region of lung, the connections between the pulmonary and bronchial circulations allow blood to enter that region from the bronchial circulation thereby tending to reduce the chance of ischemia.

The bronchial circulation is involved in inflammation, healing and remodeling of the lung. In horses with lesions of exercise-induced pulmonary hemorrhage, the bronchial circulation proliferates in the walls of the inflamed airways (O'Callaghan et al 1987). In humans, this neovascularization can be a cause of hemoptysis.

The Thorax

The horse's thorax has 18 ribs that form a fairly rigid protection for the intrathoracic organs. Each rib articulates dorsally with the vertebral column and is extended ventrally by a costal cartilage. The costochondral junctions can be palpated beneath the skin in a line that follows roughly a line drawn between the tuber sacrale and the elbow. The first eight ribs articulate directly with the sternum. The costal cartilages of ribs 9 to 17 are attached to each other by elastic tissue to form the costal arch. The last rib is a floating rib. The thorax is long and laterally compressed at its anterior end. Because of the downward curvature of the vertebra in the anterior thorax, the thoracic inlet is only 18–20 cm high and it is about 10 cm wide. The proximal forelimb covers the cranial part of the thoracic wall to the level of the fifth or sixth rib (Fig 1.11), while the thinner serratus ventralis thoracis muscle covers much of the first eight or nine ribs. The thoracic wall con-

sists of the skin, subcutaneous tissues, intercostal muscles, ribs, parietal pleura, sternum and thoracic vertebrae.

The diaphragm is attached to the thoracic wall along the eighth to tenth costal cartilages, then to the costochondral junctions of ribs 10 to 13 and then to the ribs at increasing distances from their costochondral junctions until it reaches the last intercostal space. In the median plane, each hemidiaphragm extends cranially to the level of the fifth or sixth rib, which approximates to the level of the olecranon in the standing horse. Because the cupola of the diaphragm extends so far cranially and its attachments are to each rib, much of the thoracic cavity is lateral to or above the abdomen (Figs 1.11 and 1.13). For this reason, borborygmi originating in the intestines are frequently heard during auscultation of the lung. The diaphragm consists of a tendinous center through which passes the vena cava. The striated muscle is arranged around the diaphragm's periphery. It consists of the pars costalis, which originates from the ribs and inserts into the central tendinous portion, and the left and right crura, which are connected to the vertebrae by tendons and insert in the tendinous center of the diaphragm. The left crus is pierced by the aorta, esophagus and vagus nerve. The phrenic nerve innervates the muscles of the diaphragm.

The pleurae

The pleurae cover all of the surfaces of the thoracic cavity without interruption. The visceral pleura covers the lungs and joins the mediastinal pleura at the hilar region. The

parietal pleura is described as costal, diaphragmatic and mediastinal where it covers the ribs, diaphragm and mediastinal structures, respectively. In some horses the two pleural sacs communicate via small fenestrations where the two thin layers of mediastinal pleura are apposed in the caudal mediastinum. The pleural and peritoneal cavities communicate via diaphragmatic pores and lymphatics. The parietal pleura has numerous stomata connected to subpleural lymphatics that serve to remove excessive pleural fluid, protein, and cells. The pleural cavity is a potential space that normally contains only a small volume of clear to slightly turbid yellowish, non-clotting pleural fluid. The chest wall and parietal pleura, but not the visceral pleura, are well endowed with sensory nerve fibers from the intercostal nerves, and consequently pleural inflammation can cause overt pleurodynia (thoracic pain). The parietal pleura receives its blood supply from the intercostal vessels, with the principal vessels running immediately caudal to each rib. Blood supply to the visceral pleura is from the pulmonary and bronchial vasculature with capillary loops occupying the deep aspect of the pleura.

The pleurae comprise a single layer of mesothelial cells and underlying connective tissue. Mesothelial cells not only act as an envelope lining the pleural cavities but also have an active role in trans-serosal transport of fluid and electrolytes, aided by apical microvilli that increase their surface area for absorption. Mesothelial cells also synthesize components of the underlying connective tissue, cytokines, growth peptides and chemotaxins. Inflammatory stimuli activate mesothelial cells leading to increased production of biologically active compounds, fibrinolysis and possibly phagocytic function.

REFERENCES

Art T, Lekeux P 1991a The effect of shape, age and extension on the compliance of equine tracheal segments. Veterinary Research Communications 15: 135–146

Art T, Lekeux P 1991b Mechanical properties of the isolated equine trachea. Research in Veterinary Science 51: 55–60

Baptiste KE, Naylor JM, Bailey J et al 2000 A function for guttural pouches in the horse. Nature 403: 382–383

Barakzai S 2004 The equine paranasal sinuses. Part 1: Anatomy. UK Vet 9: 1–3

Bell BT, Baker GJ, Abbott LC et al 1995 The macroscopic vascular anatomy of the equine ethmoidal area. Anatomy Histology and Embryology 24: 39–45

Broadstone RV, Scott JS, Derksen FJ et al 1991 *In vitro* response of airway smooth muscle from horses with recurrent airway obstruction. Pulmonary Pharmacology 4: 191–202

Gehr P, Erni H 1980 Morphometric estimation of pulmonary diffusion capacity in two horse lungs. Respiration Physiology 41: 199–210

Holcombe SJ, Derksen FJ, Stick JA et al 1997 Effect of bilateral tenectomy of the tensor veli palatini muscle on soft palate function in horses. American Journal of Veterinary Research 58: 317–321

Holcombe SJ, Derksen FJ, Stick JA et al 1998 Effect of bilateral blockade of the pharyngeal branch of the vagus nerve on soft palate function in horses. American Journal of Veterinary Research 59: 504–508

Holcombe SJ, Cornelisse CJ, Berney C et al 2002 Electromyographic activity of the hyoepiglotticus muscle and control of epiglottis position in horses. American Journal of Veterinary Research 63: 1617–1621

Kaup F-J, Drommer W, Damsch S et al 1990 Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD) II: pathomorphological changes of the terminal airways and the alveolar region. Equine Veterinary Journal 22: 349–355

Kumar P, Timoney JF 2001 Light and electron microscope studies on the nasopharynx and nasopharyngeal tonsil of the horse. Anatomy Histology and Embryology 30: 77–84

Kumar P, Timoney JF 2005 Histology and ultrastructure of the equine lingual tonsil. I. Crypt epithelium and associated structures. Anatomy Histology and Embryology 34: 27–33

Kumar P, Timoney JF, Southgate HH et al 2000 Light and scanning electron microscopic studies of the nasal turbinates of the horse. Anatomy Histology and Embryology 29: 103–109

Magno M 1990 Comparative anatomy of the tracheobronchial circulation. European Respiratory Journal 12: 557s–562s; discussion 562s–563s

Manglai D, Wada R, Endo H et al 2000a Macroscopic anatomy of the auditory tube diverticulum (guttural pouch) in the thoroughbred equine – a silicon mold approach. Okajimas Folia Anatomica Japonica 76: 335–346

Manglai D, Wada R, Kurohmaru M et al 2000b Histological and morphometrical studies on the mucosa of the equine guttural pouch (auditory tube diverticulum). Okajimas Folia Anatomica Japonica 77: 69–76

Manohar M, Duren SE, Sikkes BP et al 1992 Bronchial circulation during prolonged exercise in ponies. American Journal of Veterinary Research 53: 925–929

McCann JL, Dixon PM, Mayhew IG 2004 Clinical anatomy of the equine sphenopalatine sinus. Equine Veterinary Journal 36: 466–472

McLaughlin RF, Tyler WS, Canada RO 1961 A study of the subgross pulmonary anatomy in various mammals. American Journal of Anatomy 108: 149

O'Callaghan MW, Pascoe JR, Tyler WS et al 1987 Exercise-induced pulmonary haemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. III. Subgross findings in lungs subjected to latex perfusions of the bronchial and pulmonary arteries. Equine Veterinary Journal 19: 394–404

Plopper CG, Mariassy AT, Hill LH 1980 Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung: II. A comparison of horse, steer, sheep, dog, and cat. Experimental Lung Research 1: 155–169

Robinson NE 1982 Some functional consequences of species differences in lung anatomy. Advances in Veterinary Science and Comparative Medicine 26: 1–33

Robinson NE, Sorenson PR 1978 Collateral flow resistance and time constants in dog and horse lungs. Journal of Applied Physiology 44: 63–68

Smith BL, Aguilera-Tejero E, Tyler WS et al 1994 Endoscopic anatomy and map of the equine bronchial tree. Equine Veterinary Journal 26: 283–290

Sonea IM, Bowker RM, Broadstone RV et al 1993a Adrenergic and peptidergic innervation of the trachealis muscle in the normal horse: a preliminary report. Research in Veterinary Science 54: 335–339

Sonea IM, Bowker RM, Robinson NE et al 1993b Distribution of dopamine β-hydroxylase and neuropeptide Y immuno-reactive nerves in healthy equine lungs. American Journal of Veterinary Research 54: 507–513

Sonea IM, Bowker RM, Robinson NE et al 1994a Substance P and calcitonin gene-related peptide-like immunoreactive nerve fibers in lungs from adult equids. American Journal of Veterinary Research 55: 1066–1074

Sonea IM, Bowker RM, Robinson NE et al 1994b Distribution of SP- and CGRP-like immunoreactive nerve fibers in the lower respiratory tract of neonatal foals: evidence for loss during development. Anatomy and Embryology (Berlin) 190: 469–477

Sonea IM, Bowker RM, Robinson NE 1999 Distribution of substance P binding sites in equine airways. Equine Veterinary Journal 31: 238–242

Stone KC, Mercer RR, Gehr P et al 1992 Allometric relation-ships of cell numbers and size in the mammalian lung. American Journal of Respiratory Cell and Molecular Biology 6: 235–243

Tessier C, Holcombe SJ, Derksen FJ et al 2004 Effects of stylo-pharyngeus muscle dysfunction on the nasopharynx in exercising horses. Equine Veterinary Journal 36: 318–323

Tyler WS, Gillespie JR, Nowell JA 1971 Modern functional morphology of the equine lung. Equine Veterinary Journal 3: 84–94

Widdicombe JH, Pecson IS 2002 Distribution and numbers of mucous glands in the horse trachea. Equine Veterinary Journal 34: 630–633

Yu M, Robinson NE, Wang Z et al 1992 Muscarinic receptor subtypes in equine tracheal smooth muscle. Veterinary Research Communications 16: 301–310

Yu M, Wang Z, Robinson NE et al 1994 Inhibitory nerve distribution and mediation of NANC relaxation by nitric oxide in horse airways. Journal of Applied Physiology 76: 339–344

How Horses Breathe: the Respiratory Muscles and the Airways

N Edward Robinson

A maximally exercising thoroughbred horse consumes 75 liters or 19 gallons of oxygen per minute. This oxygen consumption of $150 \ ml \cdot kg^{-1} \cdot min^{-1}$, which is one of the highest among mammals, is the result of densely packed mitochondria in the skeletal muscles (Kayar et al 1989). The delivery of oxygen from the air to the mitochondria begins with ventilation, which is the process that moves air into and out of the lung. To supply its oxygen demand, the galloping thoroughbred inhales a tidal volume of 13 liters about 120 times per minute, which results in a minute ventilation of approximately 1400 liters/min. Moving 13 liters of air into and out of the lung within half a second requires peak airflows of up to 90 liters/second.

The Pathway for Oxygen

Oxygen reaches the mitochondria from, and carbon dioxide is eliminated to, the atmosphere by processes of bulk flow, diffusion and transport in the blood. At all stages of delivery, oxygen is moving down a concentration gradient that is measured as the oxygen partial pressure (P_{O_2}) (Fig. 2.1). Atmospheric air enters the nares and is distributed among the various branches of the tracheobron-
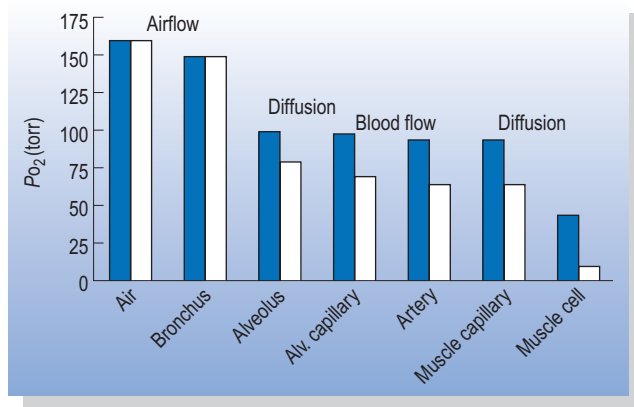
chial tree until it reaches the alveoli. Here, by diffusion, oxygen passes into the pulmonary capillary blood where it combines with hemoglobin to be transported to the tissues, for example, exercising muscle. Because the exercising muscle is continually consuming oxygen, the local tissue P_{O_2} is lower than that of arterial blood. This allows diffusion of oxygen from hemoglobin through plasma into the tissues. At the same time, carbon dioxide is leaving the tissues and entering the blood to be returned to the lungs and eliminated by ventilation. This whole process is carefully regulated so that ventilation, blood flow through the lungs to the tissues, and gas transport in the blood are adjusted to meet the needs of exercise and other metabolic demands. This chapter describes the processes involved in ventilation. Pulmonary blood flow and gas exchange are described in Chapters 3 and 4, respectively.

What are Airways?

The airways begin at the external nares and terminate at the alveoli. Between these points is a series of branching tubes of decreasing diameter (see Chapter 1), the main purpose of which is to conduct air into and out of the alveoli. Secondary, but also vital, functions of the airways include defense of the lungs against airborne irritants, infectious agents, and toxins, and warming and humidification of air. These functions occur to varying degrees at different levels of the airways (Table 2.1).

Replenishing the Air in the Lungs

Once the newborn foal takes its first breath, the lungs never empty completely. At all times, the lungs contain a large volume of air that allows gas exchange to continue as some of the used air is eliminated during exhalation and some new air is brought in during inhalation.

Volume expansion in the lung occurs in the alveolar ducts and alveoli

During inhalation, the increased capacity of the lung is brought about by expansion of the alveoli and alveolar ducts. A network of elastic fibers ramifying throughout the alveolar septa and walls of the terminal airways and

Fig. 2.1. Oxygen is delivered from the atmosphere to the muscle cells down a partial pressure (P_{O_2}) gradient that is steeper during exercise (white bars) than at rest (blue bars). Oxygen is transported by airflow from the atmosphere into the smaller airways, by diffusion within the alveoli and into the blood, by vascular transport to the capillaries, and then by diffusion into the tissues.

Table 2.1. Major functions of the divisions of the airways	
Airway	**Functions**
Nasal cavity	High-velocity airflow Warming and humidification of air Thermoregulation by evaporative heat loss Removal of large particulates Removal of soluble pollutants
Pharynx	High-velocity airflow Separation of air and food
Larynx	High-velocity airflow Regulation of airflow Separation of air and food Phonation
Trachea	High-velocity airflow Warming and humidification Removal of moderate-sized particulates Removal of soluble pollutants Initiation of cough
Bronchi	Moderate-velocity airflow Distribution of air within the lung Removal of moderate-sized particulates Initiation of cough
Bronchioles	Low-velocity airflow Distribution of air to gas exchange units Removal of small particulates
Alveoli	Air moves by diffusion Gas exchange with pulmonary capillary blood Phagocytosis of small particulates

connected to the pleura and larger bronchi provides this part of the respiratory tract with elasticity. In addition, the air–liquid interface in the alveoli generates a surface tension that is also responsible for lung elasticity.

Pulmonary surfactant greatly reduces the surface tension of the alveolar lining

Type II alveolar epithelial cells produce pulmonary surfactant without which the surface tension of the lung lining fluid would be so high that the lung would collapse. Surfactant is a mixture of phospholipids, primarily dipalmitoyl phosphatidylcholine, and proteins. It seeks the surface of the alveolar lining fluid and by displacing water molecules it reduces the surface tension. As the lung decreases in volume during exhalation, the surfactant molecules became more concentrated on the surface and further reduce surface tension, which makes the alveoli very stable at low lung volumes. The proteins in surfactant have a variety of functions concerned with recruitment of surfactant to the lung surface, recycling of surfactant into type II cells, and antibacterial defense. In horses, changes in surfactant activity have been described after exercise (Morrison et al 1999) and changes in surfactant content

have been reported after transportation (Hobo et al 1997). The functional significance of these changes awaits further investigation.

The presence of adequate amounts of surfactant is essential for lung stability after birth. While the use of naturally derived and synthetic surfactants is routine procedure in the treatment of premature humans, their use is only beginning to be explored in neonatal and premature foals.

Lung volumes

The volume of air inhaled in each breath is known as a tidal volume. In a resting 500-kg horse, tidal volume averages 4–5 liters. At the end of a tidal exhalation, the lung still contains about 20 liters of air (approximately 40 ml/kg) (Aguilera-Tejero et al 1993), about 45% of its total lung capacity. This volume of air, known as the functional residual capacity, provides a reservoir so that gas exchange continues unabated between each breath. Maintaining a functional residual capacity also ensures that the airways and alveoli stay open, which decreases the work required to inhale. In many mammals, including humans, functional residual capacity represents the mechanical equilibrium of the respiratory system where the inward pull of the lungs is balanced by the outward recoil of the rib cage. By contrast, in horses, the mechanical equilibrium of the respiratory system occurs in the midst of the tidal volume. This has important consequences for the strategy that horses use to breathe (see below).

Total lung capacity is the maximal volume of air that can be held by the respiratory system and residual volume is the air remaining after a maximal exhalation. These two volumes are determined by the mechanical limits of the lung and chest wall. Vital capacity is the difference between the two and is the maximal possible tidal volume. In horses, vital capacity measured by forcefully inflating and then deflating the lungs is about 42 liters (Couetil et al 2000). It is unlikely that a horse ever takes a breath equal to the vital capacity.

Breathing in the Resting Horse

Because the mechanical equilibrium of the respiratory system of the horse lies at the midpoint of a normal tidal volume, the resting horse uses a biphasic pattern of breathing with passive and active components to both inhalation and exhalation (Fig. 2.2) (Koterba et al 1988). This pattern of breathing can be easily observed by watching a horse's flanks. The lung volume at end exhalation is below the equilibrium position of the respiratory system so that inhalation begins by allowing the respiratory system to passively expand back to the equilibrium position. This can be seen as a rapid outward movement of the lower abdomen. The second part of inhalation involves active
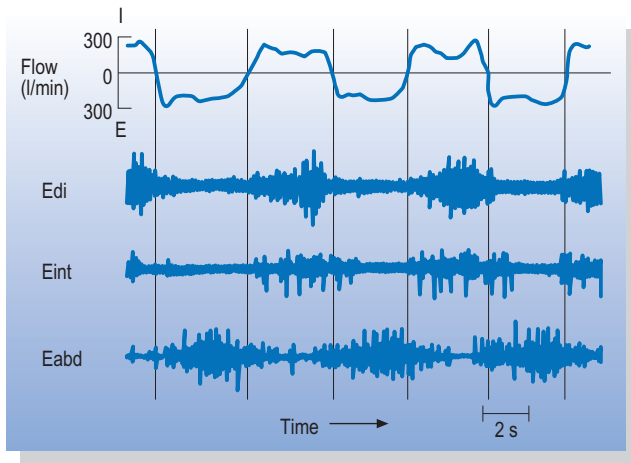
Fig. 2.2. Airflow and respiratory muscle EMG activity in a resting horse. The upper trace shows airflow with inhalation upwards and exhalation downwards. Note that inhalation and to a lesser extent exhalation are biphasic. Electrical activity in the diaphragm (Edi) is greatest during the second peak of inhalation. Intercostals (Eint) are active during inhalation and the early exhalation. Abdominal muscle activity (Eabd) peaks in the latter half of exhalation but persists somewhat into early inhalation. Redrawn from Koterba et al, 1988, Fig. 5, with permission.

Fig. 2.3. Tidal volume and ventilation in exercising horses. (A) Tidal volume (height of total bar), physiological dead space volume (height of gray bar), and alveolar volume (height of blue bar). (B) Minute ventilation (height of total bar), dead space ventilation (height of gray bar), and alveolar ventilation (height of blue bar). As exercise intensity increases, tidal volume and minute ventilation increase but the physiological dead space remains constant so that more of each breath participates in gas exchange (alveolar ventilation). The dead space to tidal volume ratio is indicated above each bar. Drawn from data in Pelletier & Leith, 1995, with permission.

Fig. 2.4. Effect of prolonged heavy exercise on minute and alveolar ventilation. As exercise at a constant workload progresses, alveolar ventilation remains virtually constant but minute ventilation increases because the horse requires more dead space ventilation for thermoregulation. Redrawn from Hopkins et al, 1998, Fig. 1, with permission.

contraction of the diaphragm and other inspiratory muscles that is visible as an expansion of the caudal part of the rib cage. Exhalation begins with passive recoil of the respiratory system down to its equilibrium position (inward movement of the caudal ribs) and ends with contraction of the abdominal muscles that is visible as an upward movement of the abdomen. The latter forces the respiratory system below its equilibrium so that the next inhalation begins with relaxation of the abdomen. The combination of both passive and active components to inhalation and exhalation results in a biphasic pattern of airflow in the resting horse.

Not all the air entering the lungs participates in gas exchange

In the resting horse, only one-third of each breath participates in gas exchange by reaching the alveoli (Pelletier & Leith 1995, Hopkins et al 1998). The rest of the breath occupies the dead space, that is the conducting airways and some unperfused or poorly perfused alveoli. Thus out of the total minute ventilation of 60–100 liters/min, one-third is alveolar ventilation and two-thirds is dead space ventilation (Fig. 2.3).

Ventilation Increases During Exercise

As the gas exchange demands increase during exercise, there is a need for more minute ventilation; this increase is accomplished by an increase in both tidal volume and respiratory frequency. Unlike the situation in the resting
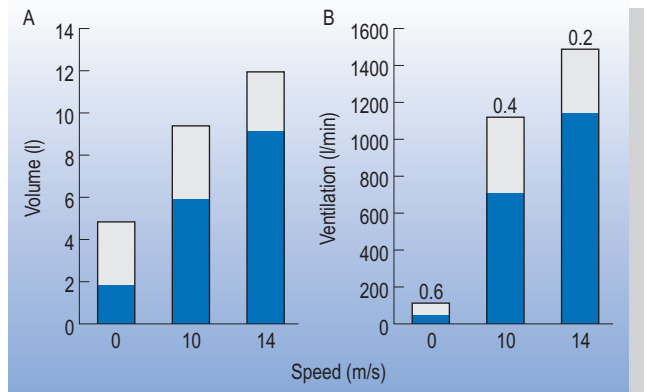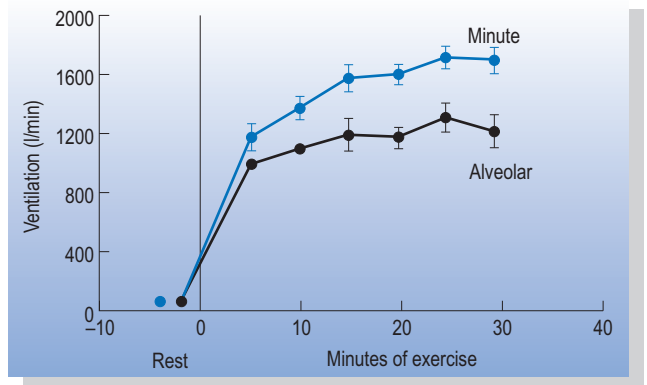
horse, most of the minute ventilation participates in gas exchange as alveolar ventilation increases and the dead space to tidal volume ratio decreases during exercise (Fig. 2.3). As the horse exercises for longer periods, however, its dead space ventilation increases to meet the demands of thermoregulation (Fig. 2.4) (Hopkins et al 1998).

Breathing pattern during exercise

At the walk and trot, there is no clear relationship between the frequency of breathing and footfall (Hornicke et al 1982). However, at the canter and the gallop, breathing is
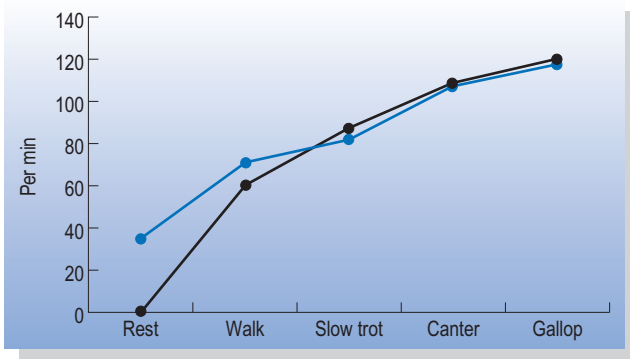
Fig. 2.5. Relationship between respiratory and step frequencies. At slow gaits, step frequency (black line) is unrelated to breathing frequency (blue line). At the canter and gallop, the two frequencies are identical. Drawn from data in Hornicke et al, 1982, with permission.

synchronized with gait (Fig. 2.5), so that inhalation occurs when the forelimbs extend and exhalation occurs when the forelimbs are on the ground and hind limbs are moving forwards. The horse usually takes one breath per stride but when it sighs, it takes two strides to inhale the larger tidal volume. The coordination between gait and breathing is also altered if the horse is having difficulty breathing, for example, as the result of an airway obstruction. In the latter situation, the horse may need more time to inhale and may therefore take two strides to inhale.

The coordination of gait and breathing can help in the diagnosis of airway obstructions

Certain types of upper airway obstructions, for example recurrent laryngeal neuropathy, cause dynamic collapse of the airway during inhalation while others, for example dorsal displacement of the soft palate, cause obstruction during exhalation. Temporally relating the occurrence of the abnormal sounds to the phase of the stride can help to determine if the obstruction is occurring during inhalation or exhalation. For example, if the abnormal sound occurs while the forelimbs are being extended, the obstruction is occurring during inhalation. If it occurs when the forelimbs are weight bearing, the obstruction is occurring during exhalation.

The Respiratory Muscles

The diaphragm is the principal inspiratory muscle

Contraction of the respiratory muscles provides the energy necessary to move air between the atmosphere and the lungs. The diaphragm, which is the principal inspiratory muscle, is dome-shaped with a tendinous center that reaches forward to contact the heart. The muscles of the diaphragm are at its margin and muscle fibers are aligned
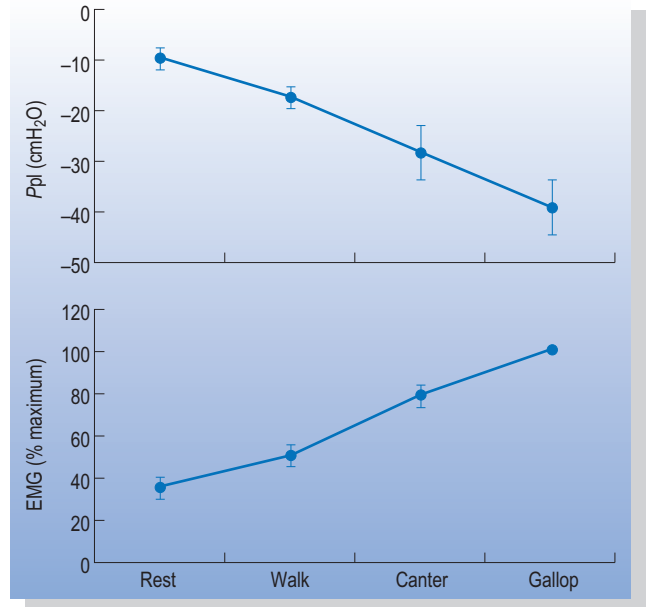

Fig. 2.6. Effect of exercise on pleural pressure change during breathing (*P*pl) and on diaphragmatic EMG activity. As exercise intensity increases, the diaphragm becomes increasingly active and this leads to a greater decrease in pleural pressure. Redrawn from Ainsworth et al, 1996, with permission.
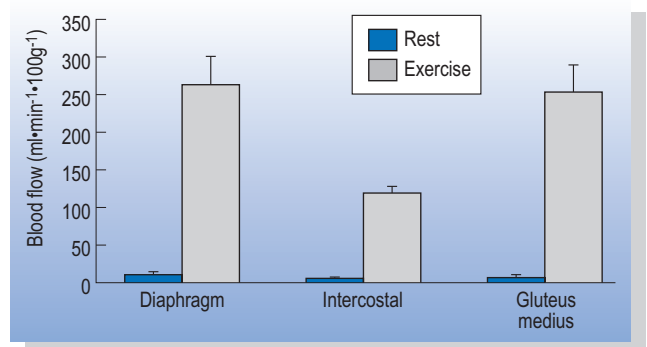

Fig. 2.7. Effect of exercise on blood flow in respiratory and locomotor muscles. Diaphragmatic and gluteal (locomotor) blood flow increase to similar degrees and more than intercostal blood flow. Drawn from data in Manohar, 1986, with permission.

so that contraction pulls the tendinous center of the diaphragm backwards, thus making it flatter and thereby enlarging the thorax and compressing the abdominal contents. Diaphragmatic activity increases as exercise intensity increases and concurrently the magnitude of the decrease in pleural pressure also increases (Fig. 2.6) (Ainsworth et al 1996). The increase in diaphragmatic blood flow during exercise is dramatic and similar to that in the muscles of locomotion (Fig. 2.7) (Manohar 1986). Diaphragmatic activity also increases during the hyperpnea associated with hypoxia or hypercapnia and when the

horse breathes against an airway obstruction such as that provided by laryngeal hemiplegia. The diaphragm has a large functional reserve of oxidative capacity so that even during heavy work it seems able to generate its energy by aerobic metabolism (Manohar et al 1988, 1992, Poole et al 2002). The hypoventilation that occurs in cases of diaphragmatic paralysis indicates the importance of the diaphragm as a respiratory muscle (Amory et al 1994).

Intercostals are active during inhalation

In most mammals, the external intercostal muscles are active during inhalation and serve to move the ribs forwards and outwards, so enlarging the thorax. In the horse, there is very little change in chest circumference during quiet breathing and therefore the contribution of the external intercostal muscles to thoracic enlargement is probably quite small. Under certain circumstances, for example in post-exercise hyperpnea, chest circumference increases during inhalation. Surprisingly, during exercise, the chest circumference decreases slightly rather than increasing during inhalation and therefore contributes nothing to the large tidal volume (Marlin et al 2002). This decrease in circumference is probably a result of the large negative pressures being generated in the thorax by contraction of the diaphragm. Even though the chest circumference does not increase, the increase in intercostal muscle blood flow during exercise (Manohar 1986) indicates that these muscles are active and probably stiffening the rib cage to resist the deformation caused by the change in pleural pressure. Not only does the chest circumference not change much during exercise, neither does the abdominal circumference. This leads to the conclusion that most of the large tidal volume taken in during exercise is accommodated by the elongation of the thoracoabdominal segment (Young et al 1992, Marlin et al 2002) that is occurring in synchrony with gait and with diaphragmatic contraction.

Other muscles that are active during inhalation

Other muscles that are active during inhalation include the abductor muscles of the larynx, pharynx, and external nares. Activity in the latter group of abductor muscles begins late in exhalation and peaks early in inhalation so that the upper airway is dilated and stiffened before it is exposed to the subatmospheric pressures that are generated during inhalation. Failure of the abductor muscles to activate during inhalation can give rise to dynamic collapse of the upper airway. Laryngeal hemiplegia, which results from recurrent laryngeal neuropathy, is a prime example of dynamic collapse that is a result of the failure of abductor muscle function, in this case the cricoarytenoideus dorsalis. In other species, the muscles that connect the sternum to the head, for example the sternocephalicus, become active during intense exercise and serve to pull the sternum forward.

Exhalation involves contraction of abdominal muscles

As noted above, exhalation results in part from the elastic recoil of the respiratory system towards its equilibrium position but also from activity of expiratory muscles, primarily the abdominal muscles (Koterba et al 1988). Measurements of blood flow during exercise suggest that the internal abdominal oblique muscle is more active than the transversus abdominis, rectus abdominis, or external abdominal oblique muscles (Manohar et al 1992). When the abdominal muscles contract, abdominal pressure increases and the diaphragm is pushed forwards, thus reducing the volume of the thorax. The internal intercostal muscles are active during exhalation. Because there is little change in chest circumference, these muscles probably stiffen the ribs to resist the increase in intrathoracic pressure caused by cranial movement of the diaphragm. Muscular activity is not required to keep the upper airway open during exhalation because pressure within the airway is greater than atmospheric pressure and therefore passive airway dilatation occurs.

During Breathing there are Cyclic Changes in Pleural Pressure

During inhalation, contraction of the respiratory muscles enlarges the thorax and this causes a decrease in pleural pressure (Ainsworth et al 1996) (Fig. 2.6) that is used to stretch the lung, to generate airflow against the frictional resistance provided by the tracheobronchial tree, and to accelerate and decelerate the inspired air. During exhalation, the energy stored in the stretched lung plus that generated by the expiratory muscles increases the pleural pressure and generates airflow against the airway resistance.

At the end of exhalation in the resting horse, pleural pressure is between -3 and $-5\,\mathrm{cmH_2O}$, that is, 3 to $5\,\mathrm{cmH_2O}$ below atmospheric pressure. This subatmospheric pressure occurs because the lung is stretched and therefore trying to collapse away from the rib cage. If the rib cage is opened, the subatmospheric pressure causes air to rush into the pleural cavity and the lung collapses (Fig. 2.8).

When the inspiratory muscles contract and the thorax enlarges, pressure in the pleural cavity decreases, that is, it becomes more subatmospheric. The lung is expanded by this decrease in pleural pressure and expansion decreases the pressure within the alveoli so that air flows into the lung. In the resting horse, pleural pressure decreases to about $-10\,\mathrm{cmH_2O}$ but, during intense exercise, pleural pressure decreases to $-30\,\mathrm{cmH_2O}$ or less (Fig. 2.6). During exhalation, pleural pressure increases (becomes less negative) and, even in the resting horse, transiently may become slightly positive with respect to atmospheric pressure. As exercise intensity increases and expiratory muscles
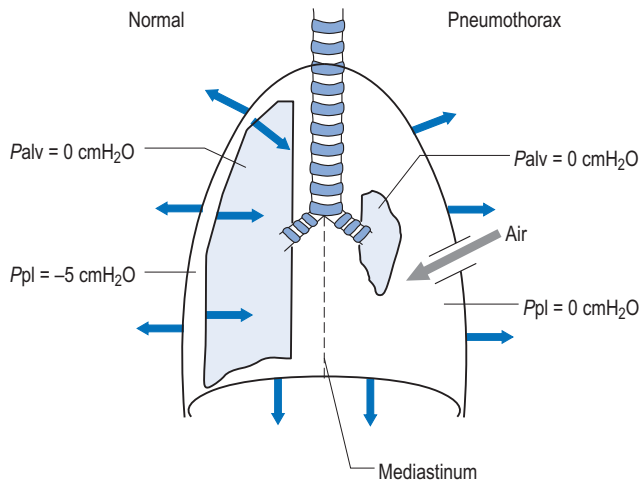
Fig. 2.8. Mechanisms of pneumothorax. On the left is the normal hemithorax in which the lung is inflated. The outward recoil of the thoracic cage (blue outward arrows) balances the inward recoil of the lung (blue inward arrows). As a result of these opposing forces, there is a slightly subatmospheric (–5 cmH$_2$O) pleural pressure (Ppl). The alveolar pressure (Palv) is atmospheric (zero) because the alveoli are connected to the atmosphere by the tracheobronchial tree. On the right side, the injury to the thorax allows air to rush into the thorax until the pleural pressure equals atmospheric pressure. The loss of the negative pleural pressure allows the lung to collapse until it has no more elastic recoil.

Fig. 2.9. Schematic representation of the tracheobronchial tree showing the increase in total cross-sectional area between the trachea and bronchioles. The velocity of airflow is represented by the triangle that shows the highest velocity in the trachea and decreasing velocity toward the peripheral airways.

are recruited, pleural pressure becomes increasingly positive during exhalation, reaching a value of about +30 cmH$_2$O at the gallop (Slocombe et al 1991).

What is ΔPpl_{max}?

The ΔPpl_{max} is the maximal change in pleural pressure during tidal breathing and is the difference between peak inspiratory and peak expiratory pleural pressure. In the resting healthy horse ΔPpl_{max} averages less than 10 cmH$_2$O; with exercise it increases to 60 cmH$_2$O or more (Slocombe et al 1991) to cause greater enlargement of the lung and generate the higher airflow that occurs during exercise.

Measurement of the ΔPpl_{max} can be used as a simple indicator of lung function (Robinson et al 1999). An increase in ΔPpl_{max} is simply an indicator that the respiratory muscles are working harder. Such an increase in ΔPpl_{max} can simply be a result of a large tidal volume or high airflow rates but it can also indicate that the lung is more difficult to inflate because the airways are obstructed or the lung parenchyma is stiffer than normal, that is the lung has a low compliance.

Frictional Resistance of the Air Passages Opposes Airflow

Frictional resistance opposes movement of any fluid such as air when it moves through a series of tubes. In the resting horse, about half of the frictional resistance to
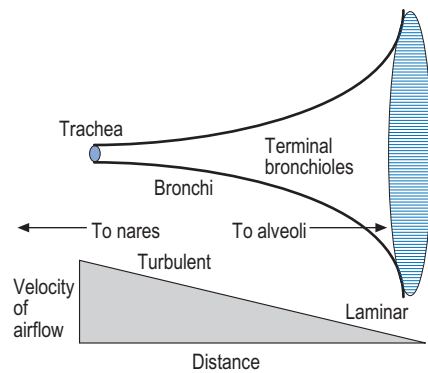
breathing is provided by the nasal cavity, one-quarter by the trachea and the remainder by the bronchi and bronchioles (Art et al 1988). Within the nose, the greatest point of resistance is just inside the nares at the nasal valve and this is the part of the respiratory tract that is dilated by application of the commercially available strip (Holcombe et al 2002).

The magnitude of the resistance of a tube is determined primarily by the tube's diameter and to a much lesser extent by the tube's length. This can be seen in the following equation:

$$R = 8\,u\,l/r^4$$

where R is the resistance, u is the viscosity of air, l is the length of the airway and r is the radius of the airway.

Using this formula, it is possible to compare the resistance of an individual bronchiole with that of the trachea. A bronchiole with a length of 1 cm and a radius of 1 mm has a resistance that is almost 400 times greater than the trachea with a length of 1 m and a radius of 2.5 cm. When trying to understand the distribution of resistance within the tracheobronchial tree, it is important to realize that the number of airways doubles at each bifurcation so that while there is only one trachea, there are 1,024 tenth-generation bronchi. Although the exact number of generations of bronchi and bronchioles has not been determined in horses, it approaches 50. This means that there are many thousands of bronchioles running in parallel in the periphery of the lung. The sum total of the cross-sectional area of all these bronchioles is much greater than the cross-sectional area of the trachea and for this reason the resistance contributed by the bronchioles is much less than that contributed by the larger airways (Fig. 2.9). The type of airflow occurring within a tube also determines resistance and is least when flow is laminar and greatest when flow is turbulent. In the

respiratory system flow is turbulent in the larger airways, that is, in the upper airway, trachea and larger bronchi. Flow becomes laminar in the more peripheral airways, including the bronchioles. This further accentuates the contribution of the larger airways to airway resistance.

Flow patterns are responsible for the generation of respiratory sounds

Turbulent airflow generates the breath sounds audible through a stethoscope. In the normal horse, breath sounds are loudest over the trachea and large bronchi because turbulence is greatest in these airways (Fig. 2.9). Breath sounds are least in the lung periphery because flow is laminar. Exercising a horse before auscultation of the lungs accentuates breath sounds because airflow velocity is increased at all levels of the airways.

In disease, any narrowing of the airways tends to increase airflow velocity at the site of obstruction. These increases in velocity, which can be quite local, promote turbulence and lead to the abnormal breath sounds.

Clinical consequences of the distribution of resistance

Because the upper airway and trachea provide most of the frictional resistance, obstruction of these airways can result in dramatic clinical signs and even cause hypoventilation. For example in a foal with guttural pouch tympany, the obstruction of the pharynx causes severe respiratory distress even in the resting animal. By contrast, a horse with bronchiolitis and mucus accumulation in these small airways may show few clinical signs of obstruction at rest. It may only be when the horse is required to exercise that bronchiolar obstruction becomes evident as reduced exercise tolerance. However, extensive and diffuse obstruction of the bronchioles, such as occurs in severe heaves, causes clinical respiratory distress in the resting horse.

Upper Airway Resistance is Actively Regulated

During exercise, many mammals reduce their upper airway resistance by breathing through their mouths, which bypasses the resistance provided by the nasal cavity. Because the horse is an obligate nose breather and does not have the option of breathing through its mouth, it uses other methods to decrease the resistance provided by its nasal cavity. Dilatation of the external nares by abduction of the nasal cartilages, and opening of the nasal valve by contraction of the lateralis nasi muscle both reduce the resistance of the nasal opening. In addition, catecholamines released during exercise cause contraction of the vasculature within the nasal mucosa, which shrinks the mucosa and dilates the nasal cavity.

The pharynx dilates during exercise as a result of the coordinated contraction of pharyngeal dilator muscles. These include the stylopharyngeus that lifts the roof of the pharynx, tensor veli palatini that prevents dorsal bulging of the soft palate, and hyoepiglotticus that pulls the epiglottis ventrally. Overall, the pharynx takes on an almost rectangular cross-section during intense exercise. In addition, contraction of the abductor muscle of the larynx, specifically the dorsal cricoarytenoid muscle, opens the glottis and reduces the resistance provided by the larynx.

Head position affects upper airway resistance

When a horse exercises unrestrained, it extends its head so that there are fewer bends in the airways between the external nares and the lung. During many of the sports activities in which horses are used, the horse is restrained so that its head cannot be extended. Flexion of the head and neck impedes the normal dilatation of the naso-pharynx that occurs during exercise and may tend to accentuate some of the dynamic problems of the upper airway that are described below (Petsche et al 1995).

Some diseases increase upper airway resistance

Several disease processes can increase the resistance of the upper airway by decreasing its diameter. Foreign bodies, neoplasms, abscesses, or distended guttural pouches that impinge on the upper airway can cause severe obstruction. In the nasal cavity, engorgement of the submucosal vascular sinuses can narrow the nasal cavity and obstruct airflow. This can occur in association with Horner syndrome, when loss of sympathetic innervation leads to vascular engorgement in the nasal cavity. These types of problems provide a fixed obstruction that is present during both inhalation and exhalation.

Upper airway obstructions are usually dynamic

Failure of the abductor muscles of the pharynx and larynx to contract during inhalation can also increase the frictional resistance to breathing but, in these cases, the obstruction is dynamic and is present only during inhalation. Dynamic obstructions are a result of pressure changes within the airway acting on poorly supported tissue. They occur primarily during inhalation and during exercise because at this time pressure within the lumen of the airway is subatmospheric and therefore most likely to displace the unsupported tissue into the airway lumen. The displaced tissue reduces the diameter of the airway, which causes airflow velocity to increase at the point of obstruction, just as a river flows most rapidly through a narrow gorge. As a result of the Bernouilli principle, the high-velocity airflow further decreases pressure within the airway lumen and accentuates the obstruction. Recurrent laryngeal neuropathy provides the best example of this
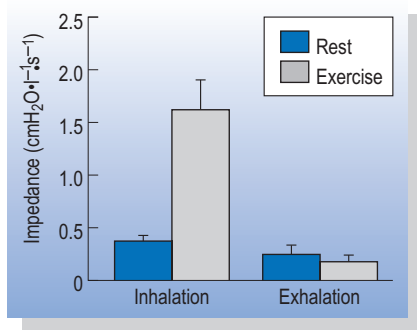
Fig. 2.10. Effect of exercise on upper airway impedance in horses with laryngeal hemiplegia. During exercise impedance to inhalation increases as a consequence of the dynamic collapse of the vocal fold. Impedance during exhalation is unaffected. Drawn from data in Lumsden et al, 1993, with permission.

type of problem (Lumsden et al 1993). The paralyzed dorsal cricoarytenoid muscle cannot abduct the arytenoid cartilage and so the unsupported vocal fold is sucked into the airway lumen during inhalation, thereby increasing the impedance to airflow (Fig. 2.10). Horses with recurrent laryngeal neuropathy have no problem exhaling. Other dynamic inspiratory obstructions include pharyngeal collapse, and collapse of the aryepiglottic folds.

Control of the upper airway muscles

Because failure of the abductor muscles of the upper airway is such an important cause of upper airway obstruction in the horse, it is useful to know how these airways become activated during exercise. The motor nerve supply to the muscles of the pharynx and larynx is provided through cranial nerves IX (glossopharyngeal) and X (vagus). During exercise, neural activity to these muscles increases along with neural activity to other respiratory muscles such as the diaphragm and intercostals. In addition, further activity can be generated when receptors in the pharynx are activated (Sant'Ambrogio et al 1995). These receptors respond to changes in pressure and flow and to cooling. If for example the nose is obstructed, there is less flow of air through the pharynx and/or pressure decreases more than normal within the pharynx. Both of these actions can initiate a reflex increase in the activity of the abductor muscles of the upper airway. Abductor muscle failure could therefore be a result of a lesion within the muscle itself, its motor nerve supply, the sensory receptor, or the sensory nerve (Holcombe et al 2001).

Tracheobronchial Tree Diameter is Affected by Passive and Active Factors

The diameter of the trachea, bronchi, and bronchioles changes passively during breathing and is actively regulated by contraction of smooth muscle. In addition, mucoid

secretions, exudates and mucosal thickening, which are a consequence of inflammation, can narrow the lumen.

Clinical consequences of changes in airway diameter during breathing

The expansion of the lung during inhalation causes dilatation of the intrapulmonary airways. Conversely, during exhalation, the decrease in lung volume reduces the diameter of the airways. These changes in diameter have some important clinical consequences. Wheezes tend to occur at the end of exhalation because, at this point in the respiratory cycle, the airways are narrowed so that obstructions by mucus or bronchospasm are accentuated. Turbulent airflow across the obstruction gives rise to wheezes. Horses with heaves have the highest airflow rate at the end of inhalation and the start of exhalation because this is when the airways have the widest diameter. The typical heave occurs at end exhalation as the horse tries to push air out through very narrowed airways.

Intrathoracic airways can undergo dynamic collapse

Dynamic collapse of intrapulmonary airways normally occurs during coughing when the intrapleural pressure becomes greatly positive and thereby compresses the larger bronchi and trachea. This narrows the lumen of the airway so that air must flow with a high velocity through the narrowed portion and thereby displace any accumulated mucus. Dynamic collapse also occurs during the forced exhalation that is typical of horses with heaves. Under these conditions, the horse activates its expiratory muscles to speed exhalation but by so doing increases the pleural pressure, compresses the airways, increases airway resistance and reduces airflow at the end of exhalation.

Smooth muscle contraction regulates the diameter of the tracheobronchial tree

The most important factor actively regulating tracheobronchial resistance is airway smooth muscle. Airway smooth muscle occurs in the walls of airways from the trachea to the alveolar ducts. In the trachea, the smooth muscle occurs only between the tips of the tracheal cartilages. In the bronchi and bronchioles, a layer of smooth muscle encircles the airways beneath the mucosa. In the alveolar ducts, smooth muscle encircles the mouths of the alveoli. When airway smooth muscle contracts the main result is narrowing of the diameter of the airways.

The purpose of airway smooth muscle is still unknown. It has been suggested that it regulates the amount of anatomic dead space, performs a defensive function to prevent entry of foreign materials into the lung, or that it undergoes peristalsis that helps move mucus. Recently, it was suggested that smooth muscle may have no normal
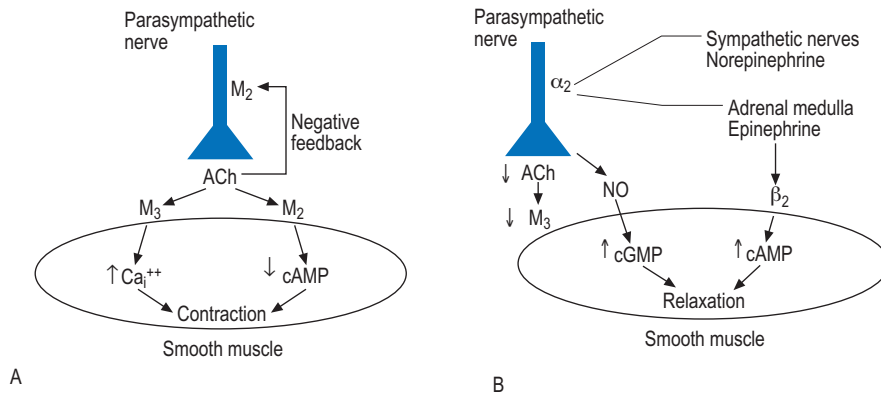
Fig. 2.11. Autonomic regulation of smooth muscle contraction in the airways. (A) Postganglionic parasympathetic fibers release acetylcholine (Ach) that activates the M_3 muscarinic receptor on the smooth muscle membrane, which releases intracellular calcium and causes contraction. In addition, Ach activates M_2 receptors on the smooth muscle cell. This inhibits the production of cAMP, which facilitates contraction. On the nerve fiber, activation of this receptor inhibits Ach release. (B) Inhibition of smooth muscle contraction can occur by activation of β_2-adrenoceptors or by release of nitric oxide (NO) from nerve fibers in the parasympathetic system. Activation of β_2-adrenoceptors, which is primarily by epinephrine that is released from the adrenal medulla, leads to an increase in cAMP. Release of nitric oxide increases cGMP. Both cAMP and cGMP cause smooth muscle relaxation.

physiological function and may simply be in the airways because the lung originates as an outpouching of the foregut (Mitzner 2004). In horses with heaves and in people with asthma, severe contraction (bronchospasm) of airway smooth muscle occurs when the airways become inflamed. For this reason, there has been extensive investigation of the neural regulation of airway smooth muscle.

Activation of parasympathetic nerves contracts airway smooth muscle

The principal excitatory innervation of equine airway smooth muscle is the parasympathetic nervous system, which reaches the lung in the vagus nerve. Autonomic ganglia of the parasympathetic system are located in the walls of the larger airways and postganglionic fibers extend to smooth muscle where they release acetylcholine, which binds to muscarinic receptors to cause contraction. Airway smooth muscle possesses both M_2 and M_3 muscarinic receptors. Activation of the M_3 receptors releases calcium from intracellular stores to cause contraction of smooth muscle (Fig. 2.11). Activation of the M_2 receptors decreases the production of the muscle-relaxing cAMP and thereby facilitates contraction. Blockade of the muscarinic receptors by the non-specific antagonist atropine prevents or treats bronchospasm (Broadstone et al 1988). There are also M_2 receptors on postganglionic parasympathetic nerve fibers and activation of these receptors inhibits the release of acetylcholine (Wang et al 1995). There is evidence that inflammatory airway diseases cause dysfunction of these prejunctional receptors, which facilitates the release of acetylcholine and leads to bronchospasm (Coulson & Fryer 2003).

Activation of β-adrenoceptors relaxes airway smooth muscle

Activation of the numerous β_2-adrenergic receptors on equine airway smooth muscle increases the production of cAMP, which causes muscle relaxation (Torneke et al 1997). There are few sympathetic nerves in equine airway smooth muscle (Sonea et al 1993) and the norepinephrine that they release is not a strong agonist for the β_2-adrenoceptor. The principal agonist for this receptor is circulating epinephrine, which is released from the adrenal gland at times of stress and excitement. Many of the drugs used to treat bronchospasm, including clenbuterol (Erichsen et al 1994), albuterol (Derksen et al 1999), and salmeterol (Henrikson and Rush 2001), are β_2-adrenergic agonists. However, it is important to realize that in the normal horse, airway smooth muscle is usually relaxed and almost certainly the airways are maximally dilated during exercise. For this reason administration of bronchodilators is unlikely to increase airflow during exercise in the normal horse.

Several other types of nerves also have effects on smooth muscle

There are two other components to the nerve supply of the tracheobronchial tree. These are the non-adrenergic non-cholinergic inhibitory and excitatory nervous systems (iNANC and eNANC, respectively). The iNANC system supplies the airway smooth muscle in the larger bronchi of horses (Yu et al 1994a). When activated, it releases nitric oxide that relaxes airway smooth muscle (Sweeney et al 1999). This system seems to be dysfunctional in horses with heaves (Broadstone et al 1991, Yu et al 1994b), and

interestingly, drugs that provide a source of nitric oxide were used to treat heaves in the 19th century.

The eNANC system is composed of small non-myelinated sensory nerves that contain neuropeptides such as substance P (Sonea et al 1994). This system is activated by inhalation of irritating materials, for example ammonia. When this occurs, sensory information is sent to the central nervous system but in addition, neuropeptides are released locally in the walls of the airways where they can initiate inflammation, edema formation, and mucus secretion. The physiological importance of the system in the horse is unknown.

Inflammatory mediators can also cause smooth muscle contraction

Many inflammatory mediators, such as histamine, sero-tonin, and leukotriene D_4, can also cause smooth muscle contraction while others, such as prostaglandin E_2, cause relaxation. The individual roles of such agonists in the bronchospasm of heaves are unclear. Present evidence suggests that these inflammatory mediators may act indirectly by facilitating the release of acetylcholine, which then binds to the muscarinic receptors to cause broncho-spasm (Olszewski et al 1999a,b). That is why atropine (Broadstone et al 1988) and other anticholinergic agents (Robinson et al 1993, Duvivier et al 1999) are such potent bronchodilators in horses with heaves.

Airway Hyperresponsiveness

Exaggerated airway narrowing in response to a variety of endogenous and exogenous stimuli is described as non-specific airway hyperresponsiveness. Such stimuli would not cause respiratory distress in a normal horse but result in extreme airway narrowing and difficult breathing in a hyperresponsive horse. Hyperresponsiveness is associated with inflammation and occurs in horses with heaves (Derksen et al 1985a,b, Klein & Deegen 1986) and also is associated with viral respiratory infections, especially influenza. Airway inflammation leads to hyperrespon-siveness by a variety of mechanisms. Hypertrophy of the epithelium and mucus-producing cells causes thickening of the airway wall internal to the smooth muscle layer so that a small contraction of the smooth muscle causes greater narrowing of the airway lumen than normal (Moreno et al 1986). Other causes of hyperresponsiveness include facilitation of acetylcholine release by inflammatory mediators (Olszewski et al 1999a,b), reduced inhibition of smooth muscle contraction as a result of decreased production of prostaglandin E_2 (Gray et al 1989, Yu et al 1994b), lack of iNANC function (Yu et al 1994a), and decreased activity of β-adrenoceptors.

In horses with influenza, airway hyperresponsiveness persists for several weeks after the clinical signs abate. For this reason, such horses should be allowed adequate time for healing and should be kept in an environment that is free of irritants such as dusts and ammonia. In heaves-susceptible horses, exposure to allergens and dusts for just a few hours can cause hyperresponsiveness that persists for days (Fairbairn et al 1993). This makes the horse prone to bronchospasm and is one reason that owners tend to fail when using environmental control to treat heaves. They bring their horses into pro-inflammatory environments for grooming or overnight stabling and this is sufficient to keep the airways hyperresponsive.

In horses with heaves, there is increasing evidence that airway hyperresponsiveness can persist even with environmental treatments that greatly reduce overt clinical signs of disease. When heaves-affected horses are fed silage (Vandenput et al 1998), their lung function improves but they remain hyperresponsive. Similarly, with pelleted feed (Jackson et al 2000) or when fed soaked hay, low-level airway inflammation can persist and can be associated with airway hyperresponsiveness. Under these conditions, exposure to inhaled irritants may precipitate overt signs of airway obstruction.

Distribution of Ventilation within the Lung

For optimal gas exchange in the lung, the air and blood must be delivered to the alveoli in approximately equal amounts. In healthy horses, the average ventilation to blood flow ($\dot{V}/\dot{Q}$) ratio of 1.0 and the narrow spread of such ratios indicates that this is so (Hedenstierna et al 1987). In humans, there are gravitational effects on both ventilation and blood flow so that the lower parts of the lung receive more ventilation and blood flow per unit of lung volume than does the top part of the lung. Recent studies suggest that gravity plays only a small part in the distribution of blood flow in horse lungs (Hlastala et al 1996) and so, if $\dot{V}/\dot{Q}$ ratios average 1.0, gravity also should play only a small part in the distribution of ventilation. This would be in conflict with earlier observations that the lower parts of the horse lung receive more ventilation than the upper portions (Amis et al 1984) because of regional differences in pleural pressure (Derksen & Robinson 1980). Clearly there is a need for further investigation.

In diseased lungs, uneven distribution of ventilation is caused by airway obstruction and by restriction of lung inflation in regions with low compliance. This abnormal distribution of ventilation, which has been demonstrated in horses with chronic airway disease (Rush et al 1999, Votion et al 1999), can be partially reversed by use of a
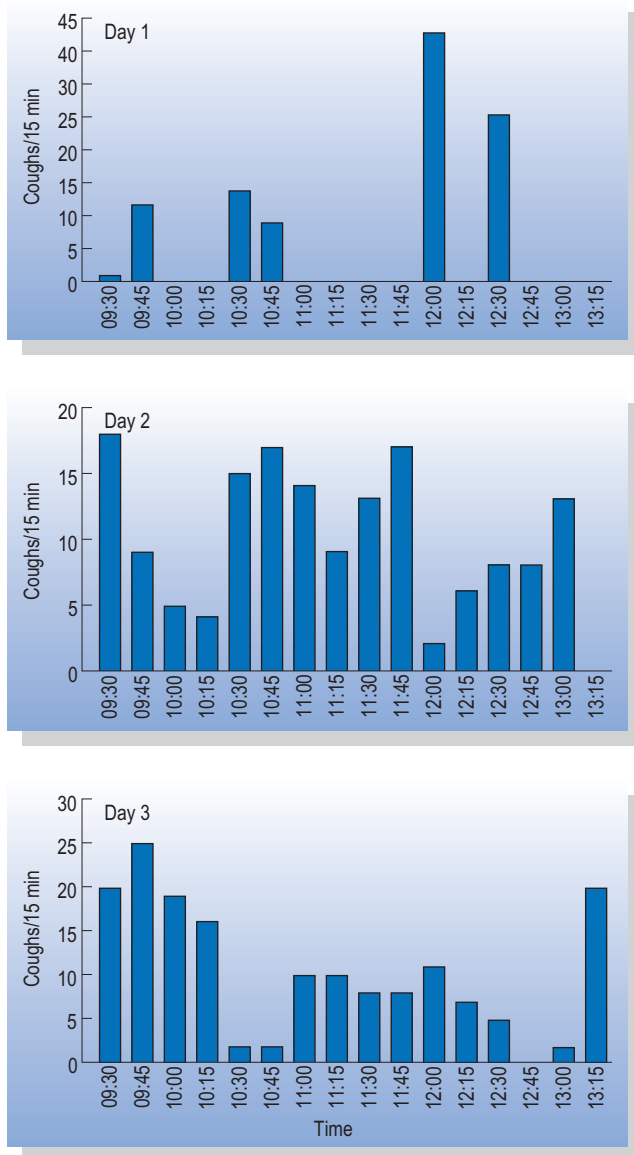
Fig. 2.12. Coughs per 15 min in a horse with heaves during the first, second and third days of stabling. Cough frequency increases from day 1 to 3 but the number of coughs per 15 min is very variable. Redrawn from Robinson et al, 2003, with permission.

bronchodilator (Rush et al 1999). Horses with such airway disease have an abnormal distribution of $\dot{V}/\dot{Q}$ ratios (Nyman et al 1991).

Cough

Cough is a forced exhalation that clears mucus and foreign material from the larger bronchi. Cough is initiated by activation of irritant receptors that are located in the mucosa of the bronchi (Widdicombe 1996). The stimulus for these receptors can be mechanical, such as a foreign body or mucus, or can be a change in osmolarity or release of inflammatory mediators. Cough begins with a full inhalation followed by exhalation against a closed glottis. This action increases intrathoracic pressure so that when the glottis opens there is a high driving pressure that causes high-velocity airflow. In addition, the elevated intrathoracic pressure dynamically compresses the larger intrathoracic airways so that the high airflow passes through narrowed airways. The resultant high-velocity air movement dislodges mucus and other foreign materials.

Coughing is a sign of inflammation

Healthy horses rarely, if ever, cough. Coughing frequency increases whenever the airways become inflamed but the magnitude of the increase in frequency varies greatly (Xiang et al 1998). In horses with heaves for example, some horses may cough 40 times per hour while others with similar severity of inflammation may cough less than 10 times per hour (Robinson et al 2003). Coughing is usually paroxysmal. A horse may not cough for several hours and then may cough 40 times in the next 15 minutes (Fig. 2.12). For this reason, horse owners may not notice that their horse is coughing if they only go to the stable to feed their horse twice a day.

The mechanisms whereby inflammation increases cough sensitivity are not well understood. It is clear that prostanoids, such as prostaglandin E_2 and thromboxane, increase cough sensitivity and that neuropeptides may also be involved. This is leading to a search for more effective methods to treat cough (Belvisi & Geppetti 2004).

REFERENCES

Aguilera-Tejero E, Pascoe JR, Amis TC et al 1993 Measurement of pulmonary diffusing capacity for carbon monoxide and functional residual capacity during rebreathing in conscious thoroughbreds. American Journal of Veterinary Research 54: 1752–1757

Ainsworth DM, Eicker SW, Nalevanko ME et al 1996 The effect of exercise on diaphragmatic activation in horses. Respiration Physiology 106: 35–46

Amis TC, Pascoe JR, Hornof W 1984 Topographic distribution of pulmonary ventilation and perfusion in the horse. American Journal of Veterinary Research 45: 1597–1601

Amory H, Lomba F, Lekeux PM et al 1994 Bilateral diaphragmatic paralysis in a pony. Journal of the American Veterinary Medical Association 205: 587–591

Art T, Serteyn D, Lekeux P 1988 Effect of exercise on the partitioning of equine respiratory resistance. Equine Veterinary Journal 20: 268–273

Belvisi MG, Geppetti P 2004 Cough. 7: Current and future drugs for the treatment of chronic cough. Thorax 59: 438–440

Broadstone RV, Scott JS, Derksen FJ et al 1988 Effects of atropine in ponies with recurrent airway obstruction. Journal of Applied Physiology 65: 2720–2725

Broadstone RV, Scott JS, Derksen FJ et al 1991 In vitro response of airway smooth muscle from horses with recurrent airway obstruction. Pulmonary Pharmacology 4: 191–202

Couetil LL, Rosenthal FS, Simpson CM 2000 Forced expiration: a test for airflow obstruction in horses. Journal of Applied Physiology 88: 1870–1809

Coulson FR, Fryer AD 2003 Muscarinic acetylcholine receptors and airway diseases. Pharmacology and Therapeutics 98: 59–69

Derksen FJ, Robinson NE 1980 Esophageal and intrapleural pressures in the healthy conscious pony. American Journal of Veterinary Research 41: 1756–1761

Derksen FJ, Robinson NE, Armstrong PJ et al 1985a Airway reactivity in ponies with recurrent airway obstruction (heaves). Journal of Applied Physiology 58: 598–604

Derksen FJ, Scott JS, Miller DC et al 1985b Bronchoalveolar lavage in ponies with recurrent airway obstruction (heaves). American Review of Respiratory Disease 132: 1066–1070

Derksen FJ, Olszewski MA, Robinson NE et al 1999 Aerosolized albuterol sulfate used as a bronchodilator in horses with recurrent airway obstruction. American Journal of Veterinary Research 60: 689–693

Duvivier DH, Bayly WM, Votion D et al 1999 Effects of inhaled dry powder ipratropium bromide on recovery from exercise of horses with COPD. Equine Veterinary Journal 31: 20–24

Erichsen DF, Aviad AD, Schultz RH et al 1994 Clinical efficacy and safety of clenbuterol HCl when administered to effect in horses with chronic obstructive pulmonary disease. Equine Veterinary Journal 26: 331–336

Fairbairn SM, Lees P, Page CP et al 1993 Duration of antigen-induced hyperresponsiveness in horses with allergic respiratory disease and possible links with early airway obstruction. Journal of Veterinary Pharmacology and Therapeutics 16: 469–476

Gray PR, Derksen FJ, Robinson NE et al 1989 Decreased airway mucosal prostaglandin E2 production during airway obstruction in an animal model of asthma. American Review of Respiratory Disease 146: 586–691

Hedenstierna G, Nyman G, Kvart C et al 1987 Ventilation-perfusion relationships in the standing horse: an inert elimination study. American Journal of Veterinary Research 47: 277–282

Henrikson SL, Rush BR 2001 Efficacy of salmeterol xinafoate in horses with recurrent airway obstruction. Journal of the American Veterinary Medical Association 218: 1961–1965

Hlastala MP, Bernard SL, Erickson HH et al 1996 Pulmonary blood flow distribution in standing horses is not dominated by gravity. Journal of Applied Physiology 81: 1051–1061

Hobo S, Oikawa M, Kuwano A et al 1997 Effect of transportation on the composition of bronchoalveolar lavage fluid obtained from horses. American Journal of Veterinary Research 58: 531–534

Holcombe SJ, Derksen FJ, Berney C et al 2001 Effect of topical anesthesia of the laryngeal mucosa on upper airway mechanics in exercising horses. American Journal of Veterinary Research 62: 1706–1710

Holcombe SJ, Berney C, Cornelisse CJ et al 2002 Effect of commercially available nasal strips on airway resistance in exercising horses. American Journal of Veterinary Research 63: 1101–1105

Hopkins SR, Bayly WM, Slocombe RF et al 1998 Effect of prolonged heavy exercise on pulmonary gas exchange in horses. Journal of Applied Physiology 84: 1723–1730

Hornicke H, Meixner R, Pollmann U 1982 Respiration in exercising horses. In Snow DH, Persson SGB, Rose RJ (editors) Equine Exercise Physiology: Proceedings of the first international conference, Oxford, Granta Editions, pp.7–16.

Jackson CA, Berney C, Jefcoat AM et al 2000 Environment and prednisone interactions in the treatment of recurrent airway obstruction (heaves). Equine Veterinary Journal 32: 432–438

Kayar SR, Hoppeler H, Lindstedt SL et al 1989 Total muscle mitochondrial volume in relation to aerobic capacity of horses and steers. Pflügers Archiv European Journal of Physiology 413: 343–347

Klein H-J, Deegen E 1986 Histamine inhalation provocation test: Method to identify nonspecific airway reactivity in equine. American Journal of Veterinary Research 47: 1796–1800

Koterba AM, Kosch PC, Beech J et al 1988 Breathing strategy of the adult horse (*Equus caballus*) at rest. Journal of Applied Physiology 64: 337–346

Lumsden JM, Derksen FJ, Stick JA et al 1993 Use of flow-volume loops to evaluate upper airway obstruction in exercising standardbreds. American Journal of Veterinary Research 54: 766–775

Manohar M 1986 Vasodilator reserve in respiratory muscles during maximal exertion in ponies. Journal of Applied Physiology 60: 1571–1577

Manohar M, Goetz TE, Nganwa D 1988 Costal diaphragmatic O_2 and lactate extraction in laryngeal hemiplegic ponies during exercise. Journal of Applied Physiology 65: 1723–1728

Manohar M, Duren S E, Sikkes B et al 1992 Respiratory muscle perfusion in ponies during prolonged submaximal exercise in thermoneutral environment. American Journal of Veterinary Research 53: 558–562

Marlin DJ, Schroter RC, Cashmann PMM et al 2002 Movements of thoracic and abdominal compartments during ventilation at rest and during exercise. Equine Veterinary Journal Supplement 34: 384–390

Mitzner W 2004 Airway smooth muscle: the appendix of the lung. American Journal of Respiratory and Critical Care Medicine 169: 787–790

Moreno RH, Hogg JC, Pare PD 1986 Mechanics of airway narrowing. American Review of Respiratory Disease 133: 1171–1180

Morrison KE, Slocombe RF, McKane SA et al 1999 Functional and compositional changes in pulmonary surfactant in response to exercise. Equine Veterinary Journal Supplement 30: 62–66

Nyman G, Lindberg R, Weckner D et al 1991 Pulmonary gas exchange correlated to clinical signs and lung pathology in horses with chronic bronchiolitis. Equine Veterinary Journal 23: 253–260

Olszewski MA, Robinson NE, Zhou FX et al 1999a Mediators of anaphylaxis but not activated neutrophils augment cholinergic responses of equine small airways. American Journal of Physiology 276: L522–L529

Olszewski MA, Zhang XY, Robinson NE 1999b Pre- and post-junctional effects of inflammatory mediators in horse airways. American Journal of Physiology 277: L327–L333

Pelletier N, Leith DE 1995 Ventilation and carbon dioxide exchange in exercising horses: effect of inspired oxygen fraction. Journal of Applied Physiology 78: 654–662

Petsche VM, Derksen FJ, Berney CE et al 1995 Effect of head position on upper airway function in exercising horses. Equine Veterinary Journal Supplement 18: 18–22

Poole DC, Petrisko RN, Anderson L et al 2002 Structural and oxidative enzyme characteristics of the diaphragm. Equine Veterinary Journal Supplement 34: 459–463

Robinson NE, Derksen FJ, Berney C et al 1993 The airway response of horses with recurrent airway obstruction (heaves) to aerosol administration of ipratropium bromide. Equine Veterinary Journal 25: 299–303

Robinson NE, Berney C, Olszewski M et al 1999 Determinants of maximal changes in pleural pressure in horses during tidal breathing in COPD-affected horses. Veterinary Journal 157: 160–165

Robinson NE, Berney C, DeFeijter-Rupp H et al 2003 Cough, mucus, airway obstruction, and inflammation in heaves-affected and control horses. American Journal of Veterinary Research 64:550–557

Rush BR, Hoskinson JJ, Davis EG et al 1999 Pulmonary distribution of aerosolized technetium Tc 99m pentetate after administration of a single dose of aerosolized albuterol sulfate in horses with recurrent airway obstruction. American Journal of Veterinary Research 60: 764–769

Sant'Ambrogio G, Tsubone H, Sant'Ambrogio FB 1995 Sensory information from the upper airway: role in the control of breathing. Respiration Physiology 102: 1–16

Slocombe RF, Brock K, Covelli G et al 1991 Effect of treadmill exercise on intrapleural, transdiaphragmatic and intra-abdominal pressures in standardbred horses. In: Persson SGB, Lindholm A, Jeffcott LB (editors) Equine Exercise Physiology 3: Proceedings of the Third International Conference on Equine Exercise Physiology, Uppsala, ICEEP Publications, pp.83–91

Sonea IM, Bowker RM, Broadstone RV et al 1993 Adrenergic and peptidergic innervation of the trachealis muscle in the normal horse: a preliminary report. Research in Veterinary Science 54: 335–339

Sonea IM, Bowker RM, Robinson NE et al 1994 Substance P and calcitonin gene-related peptide-like immunoreactive nerve fibers in lungs from adult equids. American Journal of Veterinary Research 55: 1066–1074

Sweeney CR, Tomasic M, Russell GE 1999 A preliminary study of the effect of inhaled nitric oxide on lung mechanics in the standing horse with histamine-induced bronchoconstriction. Equine Veterinary Journal Supplement 30: 67–70

Torneke K, Larsson CI, Appelgren LE 1997 Relaxation of equine tracheal muscle *in vitro* by different adreno-ceptor drugs. Journal of Veterinary Pharmacology and Therapeutics 20: 216–219

Vandenput S, Votion D, Duvivier D et al 1998 Effect of a set stabled environmental control on pulmonary function and airway reactivity of COPD affected horses. Veterinary Journal 155: 189–195

Votion D, Ghafir Y, Vandenput S et al 1999 Analysis of scintigraphical lung images before and after treatment of horses suffering from chronic pulmonary disease. Veterinary Record 144: 232–236

Wang Z-W, Yu M-F, Robinson NE 1995 Prejunctional muscarinic autoreceptors on horse airway cholinergic nerves. Life Sciences 56: 2255–2262

Widdicombe JG 1996 Sensory neurophysiology of the cough reflex. Journal of Allergy and Clinical Immunology 98: S84-89; discussion S89–S90

Xiang A, Uchida Y, Nomura A et al 1998 Effects of airway inflammation on cough response in the guinea pig. Journal of Applied Physiology 85: 1847–1854

Young IS, Alexander R, Woakes AJ et al 1992 The synchronization of ventilation and locomotion in horses (*Equus caballus*). Journal of Experimental Biology 166: 19–31

Yu M, Wang Z, Robinson NE et al 1994a Inhibitory nerve distribution and mediation of NANC relaxation by nitric oxide in horse airways. Journal of Applied Physiology 76: 339–344

Yu M, Wang ZW, Robinson NE et al 1994b Modulation of bronchial smooth muscle function in horses with heaves. Journal of Applied Physiology 77: 2149–2154

3

Pulmonary Blood Flow

David J Marlin and Thea L Vincent

The lungs receive blood from two sources: the pulmonary and bronchial circulations. Whilst the primary function of the pulmonary circulation is gas exchange, it acts as a reservoir for blood between the right and left sides of the heart and also serves to filter thrombi and emboli. A wide range of chemically active substances, including hormones, are also either produced or removed by the pulmonary vasculature. In contrast, the role of the bronchial circulation is primarily to ensure nutrition of the airways, vessels, parenchyma, and visceral pleura. However, another important function of the bronchial circulation is its contribution, via airway mucosal perfusion, to the conditioning of inspired air and in thermoregulation as a route for heat dissipation.

The pulmonary circulation is in series with the systemic circulation and therefore receives the total cardiac output from the right side of the heart, despite the fact that it only has approximately one-tenth the capacity of the systemic circulation. The pulmonary circulation is a highly compliant, highly conductive, low-pressure system, relative to the systemic circulation. The fact that the pulmonary circulation is a low-pressure system compared with the systemic arterial circulation is also reflected in the characteristics of the ventricles and the pressures developed within them. Thus, the left ventricle has significantly more muscular walls than the right ventricle and pressures within the right heart are also considerably lower than those on the left. The right ventricle is commonly referred to as a volume pump, whilst the left ventricle is referred to as a pressure pump. The muscularity of the ventricular walls is primarily a function of the pressure they must pump against, or the after-load. In the case of the right ventricle the pressure in the pulmonary artery is low whilst for the left ventricle the aortic pressure is considerably higher. Although the absolute pressure within the pulmonary arterial circulation is considerably lower than in the systemic arterial circulation, the pressure waves, flow patterns, and cardiac valve movements of both circulations are similar.

The greater compliance of the pulmonary circulation is primarily because the arteries and arterioles are generally shorter and have less smooth muscle than those of the systemic circulation. Thus, pulmonary arteries are closer in structure to systemic veins as opposed to arteries. In the healthy resting horse, the mean pressure of large pulmonary arteries is 20–30 mmHg. During intense exercise, mean pressures may easily exceed 100 mmHg, thereby placing high stress on the vessel walls. Simultaneous measurements of pulmonary artery, aortic and esophageal pressures in horses during moderately strenuous exercise indicate transmural pressures (an index of stress on the vessel wall) of 150 mmHg for the pulmonary artery and 175 mmHg for the aorta (Erickson et al 1990). Taking into account the considerably thinner wall of the pulmonary artery, rupture of the main pulmonary arteries is perhaps surprisingly rare.

Within the lung, the pressure gradient between the pulmonary arteries and capillaries is similar to that between capillaries and pulmonary veins, which is in marked contrast to the gradients in the systemic circulation in which most of the pressure decrease occurs between arteries and capillaries. As a result, approximately half of the total pulmonary vascular resistance is precapillary and flow is pulsatile rather than constant. At rest the pulmonary circulation contains around one-fifth of the total blood volume. The volume of blood within the pulmonary capillaries is relatively small compared to that within the larger vessels. However, disturbances such as exercise or disease will increase the number of pulmonary capillaries that are recruited (perfused).

Anatomy

Pulmonary circulation

The branches of the pulmonary artery carry venous blood to the lung, accompany the bronchi (as in man and sheep), and form rich capillary plexuses on the walls of the alveoli. This allows gas exchange to take place. The blood is returned through the pulmonary veins, which run intersegmentally and amass blood from adjacent bronchopulmonary segments, to the left side of the heart. The equine pulmonary arteries adjacent to the bronchioles and the alveolar ducts have a thick medial smooth muscle layer. The main pulmonary trunk, the truncus pulmonalis, arises from the conus arteriosus of the right ventricle. From its origin, the pulmonary artery extends dorsally to the left and cranial to the aorta, exiting the pericardium at a point

above the left atrium. Here the pulmonary trunk is joined to the aorta by the ligamentum arteriosum, a remnant of the ductus arteriosus. A short distance further on from this point the first division of the truncus pulmonalis occurs to form the a.pulmonalis sinistra and the a.pulmonalis dextra, which supply the left and right lung, respectively. These vessels then divide to form numerous thinner walled branches over a relatively short distance. The transition from pulmonary arteries to pulmonary arterioles is usually considered to occur at an internal diameter of around 100 μm. At this level the vessels are almost devoid of smooth muscle.

Pulmonary capillaries form a dense network over the walls of alveoli and a single capillary network is spread over multiple alveoli. The radius of the pulmonary capillaries in horses has been reported to be ~3 μm and is therefore similar to that in rabbits and dogs. However, compared with the rabbit and dog, the horse has a thicker blood–gas barrier. These characteristics are reflected in the pressures required to cause rupture, which are in the region of 50, 90 and 130 cmH$_2$O in rabbit, dog and horse, respectively (Birks et al 1994). Flow within the pulmonary capillaries is pulsatile, in contrast to the flow in the systemic circulation, which is essentially constant. It is estimated that each red blood cell spends less than 1 second in the pulmonary capillary network, but the fact that this allows sufficient time for gas exchange illustrates the efficiency of the process.

The veins of the pulmonary circulation, in contrast to those in the systemic circulation, are devoid of valves and carry blood from the pulmonary capillaries to the left atrium. From the capillary bed, blood collects into venules, which are effectively identical to arterioles in structure. Pulmonary veins do not run adjacent to pulmonary arteries but rather lie close to the septa, which separate the different segments of the lung.

Bronchial circulation

The bronchial arterial circulation, a branch of the systemic circulation, carries arterial blood for the nutrition of the airways and other lung structures and receives around 1–2% of the total cardiac output of the left ventricle. It originates from the broncho-esophageal artery (a.broncho-esophagea) giving rise to the right, middle and left bronchial arteries (r.bronchalis, m.bronchalis and l.bronchalis). The right and left bronchial arteries supply the cranial lobes, whilst the middle bronchial artery supplies the caudal lobes, branching at the level of the tracheal bifurcation. In the horse the bronchial branches supply both the interlobular septa and the subpleural connective tissue (McLaughlin et al 1961). Bronchial arteries form a circulatory plexus in the connective tissues along the airways. Branches from this plexus penetrate the bronchial walls to form a subepithelial vascular plexus the

role of which is most likely to facilitate heat dissipation. There is also a system of branches of the bronchial circulation that follow the pulmonary arteries and provide nutrition for the alveolar capillary network. In most species, the bronchial veins drain the blood in the walls of the large bronchi; however, bronchial veins are not present in horses. The bronchial circulation of the horse is, therefore, drained by either the azygos vein (which terminates in the superior vena cava) or the pulmonary veins. At the level of the terminal bronchioles, the pulmonary and bronchial circulations anastomose. Most of these anastomoses occur at the level of the capillaries and veins rather than the arteries (McLaughlin 1983). These anastomoses have been reported to be frequent in horse lungs and provide a potential route for shunting blood flow to attenuate increases in capillary pressure that may result from increases in pulmonary arterial (e.g. during exercise) or venous (e.g. in cases of left-sided heart failure) pressure. The amount of flow through these anastomoses is determined by the pressure gradient between the bronchial and pulmonary circulations, which is also modulated by the alveolar pressure. In circumstances where the pulmonary circulation is damaged, for example in exercise-induced pulmonary hemorrhage (EIPH), the bronchial vasculature can act as an alternative supply.

Innervation of the circulation

Whilst a considerable amount is known about the innervation of the equine airways, relatively little is known about the innervation of the pulmonary vasculature. The lung is innervated by branches of the vagus and sympathetic nerves that form plexuses within the lungs and then subdivide into smaller branches to supply the blood vessels. The extent to which these nerves regulate perfusion in the horse has not been determined; however, in most species innervation appears to play only a minor role in the control of blood flow.

Pulmonary Hemodynamics

In an artificial system, the non-pulsatile flow of a homogeneous liquid through a rigid tube is a function of driving pressure, tube diameter and length, and the viscosity of the liquid; this relationship is described by Poiseuille's law (Fig. 3.1). Poiseuille's law can reasonably be applied to describe blood flow within the body, despite the fact that blood is a complex and non-homogeneous fluid, that the blood vessels vary in stiffness (dependent on both their location and the smooth muscle tone), that these vessels exhibit complex and intricate branching patterns, and that the flow of blood is pulsatile. The Poiseuille equation describes the factors contributing to resistance to flow. Ignoring the constants in the equation (π and 8), and recognizing that the only energy for flow comes from the
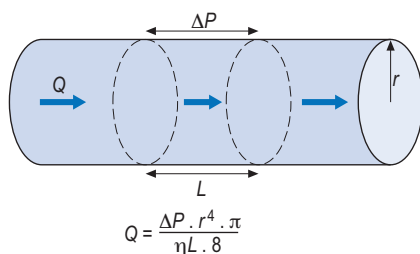
Fig. 3.1. The relationship between pressure, tube caliber and length according to Poiseuille's law. Poiseuille's law applies to the flow of a homogeneous fluid through a rigid, cylindrical tube. The flow (Q) in this system is proportional to the driving pressure along the tube (ΔP) and the fourth power of the radius (r) and inversely proportional to the length of the tube (L) and the viscosity of the liquid (η).

Fig. 3.2. Extension of Poiseuille's law to describe the relationship between flow, pressure, and resistance. The force driving flow is simply the driving pressure (ΔP), whilst the forces opposing flow can be considered the tube caliber (r), the fluid viscosity (η) and the tube length (L). These can be grouped to describe the resistance to flow (R). As flow is a function of driving pressure divided by resistance to flow, this can be arranged to solve for resistance.

driving pressure (ΔP), then the remaining factors, vessel length, caliber and fluid (blood) viscosity comprise the resistance to flow (Fig. 3.2). Thus, in the pulmonary circulation the pulmonary driving pressure divided by the pulmonary blood flow gives an index of pulmonary vascular resistance (PVR). This term is correct when mean pressure and flow values are used to calculate resistance in a "steady-state" or continuous flow system. However, where flow is more markedly pulsatile, for example in the aorta, the term "vascular impedance" is more appropriate.

There are two other important principles that should be appreciated concerning flow and resistance. Firstly, laminar flow (sometimes referred to as Poiseuille or streamline flow) requires less driving pressure than turbulent flow, at the same flow rate (Fig. 3.3). The Reynolds ratio or number (R_e), a non-dimensional index of the ratio of inertial forces to viscous forces within the fluid, predicts the type of flow at a defined velocity. Thus, in a vessel where flow is turbulent, a greater driving pressure is required to generate that flow than in a vessel in which the flow is laminar:

$$\text{Reynolds number } (R_e) = (\rho.\nu. d)/\mu$$

where ρ is density, ν is the velocity of the fluid, d is the vessel diameter and μ is the fluid viscosity. At a Reynolds number < 2000, flows of fluid other than blood are considered to be laminar. Between 2000 and 4000 flow is described as transitional, whilst when R_e > 4000 flows are considered to be turbulent. For blood the threshold for the change between laminar and transitional flow is much lower, at around 1000. Blood flow is more likely to become turbulent in large blood vessels where flow is high, such as the larger pulmonary arteries, than in smaller vessels. An important point to note is that laminar flow produces virtually no sound, whilst sound intensity increases with turbulence.

The nature of the tube in which a liquid is flowing has an important impact on the relationship between pressure and flow. Referring again to Poiseuille's law, flow will

increase in proportion to driving pressure if the diameter and length of the tube and the fluid viscosity are kept constant. However, blood vessels are not rigid and have some capacity to distend. Thus the pressure–flow relationships in blood vessels are not linear and the relationship varies according to their distensibility or compliance (Fig. 3.4). This phenomenon is extremely important in terms of the lung's ability to accommodate the large increases in pulmonary blood flow that occur with exercise, as discussed later. The shape of the curve is related to the fact that as the blood vessel distends under increasing perfusion pressure, the resulting flow will increase not in a linear fashion, but to the fourth power of the radius.

The pressures within the pulmonary circulation depend on a range of factors, including the pressures of the right
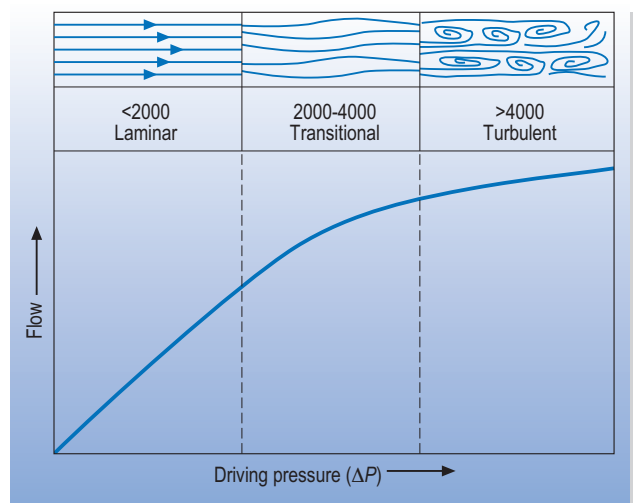

Fig. 3.3. Relationship between pressure and flow. The Reynolds number (in boxes) predicts the type of flow that is likely to predominate in rigid tubes.
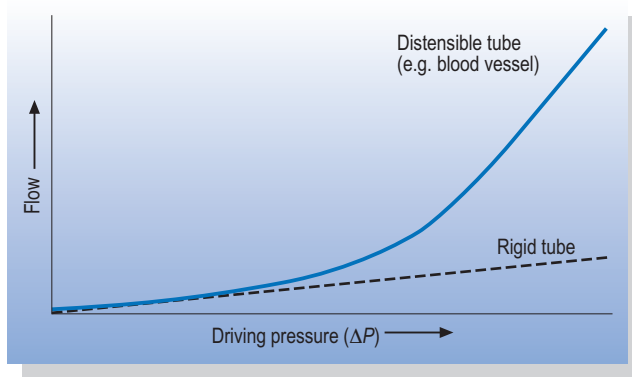
Fig. 3.4. The relationship between driving pressure and the resulting flow in rigid and distensible tubes. In a rigid tube, the flow rate increases in direct proportion to driving pressure. In a distensible tube, flow rate increases in a non-linear manner because of the increase in radius resulting from distension, according to Poiseuille's law (see Fig. 3.2).

Fig. 3.5. The effect of lung volume on blood vessel caliber and pulmonary vascular resistance (PVR). At functional residual capacity (FRC), tethering of vessels by parenchyma maintains vessel patency. At lower lung volumes up to residual volume (RV), the degree of tethering is reduced and vessels receive minimal additional support. This may be exacerbated in dependent lung regions. At high lung volumes, up to total lung capacity (TLC) and at the top of the lung gravitationally, tethering may lead to stretching and elongation of vessels. This increases PVR both by increasing the vessel length and decreasing caliber.

and left heart, size of animal, proportion of the vascular bed perfused, age, disease and activity. Right ventricular pressures in the healthy resting horse range from around 15 mmHg during diastole to approximately 50 mmHg during systole. These pressures are higher than those in humans and smaller mammals and are thought to be related to the physical size of the lungs, with longer distances for blood to travel, and the volume of the circulation containing blood that is above the height of the heart itself (i.e. an effect of hydrostatic pressure).

PVR in the horse is low. The PVR is calculated from the difference between pulmonary arterial and left atrial pressure divided by cardiac output. It has been estimated that the majority (~72%) of PVR at rest is the result of precapillary resistance (Sinha et al 1996). For calculation of PVR, direct measurements of pulmonary artery pressure can readily be obtained by passing a catheter inserted into the right or left jugular vein, through the right atrium and right ventricle and into the main pulmonary trunk. However, left atrial pressure is technically difficult to measure. Whilst a pressure catheter can be inserted into the carotid artery and passed down into the aortic arch and into the left ventricle, it cannot be readily or reliably advanced into the left atrium. For this reason, pulmonary artery wedge pressure is used as an index of left atrial pressure.

In the resting horse, mean pulmonary arterial pressure is in the region of 20–30 mmHg (Manohar & Goetz 1998) compared to around 120 mmHg in a systemic artery, such as the aorta or carotid artery (around one-fifth to one-sixth that in the systemic arterial circulation). Pulmonary arterial wedge pressure (considered to be equivalent to left atrial pressure) is around 24 mmHg (Manohar & Goetz 1998), and thus the pressure to drive the blood through the pulmonary circulation is only a few mmHg, implying

a low-resistance system (Robinson 1985). This is primarily because the pulmonary arteries are relatively short but large in diameter.

The pressure, and hence flow, within the pulmonary arterial system is not only influenced by pulmonary arterial smooth muscle tone, but also by circulatory volume, relative (tidal volume) and absolute lung volume, and the changes in intrathoracic pressure that occur with ventilation; thus, PVR changes during each respiratory cycle. When the lung inflates during inspiration, the attachments between the parenchyma and the vasculature result in radial expansion of the blood vessels, thereby decreasing PVR, whilst on expiration the vessel diameter is reduced and flow may even be prevented if vessels are compressed sufficiently. PVR therefore changes more during breathing as tidal volume becomes greater. In addition, absolute lung volume contributes to PVR. At very low lung volumes, the support of parenchyma may become negligible such that the caliber of the vessel is determined by its wall stiffness as opposed to by its tethering by lung tissue. In this situation PVR is maximal. At high lung volumes and up to total lung capacity, PVR also becomes elevated because of longitudinal stretching of smaller blood vessels in the alveolar septa. This stretching increases the vessel's length and concurrently decreases its caliber. The PVR is generally least in healthy animals when their breathing is close to the functional residual capacity (FRC; Fig. 3.5). Large increases in pulmonary arterial pressure
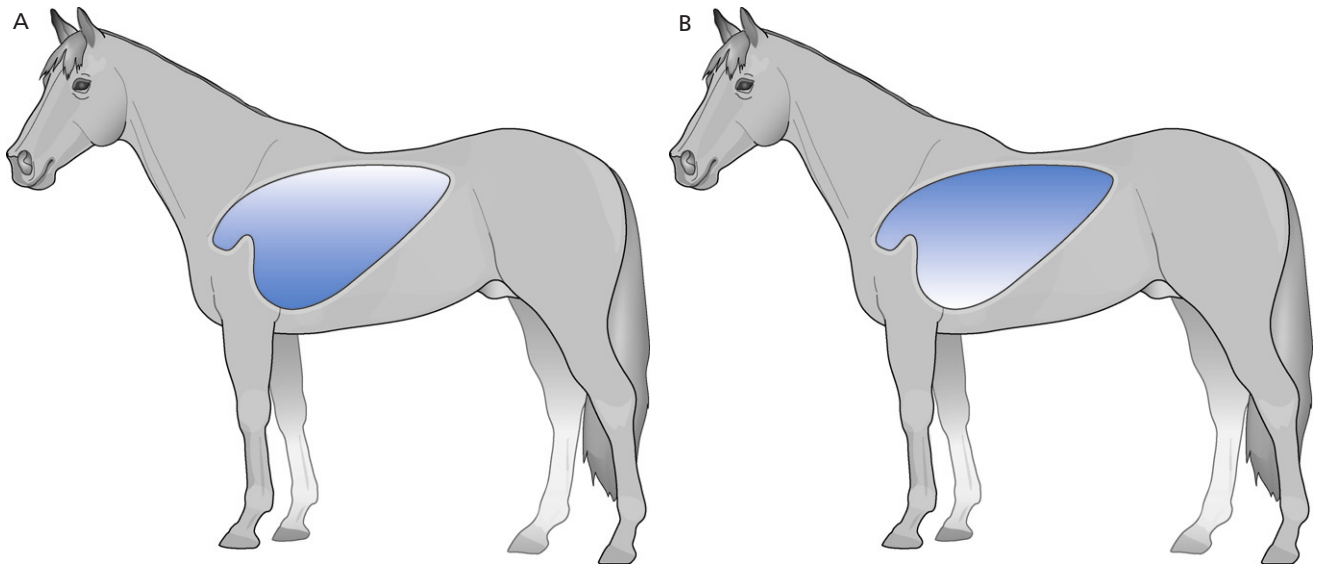
Fig. 3.6. Distribution of perfusion in the equine lung at rest according to (A) gravity-dominated and (B) gravity-independent models.

and hence PVR can also be observed during coughing because the markedly elevated intrathoracic pressure compresses some of the pulmonary vasculature.

Distribution of blood flow in the lung

In humans, blood flow distribution is mainly influenced by gravity and consequently there is a vertical gradient of pulmonary perfusion with the ventral regions receiving more perfusion per unit lung volume than the dorsal regions. This phenomenon is thought to be related to the balance between pulmonary arterial, pulmonary venous, and alveolar pressures (West et al 1964) whereby, in the upper regions of the lungs, pulmonary artery pressure may be less than the alveolar pressure. For many years, the perfusion of the equine lung was also thought to be strongly influenced by gravity. Work by Amis et al (1984) using krypton perfusion and scintigraphy confirmed the presence of a vertical gradient of pulmonary perfusion increasing from dorsal to ventral in the standing conscious horse. Puzzlingly, however, the ventilation to perfusion ratio approximated 1.0 at all heights in the lung. The results of a more recent study using microspheres (Bernard et al 1996) suggested that blood may be preferentially distributed to the dorsal regions of the lung. Furthermore, within the same vertical plane, blood flow can vary significantly. Taken together, this implies that gravitational forces are not a major determinant of equine pulmonary perfusion (Fig. 3.6). However, it should be noted that these studies were only undertaken on four horses and this phenomenon could be demonstrated in only three.

Regulation of the Pulmonary Circulation

Pulmonary blood flow is actively regulated by changes in smooth muscle tone. The equine pulmonary vasculature receives sympathetic innervation (Sonea et al 1993). Whilst it has been demonstrated that cholinergic nerves supply equine airways, little is known concerning the innervation of the pulmonary vasculature. Despite this, and in contrast to the airways, many studies suggest that these autonomic nerves play a relatively minor functional role.

A wide range of mediators released both locally and remotely can modulate smooth muscle tone leading to an increase or decrease in pulmonary blood flow, or complete closure of capillary beds and shunting. Many different drugs and mediators act on pulmonary vessel smooth muscle. Norepinephrine, epinephrine, dopamine, phenylephrine, arachidonic acid, thromboxane, leukotrienes, serotonin, histamine (via H_1 receptors), angiotensin II, endothelin and some prostaglandins (e.g. $PGF_{2\alpha}$) can produce vasoconstriction, whilst epinephrine, isoproterenol, bradykinin, prostaglandin E_1, prostacyclin, arachidonic acid, histamine (via H_2 receptors), nitric oxide (NO), aminophylline, and acetylcholine can produce vasodilatation. These effects may be mediated through α_1, α_2, β or H_1/H_2 receptors. In some instances the effects may be on specific vessels or sizes of vessels. Some mediators may also have different effects depending on other factors; epinephrine can produce vasoconstriction or vasodilatation depending on the level of smooth muscle tone.

Nitric oxide regulates vascular tone in both the pulmonary and systemic circulations. It is one of the major

vasodilators released by vascular endothelial cells and plays an important role in regulating pulmonary vascular resistance. Nitric oxide is released by the vascular endothelium in response to, amongst other stimuli, increased blood flow. Following its synthesis by endothelial nitric oxide synthase (eNOS), NO diffuses into the vessel smooth muscle cells and lumen (Lancaster 1997, Butler et al 1998). The interaction of NO with the enzyme guanylate cyclase results in increased production of cGMP, which relaxes smooth muscle cells leading to vasodilatation. In the vascular lumen, NO is either oxidized to form nitrite (Rhodes et al 1995) or is taken up by red blood cells (Liao et al 1999); the interactions of NO with hemoglobin are thought to play a regulatory role in the uptake and delivery of oxygen. In primary pulmonary hypertension and in high-altitude pulmonary edema, reduced NO availability has been highlighted as a potential cause of the excessive rise in pulmonary arterial pressure.

Evidence that NO plays a major role in modulating pulmonary vascular resistance in the horse comes from several sources. L-NAME (N$^\omega$-nitro-L-arginine methyl ester) a competitive inhibitor of eNOS, significantly increases right atrial and pulmonary arterial, capillary, and venous pressures in horses at rest (Manohar & Goetz 1998); it also increases both pulmonary and systemic vascular resistance during moderate and intense exercise (Kindig et al 2000). Furthermore, inhalation of NO during exercise produces a small, but significant, decrease in pulmonary arterial pressure (Kindig et al 2001).

Hypoxic pulmonary vasoconstriction

One of the most potent vascular smooth muscle responses of the lung is constriction in response to hypoxia, referred to as hypoxic pulmonary vasoconstriction (HPV). This is the opposite of the response of the systemic circulation, in which hypoxia produces vasodilatation. HPV is a progressive response, i.e. as the severity of alveolar hypoxia increases so does the magnitude of vasoconstriction. HPV produces a regional reduction in blood flow in the hypoxic area. By so doing, it diverts blood flow from unventilated or poorly ventilated regions of lung to those areas that are well ventilated, thereby improving overall gas exchange. This is often referred to as ventilation/perfusion matching. The mechanism of HPV is not entirely understood.

Hypoxic vasoconstriction can occur as a consequence of pulmonary disease, during exercise or at altitude. Long-term HPV as may occur with chronic pulmonary disease, such as chronic obstructive pulmonary disease in humans, can result in the development of pulmonary hypertension and lead in turn to right heart failure (cor pulmonale), although this is relatively rare in horses.

The magnitude of the HPV differs among species. The response of horses to hypoxia is less than that in cattle and pigs but greater than that in sheep and dogs. Animals with the strongest HPV response to hypoxia appear to have more smooth muscle surrounding the pulmonary arterial vessels, show well-developed interlobular connective tissue septae and have high collateral resistance to airflow.

Endothelial cells as well as other cell types release endothelin, the most potent endogenous vasoconstrictor yet identified. Its potency is around 100 times that of norepinephrine, it has a long-lasting action and has been implicated in many vascular disorders including those of the lung. Vascular smooth muscle cells also release endothelin but at a much lower level than endothelial cells. A wide range of stimuli can initiate endothelin release, including angiotensin II, catecholamines, vasopressin, bradykinin, thrombin, insulin, interleukin-1, tumor necrosis factor-α, transforming growth factor-β, endotoxin, low mechanical shear stress and thermal stress. However, it is the release of endothelin from endothelial cells in response to hypoxia that has led to a proposed role in the control of regional blood flow. Equine pulmonary arteries (2–4 mm diameter) constrict in response to endothelin *in vitro* and a vasoconstrictive response can also be observed following administration *in vivo*. In addition, there is evidence for the involvement of endothelin in the HPV response of the horse to hypoxia (~11% inspired oxygen). However, although both pulmonary arterial pressure and levels of endothelin in plasma and bronchoalveolar lavage fluid were elevated in horses with symptomatic recurrent airway obstruction (RAO), they were not correlated.

Non-respiratory Functions of the Pulmonary Circulation

Because all the output from the right ventricle passes through the pulmonary circulation (except in the case of subjects with a right-to-left shunt), it is ideally suited to perform other "processing" roles, including protection of the systemic circulation by filtration of particulates such as clots and air bubbles. In addition, a wide range of chemically active substances are produced or removed by the pulmonary circulation.

Thrombi can be eliminated faster in the lung than in any other organ because the pulmonary endothelium is rich in plasmin activator that converts plasminogen into plasmin, which in turn degrades fibrin. Paradoxically, the pulmonary vasculature is also rich in heparin and thromboplastin, which catalyze the conversion of prothrombin to thrombin. The pulmonary circulation therefore has a tremendous ability to control blood coagulation both locally and possibly also systemically.

The lung has the capacity to remove and process selective vasoactive compounds, including hormones. This role is primarily undertaken by the pulmonary vascular

endothelium in vessels of all sizes, although the smaller vessels are thought to be most active. Norepinephrine, 5-hydroxytryptamine, bradykinin, ATP, ADP, AMP, prostaglandins E_2, E_1, and $F_{2\alpha}$, and leukotrienes are essentially removed from the circulation whilst epinephrine, angiotensin II, vasopressin, isoprenaline, dopamine, histamine, and prostaglandins I_2 and A_2 all pass through the pulmonary vasculature largely unaffected. The lung is also a major site for the synthesis, metabolism and uptake and secretion of eicosanoids. Drugs that are taken up by the endothelium of the pulmonary circulation include propranolol, lignocaine, chlorpromazine, imipramine, and nortriptyline. The lung also has some ability to process anesthetic agents.

The Effect of Exercise

At the transition from rest to intense exercise, cardiac output, and therefore pulmonary blood flow, increases by around 10–12-fold. Pulmonary vascular pressures also increase both as a result of the increased blood flow and the increases in blood viscosity caused by splenic contraction. However, pulmonary vascular pressures do not increase to the same extent as blood flow, with the overall effect that PVR initially falls during the transition from rest to light exercise and is then unchanged with increasing exercise intensity, despite further increases in cardiac output (Butler et al 1993, Manohar & Goetz 1999) (Fig. 3.7). Inclined running reduces PVR even further compared with running on the flat (McDonough et al 2002,
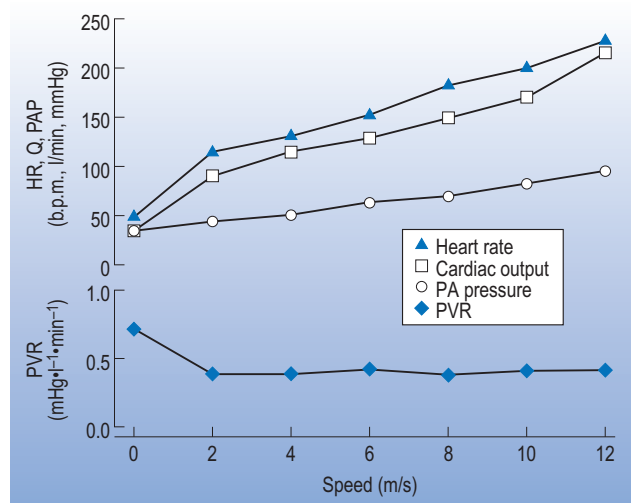
Kindig et al 2003). The reductions in PVR despite the increased cardiac output are the result of a combination of factors: recruitment of vessels previously unperfused; further active vasodilatation in vessels already perfused; and distension of vessels already maximally relaxed. As a result of the significant pressure increase in both the pulmonary artery and left atrium during exercise, capillary pressure is also greatly increased (Fig. 3.8).

Perfusion studies using microspheres (Bernard et al 1996) and macroaggregates of human albumin labeled with technetium-99m (Harmegnies et al 2002) have both shown an increase in pulmonary blood flow in the dorsal and dorsocaudal regions of the equine lung during intense exercise. The mechanism for preferential redistribution of blood flow to dorsal regions during exercise and the fact that, even at rest, blood flow is not dominated by gravity as in the conventional perfusion model (Fig. 3.6) may relate to regional variations in pulmonary arterial endothelial function. Thus, mediators that may produce vasoconstriction in pulmonary vessels from the ventral lung regions may paradoxically induce vasodilatation in vessels of the dorsal lung (Pelletier et al 1998). It appears that the regional differences in pulmonary perfusion, at least under resting conditions and in isolated vessels, may be largely determined by differences in NO release (Pelletier et al 1998). Further evidence that NO may play a role in the control of pulmonary smooth muscle tone and pulmonary perfusion during exercise comes from studies using NO administration and antagonism. Thus, there is evidence that inhaled administration of NO reduces mean pulmonary artery pressure (Mills et al 1996a, Kindig et al 2001) whilst antagonism with L-NAME increases pressure (Mills et al 1996b).

The redistribution of pulmonary blood flow to the dorsal regions of the lung during exercise almost certainly has implications when considering EIPH. However, increased
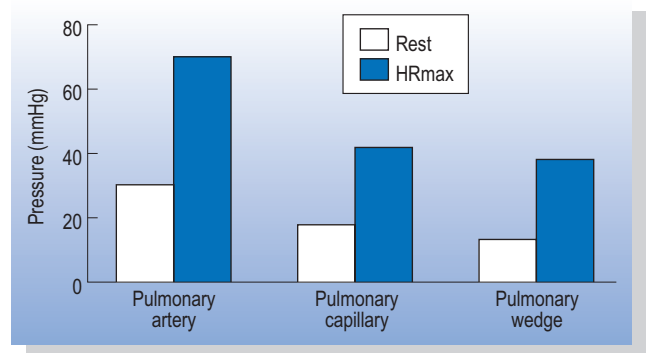

Fig. 3.7. Pulmonary hemodynamic variables in the horse at rest and during exercise. Despite increases in heart rate (HR), flow (cardiac output, Q), and pulmonary arterial pressure (PAP), pulmonary vascular resistance (PVR) falls in the transition from rest to exercise. Drawn from data in Butler et al, 1993 with permission.

Fig. 3.8. The relationship between pulmonary arterial, capillary, and wedge pressures in horses at rest and during intense exercise (HRmax – maximal heart rate). Redrawn from Sinha et al, 1996.

Conventional model:
Pulmonary vascular bed is fully dilated during exercise
(I.e. no pulmonary arterial smooth muscle tone)
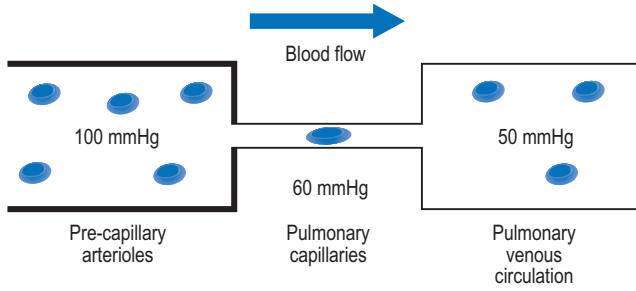
Proposed model:
Pulmonary vascular bed is not normally fully dilated during exercise
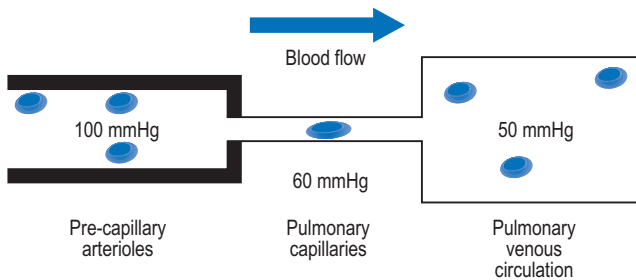
Proposed model:
Dilate arterioles (e.g. NO), pressure drop now occurs across
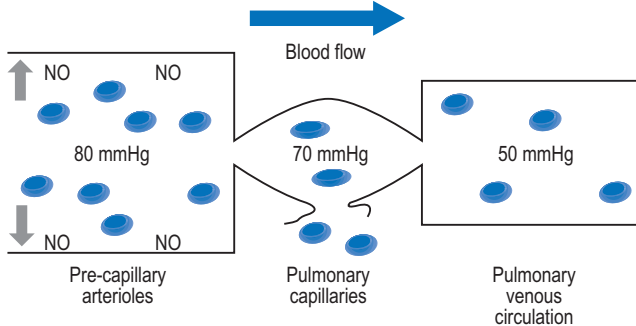pulmonary capillaries, ↑capillary pressure = ↑EIPH

Fig. 3.9. Conventional and proposed models relating to smooth muscle tone during exercise in the pulmonary vascular bed.

Fig. 3.10. The relationship between alveolar and blood oxygen tension and capillary transit time in horses at rest and during light and intense exercise. Drawn from data in Butler et al, 1993.

flow does not necessarily imply increased pressure! A simple analogy is with a garden hose. If the tap is turned on and the outflow is obstructed by the thumb, then the pressure is high but the flow is low. When the thumb is removed the flow increases but the pressure falls. The fact that NO can attenuate pulmonary vascular pressures during exercise, possibly by pulmonary endothelium-dependent vasodilatation, also goes against the conventional view of the pulmonary vascular bed being fully dilated during exercise. An explanation of why inhaled NO may increase EIPH is that it may transfer the site of pressure drop (resistance) into the capillary bed (Fig. 3.9).

As a result of exercise, the pulmonary right-to-left shunt (as a result of the coronary and bronchial circulations and $\dot{V}/\dot{Q}$ mismatch), which is only of the order of 1% of the total cardiac output at rest, is reduced to around a half (Wagner et al 1989). Even in standardbreds with mild bronchiolitis, shunt was not increased during moderate exercise compared with healthy controls (Nyman et al 1991b). In addition, despite the increases in total blood flow and redistribution during exercise and evidence for heterogeneity of intrapleural pressures (which may influence regional ventilation), $\dot{V}/\dot{Q}$ relationships during exercise appear to be similar to those at rest (Wagner et al 1989, Seaman et al 1995).

The very high pulmonary vascular pressures in the equine lung during exercise, and in particular, the pulmonary capillary pressures, might be reasonably considered to lead to an increase in extravasation of plasma into the interstitial space and airways. However, attempts to measure extravascular lung water with a dilution method failed to demonstrate a significant increase in extravascular lung fluid in horses during intense exercise (Wilkins et al 2001). In addition, although diffusion limitation has been identified as the main component of exercise-induced arterial hypoxemia in the exercising horse (Wagner et al 1989), this appears to be primarily the result of a function of short pulmonary capillary transit times rather than of thickening of the diffusion pathway by edema fluid (Fig. 3.10). However, in EIPH there is clearly evidence of disruption to the integrity of the alveolar capillary membrane leading to red blood cells being found in the interstitium. It is not inconceivable that some extravasation would occur prior to frank hemorrhage. In fact, the total protein concentration in bronchoalveolar lavage fluid collected from horses soon after exercise is often increased, even without significant evidence of EIPH (Marlin, Schroter, Brown-Feltner and Deaton, unpublished observation).

The Impact of Disease

Exercise-induced pulmonary hemorrhage

Endoscopic studies have highlighted the high prevalence of visible blood in the airways of horses following exercise. The increase in capillary pressure with strenuous and near maximal exercise is sufficient to lead to stress failure of the capillaries (Birks et al 1994, 1997) and the leakage of blood from the circulation into the lungs (Fig. 3.9).

The most widely accepted theory to explain EIPH is pulmonary capillary stress failure because of high transmural pressures (pressures or stresses acting on the pulmonary capillaries). Pulmonary capillary transmural pressure is determined by the difference between pulmonary capillary pressure and airway pressure. When the high pressures (exceeding 100 mmHg during intense exercise) distending the capillaries are opposed by highly positive airway pressures, such as occur during expiration, the transmural pressure (and by implication, wall stress) is low. However, when the distending internal vascular pressure is associated with a large negative airway pressure (as occurs during inspiration), the transmural pressure and wall stress will be high.

Lesions indicative of EIPH are predominantly present in the dorsal caudal region of the lung and it is possible that this is the result of the reported greater perfusion (Bernard et al 1996, Harmegnies et al 2002) leading to increased distension of the thin-walled capillaries in this region.

Studies in isolated, perfused horse lungs have demonstrated that significant disruption of the pulmonary capillaries occurs at pressures of approximately 80 mmHg. It has also been shown *in vivo* that there is probably a threshold mean pulmonary artery pressure of around 80–95 mmHg, above which significant hemorrhage is more likely to occur (Meyer et al 1998, Langsetmo et al 2000). Consequently, any factor that serves to increase pulmonary vascular pressures (e.g. hypervolemia) or to increase the magnitude of the negative pressures in the lung during inspiration (e.g. dynamic upper airway obstruction) would be expected to increase the severity of EIPH. Indeed, hypervolemia is known to be associated with EIPH in racing standardbreds and the severity of EIPH is reduced following phlebotomy (Funkquist et al 2001). Interestingly, upper airway obstructions (neither experimentally induced laryngeal hemiplegia nor dorsal displacement of the soft palate) have been found to increase pulmonary capillary transmural pressure (Jackson et al 1997).

Although NO administration reduces pulmonary arterial pressure during maximal exercise, the severity of EIPH is significantly greater following inhaled NO treatment (Kindig et al 2001). This finding lends weight to the theory that the high pulmonary arterial pressures observed during exercise of high intensity are in fact a protective
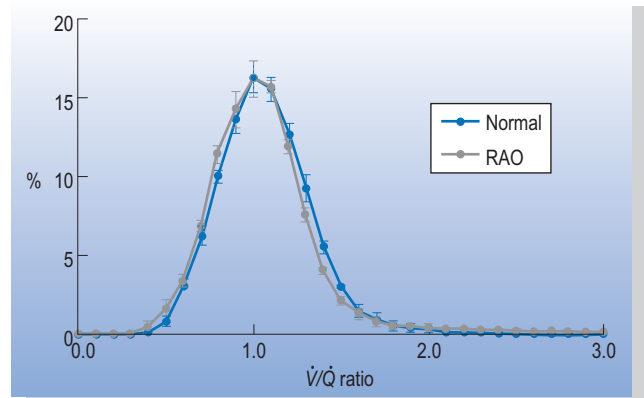

Fig. 3.11. Distribution of ventilation ($\dot{V}$) perfusion ($\dot{Q}$) ratios ($\dot{V}/\dot{Q}$) in horses affected by recurrent airway obstruction (RAO) when in a high degree of clinical remission and in healthy, normal horses determined using inhaled krypton gas in air ($\dot{V}$) and technetium-99m macroaggregates of human albumin (MAA) ($\dot{Q}$).

mechanism caused by arteriolar vasoconstriction to safeguard the capillary bed from the extremely high pressures induced by maximal exercise (Fig. 3.9).

Recurrent airway obstruction

In human patients with pulmonary diseases such as asthma and chronic obstructive pulmonary disease, pulmonary hypertension may be observed. Human patients may also develop pulmonary hypertension secondary to hypoventilation via the HPV mechanism. Pulmonary hypertension has been identified in RAO-affected horses (Dixon 1978, Littlejohn & Bowles 1980, Nyman et al 1991a, Benamou et al 1998). However, in contrast to human patients with chronic pulmonary disease, RAO-affected horses do not generally develop right heart failure secondary to primary pulmonary disease (i.e. cor pulmonale). Right ventricular hypertrophy may, however, occur in severe and chronic cases of RAO. Although pulmonary artery pressure can be elevated in RAO-affected horses, cardiac output is not, indicating that PVR is increased (Nyman et al 1991a). Hypoxic pulmonary vasoconstriction likely plays some role in the development of pulmonary hypertension in the horse as increased inspired oxygen fraction has been shown to reduce hypertension (Dixon 1978). As might be reasonably expected, RAO-affected horses show poor $\dot{V}/\dot{Q}$ matching that improves after treatment (Votion et al 1999). Asymptomatic RAO-affected horses in a high degree of clinical remission (less than 10% bronchoalveolar lavage neutrophils) and maintained at pasture have $\dot{V}/\dot{Q}$ ratio distributions that are not discernible from those of healthy controls (Fig. 3.11).

During intense exercise, RAO-affected horses have higher mean pulmonary artery pressures (of the order

of ~10 mmHg higher) both in clinical remission and during an exacerbation compared to non-RAO controls (Harmegnies et al 2002). Furthermore, RAO-affected horses also have a greater redistribution of pulmonary perfusion to the dorsal lung than non-RAO controls.

REFERENCES

Amis TC, Pascoe JR, Hornof W 1984 Topographic distribution of pulmonary ventilation and perfusion in the horse. American Journal of Veterinary Research 45: 1597–1601

Benamou AE, Art T, Marlin DJ et al 1998 Variations in systemic and pulmonary endothelin-1 in horses with recurrent airway obstruction (heaves). Pulmonary Pharmacology and Therapeutics 11: 231–235

Bernard SL, Glenny RW, Erickson HH et al 1996 Minimal redistribution of pulmonary blood flow with exercise in racehorses. Journal of Applied Physiology 81: 1062–1070

Birks EK, Mathieu-Costello O, Fu Z et al 1994 Comparative aspects of the strength of pulmonary capillaries in rabbit, dog, and horse. Respiration Physiology 97: 235–246

Birks EK, Mathieu-Costello O, Fu Z et al 1997 Very high pressures are required to cause stress failure of pulmonary capillaries in thoroughbred racehorses. Journal of Applied Physiology 82: 1584–1592

Butler AR, Megson IL, Wright PG 1998 Diffusion of nitric oxide and scavenging by blood in the vasculature. Biochimica et Biophysica Acta 1425: 168–176

Butler PJ, Woakes AJ, Smale K et al 1993 Respiratory and cardiovascular adjustments during exercise of increasing intensity and during recovery in thoroughbred racehorses. Journal of Experimental Biology 179: 159–180

Dixon PM 1978 Pulmonary artery pressures in normal horses and in horses affected with chronic obstructive pulmonary disease. Equine Veterinary Journal 10: 195–198

Erickson BK, Erickson HH, Coffman JR 1990 Pulmonary artery, aortic and oesophageal pressure changes during high intensity treadmill exercise in the horse: a possible relation to exercise-induced pulmonary haemorrhage. Equine Veterinary Journal Supplement 9: 47–52

Funkquist P, Sandhagen B, Persson SG et al 2001 Effects of phlebotomy on haemodynamic characteristics during exercise in standardbred trotters with red cell hypervolaemia. Equine Veterinary Journal 33: 417–424

Harmegnies NF, Duvivier DH, Vandenput SN et al 2002 Exercise-induced pulmonary perfusion redistribution in heaves. Equine Veterinary Journal Supplement 34: 478–484.

Jackson JA, Ducharme NG, Hackett RP et al 1997 Effects of airway obstruction on transmural pulmonary artery pressure in exercising horses. American Journal of Veterinary Research 58: 897–903

Kindig CA, Gallatin LL, Erickson HH et al 2000 Cardiorespiratory impact of the nitric oxide synthase inhibitor L-NAME in the exercising horse. Respiration Physiology 120: 151–166

Kindig CA, McDonough P, Finley MR et al 2001 NO inhalation reduces pulmonary arterial pressure but not hemorrhage in maximally exercising horses. Journal of Applied Physiology 91: 2674–2678

Kindig CA, Ramsel C, McDonough P et al 2003 Inclined running increases pulmonary haemorrhage in the Thoroughbred horse. Equine Veterinary Journal 35: 581–585

Lancaster JR 1997 A tutorial on the diffusibility and reactivity of free nitric oxide. Nitric Oxide 1: 18–30

Langsetmo I, Meyer MR, Erickson HH 2000 Relationship of pulmonary arterial pressure to pulmonary haemorrhage in exercising horses. Equine Veterinary Journal 32: 379–384

Liao JC, Hein TW, Vaughn MW et al 1999 Intravascular flow decreases erythrocyte consumption of nitric oxide. Proceedings of the National Academy of Sciences USA 96: 8757–8761

Littlejohn A, Bowles F 1980 Studies on the physiopathology of chronic obstructive pulmonary disease in the horse. II. Right heart haemodynamics. Onderstepoort Journal of Veterinary Research 47: 187–192

Manohar M, Goetz TE 1998 L-NAME does not affect exercise-induced pulmonary hypertension in thoroughbred horses. Journal of Applied Physiology 84: 1902–1908

Manohar M, Goetz TE 1999 Pulmonary vascular resistance of horses decreases with moderate exercise and remains unchanged as workload is increased to maximal exercise. Equine Veterinary Journal Supplement 30: 117–121

McDonough P, Kindig CA, Hildreth TS et al 2002 Effect of body incline on cardiac performance. Equine Veterinary Journal Supplement 34: 506–509

McLaughlin RF 1983 Bronchial artery distribution in various mammals and in humans. American Review of Respiratory Diseases 128: S57–S58

McLaughlin RF, Tyler WS, Canada RO 1961 A study of the subgross pulmonary anatomy in various mammals. American Journal of Anatomy 108: 149–165

Meyer TS, Fedde MR, Gaughan EM et al 1998 Quantification of exercise-induced pulmonary haemorrhage with bronchoalveolar lavage. Equine Veterinary Journal 30: 284–288

Mills PC, Marlin DJ, Demoncheaux E et al 1996a Nitric oxide and exercise in the horse. Journal of Physiology 495: 863–874

Mills PC, Marlin DJ, Scott CM 1996b Pulmonary artery pressure during exercise in the horse after inhibition of nitric oxide synthase. British Veterinary Journal 152: 119–122

Nyman G, Lindberg R, Weckner D et al 1991a Pulmonary gas exchange correlated to clinical signs and lung pathology in horses with chronic bronchiolitis. Equine Veterinary Journal 23: 253–260

Nyman N, Bjork M, Funkquist P 1991b Gas exchange during exercise in Standardbred trotters with mild bronchiolitis. Equine Exercise Physiology 30: 96–101

Pelletier N, Robinson NE, Kaiser L et al 1998 Regional differences in endothelial function in horse lungs: possible role in blood flow distribution? Journal of Applied Physiology 85: 537–542

Rhodes P, Leone AM, Francis PL et al 1995 The L-arginine:nitric oxide pathway is the major source of plasma nitrite in fasted humans. Biochemical and Biophysical Research Communications 209: 590–596

Robinson NE 1985 Respiratory adaptations to exercise. Veterinary Clinics of North America: Equine Practice 1: 497–512

Seaman J, Erickson BK, Kubo K et al 1995 Exercise induced ventilation/perfusion inequality in the horse. Equine Veterinary Journal 27: 104–109

Sinha AK, Gleed RD, Hakim TS et al 1996 Pulmonary capillary pressure during exercise in horses. Journal of Applied Physiology 80: 1792–1798

Sonea IM, Bowker RM, Robinson NE et al 1993 Distribution of dopamine beta-hydroxylase and neuropeptide Y-immunoreactive nerves in healthy equine lungs. American Journal of Veterinary Research 54: 507–513

Votion D, Ghafir Y, Vandenput S et al 1999 Analysis of scintigraphical lung images before and after treatment of horses suffering from chronic pulmonary disease. Veterinary Record 144: 232–236

Wagner PD, Gillespie JR, Landgren GL et al 1989 Mechanism of exercise-induced hypoxemia in horses. Journal of Applied Physiology 66: 1227–1233

West JB, Dollery CT, Naimark A 1964 Distribution of blood flow in isolated lung: relation to vascular and alveolar pressures. Journal of Applied Physiology 19: 713–724

Wilkins PA, Gleed RD, Krivitski NM et al 2001 Extravascular lung water in the exercising horse. Journal of Applied Physiology 91: 2442–2450

4

Gas Exchange in Horses

R Eddie Clutton

Oxygen (O_2) is required in aerobic organisms to oxidize energy-dense compounds like glucose to produce adenosine triphosphate (ATP), the most common energy source for biochemical reactions. Glucose oxidation involves glycolysis, in which 1 mole of glucose is converted into 2 moles of pyruvic acid and *some* ATP, and the Krebs cycle, in which each mole of pyruvate yields an additional 15 moles of ATP. Therefore, the complete oxidation of 1 mole of glucose to carbon dioxide (CO_2) and water (H_2O) yields either 36 or 38 moles of ATP.

Reaction 1. $6\,O_2 + C_6H_{12}O_6 \rightarrow 6\,CO_2 + 6\,H_2O + 36\,ATP$
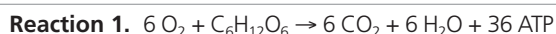
The pyruvate produced by glycolysis only enters the Krebs cycle if O_2 is available. Under anaerobic conditions, pyruvate is converted to lactic acid in a reaction that is relatively inefficient in terms of ATP production and which lowers tissue pH. These reactions occur within mitochondria, which represent the final destination of inspired O_2 in the "oxygen cascade" (Table 4.1).

The simultaneous production of CO_2 creates a diffusion gradient that initiates CO_2 movement in the opposite direction. The prompt removal of CO_2 from metabolically active tissue is important because its accumulation results in a rapid lowering of tissue pH through carbonic acid formation.

Reaction 2. $H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$
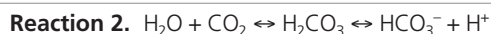
In excess, protons (H^+) denature enzymes, affect ion complex dissociation, alter biological membrane behavior, and disrupt membrane transport systems.

The ratio of CO_2 production to O_2 consumption is known as the respiratory quotient (RQ) and indicates the energy substrate being oxidized. The RQ is 1.0 for carbohydrate metabolism, 0.8 for protein metabolism and 0.7 for fat oxidation.

Units and Definitions

Gas diffusion involves random molecular movement from areas of high partial pressure to areas of low partial pressure. The rate of diffusion depends on the partial pressure (or tension) difference between each area. The partial pressure (P) of a gas in a mixture is the pressure that a given gas would exert if it alone occupied the space. Partial pressures are not the same as "contents" or "concentrations" but are important because they determine the rate and direction of gas diffusion. The concentration or content of gases in liquids (usually expressed as ml of gas per dl of blood) are important because in the final analysis, O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) occur on a ml/kg basis. The link between tension and content of a gas in a liquid is given by Henry's law, which states that the volume (V) of gas dissolving in a liquid is proportional to the partial pressure (P) of the gas:

Equation 1. $V = kP$

where k is the solubility coefficient of a specified gas in a liquid. For CO_2 in water, k = 0.07 ml·mmHg^{-1}·dl^{-1}. For O_2 in water, k = 0.0225 ml·kPa^{-1}·dl^{-1} (0.003 ml·mmHg^{-1}·dl^{-1}). Two measurement systems are used for partial pressure. These are mmHg (also known as Torr) and the SI unit kPa where 1 kPa = 7.52 mmHg. Blood gas values from horses and foals are listed in Table 14.5 in Chapter 14.

Oxygen Movement from Atmosphere to Alveolus

The partial pressure (P) of O_2 in inspired (i) air (P_IO_2) is given by $F_IO_2 \times P_B$, where F_IO_2 is the fractional concentration (F) of inspired O_2 and P_B is the barometric pressure. When breathing air (21% O_2), F_IO_2 is 0.21 given a normal barometric pressure of 101 kPa (760 mmHg), although this value changes with meteorological conditions and falls with increasing altitude. Thus, the inspired O_2 tension is 0.21 × 101 = 21.21 kPa (or 159 mmHg) and

Table 4.1. The oxygen cascade			
Site	**Notation**	**kPa**	**mmHg**
Inspired	P_IO_2	21.21	159
Alveolar	P_AO_2	13.7	103
Arterial	P_AO_2	13.3	100
Capillary	P_cO_2	6.8	51
Mitochondrial	$P_{MITO}O_2$	0.13–1.3	1–10

the overall maximum tension gradient of the O_2 cascade is $21.21 - 0.13 = 21.08$ kPa (or 158 mmHg). However, the alveolar (A) O_2 tension ($P_{A}O_2$) is less than this, because inspired air is humidified as it traverses the airways, becoming saturated with water vapor before it reaches the alveolar space. The saturated vapor pressure of water (P_{H_2O}) is 6.26 kPa (47 mmHg) at body temperature (37°C) and reduces the partial pressures of other inspired gases. Alveolar O_2 is also diluted by CO_2 evolving into the alveolar space from pulmonary capillaries. The dilution of inspired O_2 is described by the alveolar gas equation:

> **Equation 2.** $P_{A}O_2 = F_{I}O_2 (P_B - P_{H_2O}) - (P_{a}CO_2/RQ)$

where $P_{a}CO_2$ is the arterial tension of CO_2 and RQ is the respiratory quotient (see above).

Ventilation, which is the bulk movement of O_2-rich and CO_2-free gas into the alveolar space has two purposes: (1) to ensure that the alveolar O_2 tension is sufficiently greater than that in pulmonary capillary blood to maintain constant O_2 diffusion across the alveolar–capillary membrane; and (2) to maintain a sufficiently low alveolar CO_2 tension ($P_{A}CO_2$) to maintain constant CO_2 diffusion in the reverse direction. However, not all inspired gas reaches the alveolar space to fulfil these functions (see Fig. 4.1). In horses, approximately 50% of the tidal volume (V_T) remains within the upper airway and is exhaled unchanged with the next breath (Gallivan et al 1989). These regions of the tracheobronchial tree do not participate in gas exchange and gas contained within them constitutes anatomic dead space ($V_{D_{ANAT}}$). Dead space ventilation is

A. End-expiration   B. Inspiration   C. End-inspiration   D.Expiration

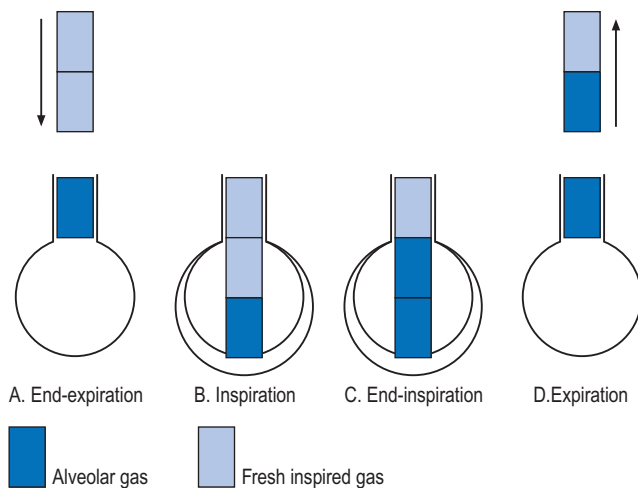Alveolar gas   Fresh inspired gas

Fig. 4.1. Dead space ventilation in the resting horse. Approximately 50% of the tidal volume resides in the anatomic dead space at the end of expiration (A). Therefore, when a 500-kg horse inspires 6 litres of air (each block represents 3 litres of gas) only 3 litres participates in gas exchange (B and C). The anatomic dead space is augmented by inspired gas distributed to underperfused alveoli, or alveolar dead-space.

probably an important means of heat dissipation in exercising horses (Pelletier 1987). The proportion of inspired gas that inflates non-perfused alveoli also fails to contribute to gas exchange and is known as alveolar dead space ($V_{D_{ALV}}$). The combination of $V_{D_{ANAT}}$ and $V_{D_{ALV}}$ is called physiological dead space ($V_{D_{PHYS}}$).

> **Equation 3.** $V_{D_{PHYS}} = V_{D_{ANAT}} + V_{D_{ALV}}$

The volume of gas reaching alveoli per minute and potentially able to participate in gas exchange is known as the alveolar minute ventilation ($\dot{V}_A$) and is given by:

> **Equation 4.** $\dot{V}_A = (V_T - V_{D_{PHYS}}) f_R$

where f_R is the respiratory rate. Alveolar ventilation ($\dot{V}_A$) is an important physiological variable, being the principal determinant of CO_2 tension in blood (see equations 1 and 2 in Chapter 14). The relationship between dead space volume and tidal volume is given by Bohr's equation:

> **Equation 5.** $V_D/V_T = (P_{a}CO_2 - P_{\bar{E}}CO_2)/P_{a}CO_2$

where $P_{a}CO_2$ is used to indicate $P_{A}CO_2$ and $P_{\bar{E}}CO_2$, the mixed expired CO_2 tension, is measured by capnometry during expiration. Ventilation ensures the bulk flow of fresh gas into the terminal bronchioles while gas movement into the alveoli occurs because of rapid diffusion.

Gas Diffusion Across the Alveolar Capillary Membrane

Alveolar gases (CO_2 and O_2) diffuse passively across the alveolar–capillary membrane in accordance with Fick's law (equation 6). The rate of gas transfer (V_{gas}) is directly proportional to the alveolar–capillary surface area available for exchange (A), the difference in gas partial pressures between the alveolus and capillary ($P_A - P_V$), and the solubility of each gas in water (s). Diffusion rate is inversely proportional to the thickness of the alveolar–capillary membrane (d) and the square root of each gas's molecular mass (RMM).

> **Equation 6.** $V_{gas} = [A (P_A - P_V) s] /d (RMM)^{1/2}$

On the basis of its relative molecular mass, O_2 diffuses more rapidly than CO_2. The diameter of the human alveolus is only 0.1 mm and differences between gas tensions at the center and periphery of the alveolus are theoretically eliminated in about a millisecond. However, in human emphysema, alveoli coalesce to form air sacs, which may themselves form bullae with diameters of 10 mm or more. In these, diffusion from the center to the periphery may be slow enough to limit V_{gas}. While this in theory may lead to CO_2 retention, hypercapnia does not occur for this reason because compensatory increases in $\dot{V}_A$ occur. Values for A in horses are high compared to other species, which satisfies their high V_{O_2} during exercise (Gehr & Erni 1980, Gehr et al 1981, Taylor et al 1981).

Oxygen and CO_2 passage across the alveolar membrane involves solution in the alveolar fluid layer and so aqueous solubility is important. CO_2 is 25 times more soluble than O_2 in the alveolar fluid layer. The relative rates of diffusion for O_2 and CO_2 between alveoli and capillaries is found by combining diffusibility and water solubility; this reveals CO_2 to be about 20 times more diffusible than O_2. For this reason, outward diffusion of CO_2 from the blood into the alveolus is rarely, if ever, a clinical problem.

The difference between alveolar and mixed venous O_2 tensions in pulmonary capillaries, i.e. the $P_{(A-V)}O_2$, is the generator for O_2 passage across the alveolar–capillary membrane and is increased when high O_2 concentrations are inspired. Its value can be calculated using the alveolar gas equation (equation 2). In the following example (equation 7) P_aCO_2 is taken as 5.33 kPa (40 mmHg), RQ is taken as 1.0, and the O_2 tension in blood returning to the lungs (P_VO_2) is 5.33 kPa (40 mmHg). Under normal conditions and when breathing room air, F_IO_2 has a value of 0.21:

Equation 7. $P_AO_2 = [0.2 (101 - 6.26)] - 5.33$
$$= 13.62 \text{ kPa (102.6 mmHg)}$$
and $P_{(A-V)}O_2 = (13.62 - 5.33)$
$$= 8.29 \text{ kPa (62 mmHg)}$$

When breathing 100% O_2 ($F_IO_2 = 1$):

Equation 8. $P_AO_2 = [1.0 (101 - 6.26)] - 5.33$
$$= 89.41 \text{ kPa (673 mmHg)}$$
and $P_{(A-V)}O_2 = (89.41 - 5.33)$
$$= 84.1 \text{ kPa (633 mmHg)}$$

Consequently, providing 100% O_2 to breathe increases the $P_{(A-V)}O_2$ tension gradient 10-fold and greatly accelerates O_2 movement into pulmonary capillaries. This contrasts with the small increase produced by controlled or voluntary hyperventilation, which increases P_AO_2 by reducing P_aCO_2. High altitude may have an incapacitating effect on alveolar O_2 diffusion, which may be important in international competition horses. At an altitude of 3048 m (10,000 feet), the barometric pressure is only 69.6 kPa (522 mmHg). According to the alveolar gas equation, at this altitude:

Equation 9. $P_AO_2 = [0.2(69.6 - 6.26)] - 5.33$
$$= 7.34 \text{ kPa (55 mmHg)}$$
and $P_{(A-V)}O_2 = (7.34 - 5.33)$
$$= 2.01 \text{ kPa (15 mmHg)},$$

which is inadequate for O_2 transfer into pulmonary capillaries (Greene et al 1999).

Oxygen uptake also depends on pulmonary capillary transit time because there is a finite time available for alveolar gases to equilibrate with pulmonary capillary blood (Fig. 4.2). Estimates in resting human beings indicate that pulmonary capillary blood remains in contact with the alveolar capillary membrane for 750 milliseconds (ms).

During this period, the O_2 tension in flowing alveolar blood rises from 5.33 towards 13.3 kPa (40–100 mmHg), with equilibration (when capillary tension ($P_{c'}O_2$) equals P_AO_2) occurring within approximately 250 ms (curve A, Fig. 4.2). This leaves an additional 500 ms for equilibration should any diffusion impediment arise. However, when diffusion is severely curtailed, e.g. at altitude, equilibration may not occur in the 750 ms available (curve B), in which case the end-capillary O_2 tension ($P_{c'}O_2$) will be lower than P_AO_2 and will increase venous admixture (see below). During strenuous exercise, pulmonary transit time may be reduced to 150 ms, which may be insufficient time for equilibration if diffusion is also retarded (curve C). The passage of large blood volumes at high rates through the lungs when there is insufficient time for $P_{(A-a)}O_2$ equilibration contributes to hypoxemia in exercising horses (Wagner et al 1989). Curve D illustrates the benefits of inspiring enriched O_2 mixtures (50% O_2 in this example). This accelerates O_2 diffusion
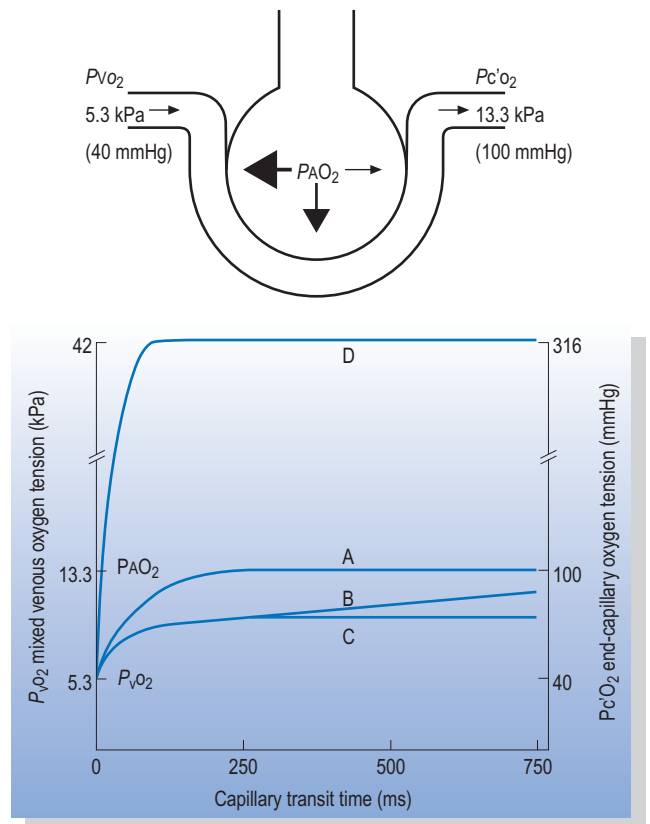
Fig. 4.2. The effects of alveolar oxygen tension and pulmonary capillary transit time on end-capillary oxygen tensions. Curve A, alveolar (P_AO_2) and end-capillary ($P_{c'}O_2$) oxygen tensions normally equilibrate within 250 ms. Curve B, diffusion impairment may prevent equilibration in which case $P_{c'}O_2$ is less than P_AO_2 and contributes to venous admixture. Curve C, short capillary transit times (250 ms) and diffusion impairment prevent alveolar and end-capillary oxygen tension equilibration. Curve D, breathing oxygen-enriched gas increases P_AO_2 and greatly accelerates alveolar and end-capillary oxygen tension equilibration.

into pulmonary capillary blood by greatly increasing the alveolar–capillary O_2 tension difference.

In diffusing from the alveolus to the erythrocyte, an O_2 molecule must cross several layers before combining with hemoglobin (Hb). These include the alveolar gas, the surfactant/fluid layer, the alveolar epithelial membrane, the capillary endothelial membrane, the plasma, the erythrocyte membrane, and the intra-erythrocytic fluid. The diffusion path length thus outlined may be increased pathologically in several ways, e.g. by edema, fibrosis, and in theory may impair diffusion. The area of perfused alveoli available for gas exchange is reduced in human emphysema when alveolar walls are broken down, and O_2 diffusion may be limited by a decrease in the area available for gas exchange.

Ventilation/Perfusion Ratios

The link between ventilation ($\dot{V}$) and lung perfusion ($\dot{Q}$) affects the efficiency of gas exchange. Breathing expends metabolic energy because work is performed in ventilating alveoli. Biological efficiency is best served when the gas volume inspired is only just sufficient to arterialize pulmonary capillary blood. In normal lungs most alveoli are adequately perfused although the best-ventilated units are not necessarily the best perfused. Alveolar O_2 concentration (C_AO_2) is a function of $\dot{V}_A$ (input) and the rate of O_2 removal by perfusion. Therefore, C_AO_2 and ultimately pulmonary capillary O_2 tension depends on the $\dot{V}/\dot{Q}$ ratio (Fig. 4.3A). Optimally, the $\dot{V}_A$ and $\dot{Q}_T$ are almost the same and so the normal ratio of $\dot{V}_A$ to $\dot{Q}_T$ is approximately 1.0. Disparity between ventilation and perfusion ($\dot{V}/\dot{Q}$ discrepancy, scatter or mismatch) is important because it represents inefficient gas exchange. $\dot{V}/\dot{Q}$ matching is profoundly altered during general anesthesia in horses when the effects of reduced cardiac output are aggravated by changes in position. It was previously believed that dorsal lung regions received less ventilation and perfusion than ventral areas in conscious standing horses (Amis et al 1984). However, more recent studies have challenged this by demonstrating that pulmonary blood flow distribution is largely independent of gravity in healthy, standing horses (Hlastala et al 1996) and that there is good ventilation/perfusion matching with $\dot{V}_A$ and $\dot{Q}_T$ being directed to units in which the $\dot{V}/\dot{Q}$ ratio is 1.0 (Hedenstierna et al 1987). Despite this, $\dot{V}/\dot{Q}$ mismatching is probably the commonest cause of low P_AO_2 in Equids and arises when pulmonary ventilation and perfusion are not distributed evenly throughout the lungs.

Dependent lung regions are better ventilated than upper zones during spontaneous breathing at normal lung volumes, irrespective of body position (see Fig. 4.4). However, when lung volume is low, as may be found in pregnant mares at term, dependent parts of the lung are poorly ventilated or not ventilated at all. The distribution of
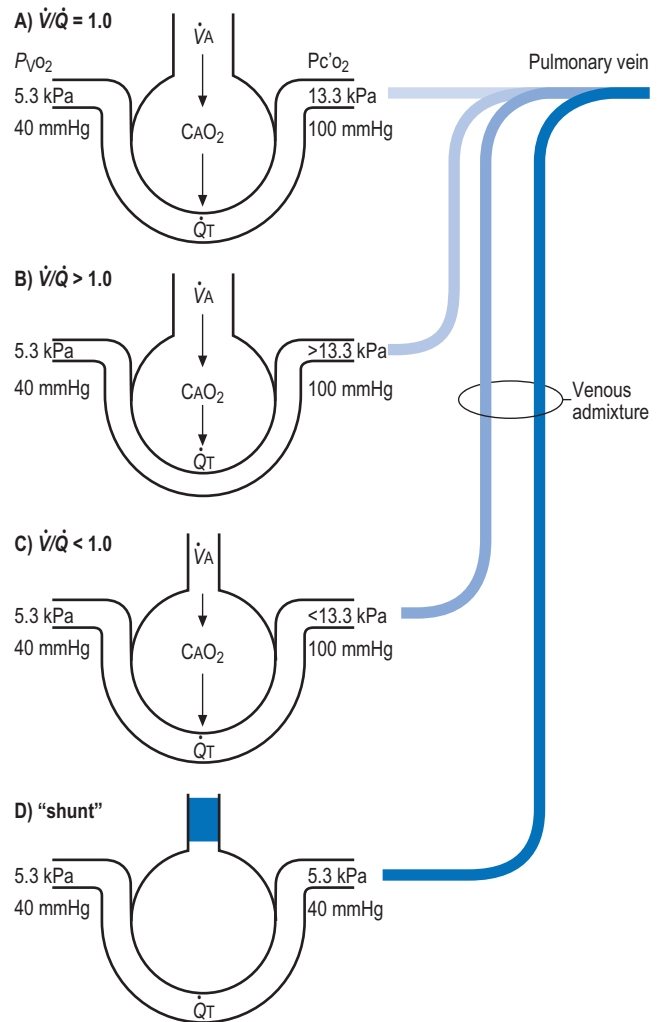
Fig. 4.3. Ventilation/perfusion ($\dot{V}/\dot{Q}$) relationships in the lung. (A) normal, (B) high $\dot{V}/\dot{Q}$, (C) low $\dot{V}/\dot{Q}$ and (D) "shunt" flow units and their effects on pulmonary venous oxygen levels. The content of oxygen in alveolar gas (C_AO_2) determines blood oxygenation, and depends on input by minute alveolar ventilation ($\dot{V}_A$) and uptake by pulmonary perfusion, which is the same as cardiac output ($\dot{Q}_T$).

ventilation is more uniform when animals are in sternal, compared to lateral or dorsal, recumbency. This suggests that the sloping diaphragm protects the lungs from compression by abdominal contents in standing animals (Sorensen & Robinson 1980). The heterogeneous distribution of inspired gas arises because the suspended lung has mass that stretches dorsal alveoli and increases their volume more than those at the base. Also, intrapleural pressure becomes less subatmospheric towards the sternum. Both effects place dorsal alveoli at the upper end of their volume–pressure curve where compliance is low. By contrast, ventral alveoli operate on the steeper portion of their curve and therefore have higher compliance. As a consequence of the differences in regional lung compliance, decrements in intrapleural pressure
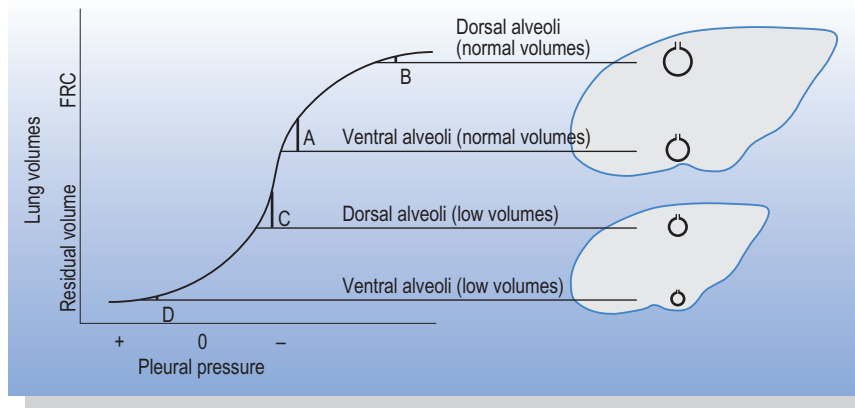
Fig. 4.4. Volume–pressure relationships for the equine lung at low and normal volumes. Ventral alveoli receive greater ventilation (curve A) than dorsal alveoli (curve B) when breathing from the resting expiratory level of functional residual capacity (FRC). This pattern is reversed at low lung volumes when ventral alveoli (curve D) may be collapsed.

during inspiration cause relatively larger increases in ventral (Fig. 4.4A) compared with dorsal alveoli (Fig. 4.4B). This is not the case when the lung is operating at low volumes, for example during anesthesia or in advanced pregnancy. Under these conditions, the pleural pressure at the lung's base can exceed atmospheric pressure so that ventral lung is under compressive rather than expansive pressures. This corresponds to the flat, left-hand portion of the volume–pressure curve. Thus, small decreases in pleural pressure cause dorsal (Fig. 4.4C), rather than ventral (Fig. 4.4D) ventilation. Only when ventral pleural pressure falls below atmospheric pressure will alveoli here begin to expand. Regional differences in alveolar size also confer different patterns of radial traction on conducting airways. Expanded alveoli at the lung's apex increase the airway diameter in this region rendering it a low-resistance pathway for inspired gas. The paucity of collateral ventilation in the equine lung aggravates the effects of uneven ventilation distribution (Robinson & Sorenson 1978).

Regional inequalities in perfusion occur in human beings in whom blood flow is greatest in dependent regions, and least in non-dependent regions. However, this pattern is less typical of horses (Hlastala et al 1996), in which perfusion is position-independent and tends to be greatest in dorsocaudal regions (Dobson et al 1985, Jarvis et al 1992). Exercise affects pulmonary blood distribution with $\dot{Q}_T$ increasing five- or six-fold and pulmonary arterial pressures reaching 100 mmHg in exercising horses (Sinha et al 1996). Both dorsal and ventral blood flows increase accordingly but the proportion of total flow reaching the dorsocaudal regions is disproportionately greater. Lung volumes also affect perfusion because pulmonary vascular resistance is high when the lung is deflated to a low volume. In human beings, uneven blood flow distribution throughout the lung results from the combined effects of gravity and the low pressures generated by the right ventricle. In addition, the thin alveolar–capillary membrane that separates air and blood in the pulmonary circulation means that hydrostatic pressures exert a major effect on small blood vessel caliber, while alveolar pressures also exert an appreciable effect on local blood flow. West (1977) summarized the effects of different pulmonary arterial, alveolar, and venous pressures on pulmonary blood flow by describing four lung zones; however, this model may be less applicable to the horse.

Two intrinsic mechanisms operate to optimize $\dot{V}/\dot{Q}$ ratios in the normal mammalian lung. The more important is known as hypoxic pulmonary vasoconstriction (HPV), which diverts blood from underventilated lung to areas where $\dot{V}_A$ is higher (Bisgard et al 1975, Marshall & Marshall 1985). Hypoxic vasoconstriction is strong in ponies compared to some other species but is incapacitated by some anesthetics and possibly by inflammatory lung disease (Robinson 1982). Hypercapnic bronchodilatation is a complementary though relatively impotent reaction compared with HPV. In hypercapnic bronchodilatation, high CO_2 concentrations from low $\dot{V}/\dot{Q}$ units induce bronchodilatation and this increases local ventilation, returning $\dot{V}/\dot{Q}$ ratios towards normal.

Ventilation/Perfusion Mismatching

Scanning studies performed in standing horses were unable to demonstrate a gradient of $\dot{V}/\dot{Q}$ ratios between dorsal and ventral regions of the lung (Amis et al 1984). More recently performed multiple inert gas studies showed that up to 10% of $\dot{Q}_T$ is directed towards high $\dot{V}/\dot{Q}$ units in the upper portions of the lung (Hedenstierna 1987). The latter indicates that $\dot{V}/\dot{Q}$ ratio scatter in conscious horses is similar to that seen in human beings (Hedenstierna et al 1987). This variation in $\dot{V}/\dot{Q}$ ratios makes regional differences in gas exchange inevitable and reduces gas exchange efficiency. Gas exchange in lungs in which $\dot{V}/\dot{Q}$ ratios are

widely dispersed is less efficient than that occurring in lungs in which $\dot{V}/\dot{Q}$ variation is low.

The effect of $\dot{V}/\dot{Q}$ mismatching in normal lungs is of little significance. $P_{A}O_2$ may be reduced by perhaps 1–2 kPa, while $P_{a}CO_2$ increases are even less. Regardless, both discrepancies are readily corrected by increased ventilation, which increases the overall $\dot{V}/\dot{Q}$ ratio. Any lung abnormality such as recurrent airway obstruction (Nyman et al 1991) can cause $\dot{V}/\dot{Q}$ inequalities and severely affect gas transfer. It is important to realize that no amount of $\dot{V}_A$ can resolve $\dot{V}/\dot{Q}$ discrepancies causing more than a 6.6 kPa (50 mmHg) depression of $P_{A}O_2$. In contrast, increasing total ventilation can reduce $P_{a}CO_2$ but this can lead to an unsustainable increase in the work of breathing, in which case chronic hypercapnia ensues.

Alveoli with high $\dot{V}/\dot{Q}$ ratios are ventilated but underperfused and are found in West's Zone 1 and periodically, in parts of Zone 2 (Fig. 4.3B). Oxygenation is usually not a problem in these units because O_2 added by ventilation exceeds that removed by blood flow. However, high $\dot{V}/\dot{Q}$ units contribute relatively little to O_2 uptake because $P_{A}O_2$ is likely to be at levels producing near-complete Hb saturation and total blood flow is low. However, this situation changes on exercise when blood flow to the dorsal lung regions increases. Areas with high $\dot{V}/\dot{Q}$ ratios effectively, though inefficiently, remove excessive CO_2. The alveolar gas equation indicates that alveolar dead space may cause hypoxemia because of alveolar CO_2 accumulation. Increasing $\dot{V}_A$ and/or increasing the inspired O_2 concentration may ameliorate this effect.

Lung units with low $\dot{V}/\dot{Q}$ ratios impair the oxygenation of blood (Fig. 4.3C). This poorly oxygenated blood dilutes oxygenated blood returning to the left heart from appropriately ventilated and perfused alveoli. This diluting action, known as venous admixture, always lowers $P_{A}O_2$. In the West model of gas exchange in human lungs (Fig. 4.3B), venous admixture comes predominantly from low $\dot{V}/\dot{Q}$ units in ventral alveoli. These units strongly influence arterial blood composition because they receive a large percentage of the pulmonary blood flow, have a low $P_{A}O_2$ and an increased $P_{A}CO_2$. It is important to point out, however, that recent studies in horses indicate that gravity is not an important determinant of blood flow distribution (Hlastala et al 1996), which questions the relevance of the West model for equine lungs.

Anatomic and pathological shunt contributes to venous admixture. "Shunt" describes the passage of blood from right (pulmonary) to left (systemic) circulations without exposure to alveolar gas (see Fig. 4.3D). Anatomic shunt refers to the small volume of Thebesian (coronary) and bronchial venous blood that normally drains into the systemic circulation. In some conditions, e.g. pleuritis, this normal fraction increases during the hyperemic response to inflammation. Pathological shunts can be either intrapulmonary (e.g. pneumonia, atelectasis, bron-

chitis, alveolitis, and neoplasia) or intracardiac (e.g. ventricular septal defects that cause blood to flow from the right to left side of the heart). The response to breathing 100% O_2 can differentiate hypoxemia caused by shunts from that caused by low $\dot{V}/\dot{Q}$ ratios. The hypoxemia caused by shunts is unresponsive to increased inspired O_2 concentration. In contrast in low $\dot{V}/\dot{Q}$ alveoli, increasing the percentage of inspired O_2 causes some improvement in $P_{A}O_2$.

With regard to O_2 exchange, blood from lung regions with high $\dot{V}/\dot{Q}$ ratios is rarely able to compensate for the venous admixture from low $\dot{V}/\dot{Q}$ regions. This is because the volume of blood is small even though its Hb is almost saturated. In the case of CO_2 exchange, this does not apply because the relationship between blood content and alveolar tension is more linear. Blood leaving high $\dot{V}/\dot{Q}$ units contains proportionately less CO_2. Increasing total lung ventilation in the presence of $\dot{V}/\dot{Q}$ abnormalities increases CO_2 excretion and thereby compensates for reduced CO_2 losses from underventilated lung. However, increased ventilation does not compensate for reduced O_2 uptake in poorly ventilated lung. Impaired pulmonary O_2 exchange may be quantified while the animal breathes air by use of the alveolar gas equation to calculate $P_{(A–a)}O_2$ (see Equation 2 and Chapter 14).

Hemoglobin Binding of Oxygen

Plasma carries only small volumes of O_2 in solution because the solubility coefficient of O_2 is low (see equation 1). At a normal $P_{A}O_2$ of 13.3 kPa (100 mmHg) each deciliter of plasma contains only 0.3 ml O_2. Hemoglobin increases the O_2 carrying capacity of blood 65-fold because each gram Hb combines with 1.34 ml of O_2. When the plasma Hb concentration is normal (15 g/dl) the volume of Hb-bound O_2 is $15 \times 1.34 \times SO_2$ ml (where SO_2 is the percentage saturation of Hb). Reference to the oxyhemoglobin (HbO_2) dissociation curve (Fig. 4.5) illustrates that SO_2 has a value of 0.97 (Hb is 97% saturated) when arterial O_2 tensions are normal [13.3 kPa (100 mmHg)]. Thus, the volume of Hb-bound O_2 under these conditions is $15 \times 1.34 \times 0.97 = 19.5$ ml/dl and the total arterial O_2 content ($C_{a}O_2$) is given by:

> **Equation 10.** $C_{a}O_2 = [(Hb) \times 1.34 \times 0.97] + (13.3 \times 0.0225)$
> $= 19.8$ ml/dl

Breathing 100% O_2 increases $P_{A}O_2$ to 89.5 kPa (673 mmHg) and the amount of O_2 in solution in plasma to 1.8 ml/dl. However, because the Hb is almost fully saturated when breathing air, breathing pure O_2 increases $C_{a}O_2$ by only 10%.

The reaction of Hb with O_2 has three important properties. First, it is rapid and reversible. Second, the molecular structure, which involves four globin chains, causes the reaction of each chain with O_2 to facilitate subsequent binding. This accounts for the sigmoid shape of
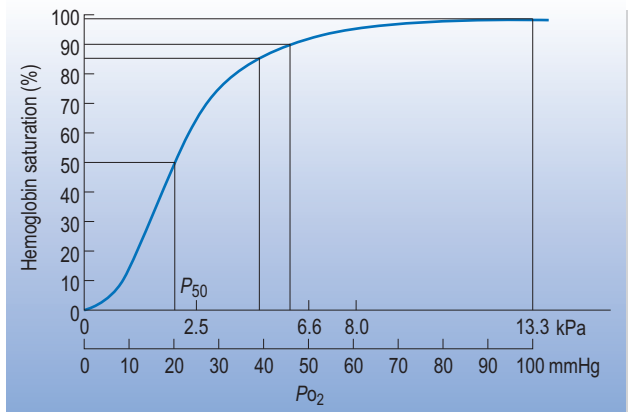
Fig. 4.5. The dissociation curve for equine hemoglobin. The oxygen tension corresponding to 50% hemoglobin saturation (P_{50}) value in horses is less than the corresponding value for human hemoglobin, indicating a "left-shifted" curve. The "shoulder" of the dissociation curve lies at a correspondingly lower value of Po_2 (40 mmHg). Drawn from data in Clerbaux et al, 1986 and Fenger et al, 2000, with permission.

the HbO_2 dissociation curve (ODC), which is required for optimal pulmonary uptake and tissue O_2 release. Third, the rate of reaction of Hb with O_2 is affected by pH.

The ODC (Fig. 4.5) depicts the relationship between plasma O_2 tension and Hb saturation (So_2). The latter is the amount of O_2 combined with Hb (O_2 content) divided by the total Hb O_2 capacity. The equine ODC lies to the left of the normal human curve. The sigmoid relationship means the slope is steep at low, and flat at high Po_2 values. The upper flat (associative) part of the curve corresponds to Po_2 values normally encountered in the lung, while the lower steep (dissociative) part covers tissue Po_2 values. The important features of the association segment are that (1) Hb is almost 100% saturated at relatively low Po_2 values; (2) increasing Po_2 much above physiological values (5.33 kPa or 100 mmHg) does not produce correspondingly larger increases in the So_2; and (3) Hb saturation does not change appreciably over the Po_2 range 6.56–13.3 kPa (50–100 mmHg).

The steep dissociative part of the ODC occurs over the range of Po_2 encountered in metabolically active tissues. In these tissues there are factors that reduce the affinity of Hb for O_2 and cause a "right-shift" in the ODC. Increased temperature, increased H^+ (decreased pH), increased Pco_2, increased ADP and increased 2,3-diphosphoglycerate all facilitate the unloading of O_2 at the tissue level. In the lung and pulmonary capillaries the opposite conditions prevail. Carbon dioxide is evolved, blood pH rises and blood is cooled. Even though all three factors increase the affinity of Hb for O_2 and augment pulmonary uptake, the effect is small over the associative part of the ODC, which is operative in the lung.

Cardiac Output and Oxygen Flux

Oxygen flux (Do_2) describes the total volume of O_2 delivered to peripheral tissue. It is equal to the product of the O_2 content of arterial blood and cardiac output:

Equation 11. $Do_2 = \dot{Q}_T \left([Hb] \times 1.34 \times S_aO_2\right) + (P_AO_2 \times 0.0225)$

where P_AO_2 is in kPa. Substituting figures for a horse with a resting cardiac output of $0.6 \text{ dl·kg}^{-1}\text{·min}^{-1}$ gives a value for Do_2 of $12.2 \text{ ml·kg}^{-1}\text{·min}^{-1}$, which considerably exceeds the resting animal's requirements ($\dot{V}o_2$) of $4 \text{ ml·kg}^{-1}\text{·min}^{-1}$ and ensures that a state of aerobic metabolism prevails. This can be expressed in terms of the "coefficient for O_2 utilization" which is given as $\dot{V}o_2/Do_2$, and in the above example is 32.7%

A consequence of the factorial nature of equation 11 is that relatively minor reductions in each factor produce a disproportionately great effect on Do_2. For example, when values for plasma Hb concentration, cardiac output and So_2 are halved, Do_2 is reduced to 12.5% of normal. Such changes culminate in critical Do_2 reductions and are commonly encountered in equine anesthesia, where low P_AO_2 values are inevitable and $\dot{Q}_T$ is often severely depressed by inhaled anesthetics. Under these conditions, mildly anemic horses may experience critical reductions in O_2 flux.

The Movement of Gas from Blood to Tissues

The tension gradient causing O_2 diffusion from plasma across capillary walls into interstitial fluid and finally into cells depends on the metabolic activity of the latter, because arteriolar Po_2 is normally constant at 13.3 kPa (100 mmHg). Metabolically active cells lower local O_2 tensions below those in the capillary, which in turn lowers plasma Po_2 and causes HbO_2 dissociation. Tissue O_2 tensions normally range from 1.33 to 5.33 kPa (10–40 mmHg).

The dissociation portion of the ODC is adapted to release O_2 at tissue level. The steep part corresponds to Po_2 values from 1.33 to 5.33 kPa and so small decrements in tissue O_2 cause disproportionately large reductions in So_2 and O_2 release. This is facilitated by the right shift in the ODC caused by the low pH and elevated temperatures found in active tissue (see previous section).

Tissue O_2 delivery depends on the rate and pattern of tissue blood flow and, while most gas exchange occurs at capillary level, considerable diffusion is possible wherever vessel walls are gas permeable and adequate transmural O_2 tension gradients exist. Tissue perfusion depends on the arteriovenous pressure gradient and vascular resistance. Arterial and venous pressures are generally maintained within a small range so the principal determinant of local perfusion is vascular resistance. Gas diffusion into the tissues is facilitated when the distance between cells

and capillaries is small. During the high oxygen demands of exercise, diffusion distance is effectively reduced by recruitment of previously unperfused capillaries and by local vasodilatation. These changes are initiated by the same factors that promote HbO_2 dissociation, i.e. local hypoxia, acidosis, hypercapnia and temperature elevation. Hemoglobin itself may contribute to blood flow control. A major portion of the vasodilator nitric oxide in the blood is bound to Hb, forming S-nitroso-Hb. On deoxygenation of Hb, the bound nitric oxide is released into the micro-circulation, causing vasodilatation.

When tissue metabolic requirements are met, O_2 extraction is fairly constant, irrespective of Do_2. When Do_2 becomes limited by inadequate flow, a linear relationship develops between O_2 delivery and O_2 extraction. This flow-limited or critical O_2 delivery system is altered by shock, when critically low Do_2 causes anaerobic metabolism.

Tissue Hypoxia

The steps of the O_2 cascade (Table 4.1) provide a sequential basis for describing the development of tissue hypoxia, which occurs when tissue O_2 requirements ($\dot{V}o_2$) fall below the rate of supply (Do_2). The earlier in the cascade when hypoxia develops, the greater the consequence at the mitochondrial level. Conversely, breathing O_2 greatly improves O_2 delivery to the tissues.

Atmospheric hypoxemia results when the O_2 tension of inspired gas (P_Io_2) is inadequate. This can occur at very high altitudes where barometric pressure is low, or in any situation where the fraction of inspired O_2 (F_Io_2) is reduced. Examples of the latter are rare, except during nitrous oxide anesthesia.

Tidal hypoxemia results from inadequate $\dot{V}_A$ and is therefore readily identified by a concurrent elevation in P_aco_2. The decrease in P_ao_2 is approximately equal to the increase in P_aco_2: increases in P_aco_2 from 5.33 to 10.66 kPa (40 to 80 mmHg) are accompanied by falls in P_Ao_2 from 13.3 to 7.99 kPa (100 to 60 mmHg). It follows that hypoventilation must be severe for O_2 tensions to fall critically. In any case, tidal hypoxemia responds readily to both ventilation and to increased O_2 administration. Tidal hypoxemia is caused by (1) suppressing neural control of breathing, e.g. heavy sedation or depression; (2) reducing respiratory muscle function, e.g. diaphragmatic hernia, tympany, pleuritis; or (3) obstructing the upper airway, e.g. pharyngeal collapse, laryngeal paresis, or tracheal obstruction.

Low P_Ao_2 despite adequate levels of inspired O_2 (P_Io_2) and $\dot{V}_A$ indicates hypoxemia arising from the alveoli. It occurs because of venous admixture, low O_2 tension in blood returning to the lung ($P_{\bar{v}}o_2$) and in theory, diffusion impairment. This alveolar hypoxemia is commonly encountered in anesthetized horses and in pulmonary disease. Foal pneumonias and neonatal maladjustment syndromes contribute to the two main causes of alveolar hypoxemia: (1) venous admixture, arising from "shunt" and/or a predominance of diseased lung with low $\dot{V}/\dot{Q}$ ratios and (2) low $P_{\bar{v}}o_2$ in the presence of shunt. A third factor, diffusion limitation, is not thought to cause arterial hypoxia in human beings or Equids (Derksen 1991) although the evidence is elusive.

In hemoglobinemic hypoxia, the O_2 carrying capacity of blood is reduced because of inadequate concentrations of functional Hb. Stagnant hypoxia reflects the inadequate delivery of oxygenated blood to meet tissue demands and can occur because of low cardiac output ($\dot{Q}_T$), increased blood viscosity or severe vasoconstriction. Histotoxic hypoxia is seldom encountered in practice, but occurs when metabolic poisons like cyanide block the enzymes involved in oxidative phosphorylation. Finally, demand hypoxia arises when O_2 delivery falls short of total body requirements. It is encountered during exercise at workloads approximating 90% $\dot{V}o_2MAX$, when horses become both hypoxemic and hypercapnic. It has been proposed that the metabolic demand of horses surpasses their ventilatory capacity to meet the O_2 demand. Ponies are less athletic and so their ventilatory responses can meet metabolic requirements (Katz et al 1999).

Carbon Dioxide Elimination

Generally O_2 consumption is matched by CO_2 production, though this depends on the metabolic substrate (carbohydrate, fat or protein). The processes involved in CO_2 elimination are the reverse of those involved in O_2 uptake. The same principles of partial pressure gradients and gas exchange apply although CO_2 is more soluble than O_2 in water and undergoes more rapid diffusion.

Carbon Dioxide Transport in Blood

Metabolically generated CO_2 diffuses from active cells into interstitial fluid and thence across capillary walls into plasma. A proportion of CO_2 diffusing into plasma enters the erythrocyte. In plasma, CO_2 undergoes one of three reactions. First, some CO_2 forms carbonic acid upon hydration (see reaction 2) although the absence of carbonic anhydrase in plasma and the accumulation of H^+ and HCO_3^- ensures that this reaction proceeds slowly. The second reaction involves physical solution and is proportional to the solubility coefficient. This process is relatively unimportant and accounts for about 6% of total CO_2 transfer. Third, some CO_2 forms carbamino compounds by combining reversibly with the terminal amino groups of plasma proteins. The total CO_2 content carried in this way is negligible, and the most important carbamino formation occurs with Hb.

Carbon dioxide entering erythrocytes dissolves, and forms both HCO_3^- and carbamino compounds. The formation of

HCO_3^- (reaction 2) proceeds approximately 13,000 times faster than in plasma because of carbonic anhydrase, and because the H^+ and HCO_3^- produced do not accumulate. Instead, Hb buffers the H^+ and the HCO_3^- diffuses out of the red cell. Electrochemical neutrality is maintained by ingress of chloride ions (the chloride or Hamburger shift). The majority of HCO_3^- formed in the erythrocyte enters the plasma. The formation of carbamino compounds does not rely on enzymatic catalysis but is nevertheless rapid. At the normal pH of the erythrocyte, the reaction proceeds promptly to the right yielding H^+ that is readily buffered by Hb.

Reaction 3.

$$Hb\ NH_2 + CO_2 \longleftrightarrow Hb\ NH\ COOH \longleftrightarrow Hb\ NH\ COO^- + H^+$$

The formation of both HCO_3^- and carbamino compounds is facilitated by the combination of H^+ with Hb. Because reduced hemoglobin is less acidic than HbO_2, it readily accepts H^+ when HbO_2 dissociates at the tissue level. This increases the production of HCO_3^- and carbamino compounds from CO_2, which in turn facilitates CO_2 uptake from the tissues. As a consequence of its large buffering capacity for H^+, reduced Hb is at least three times more capable of binding CO_2 than is HbO_2.

Carbon Dioxide Release at the Lungs

In the lung, the alveolar CO_2 tension ($P_A\text{CO}_2$) of 5.33 kPa (40 mmHg) is less than that of mixed venous blood perfusing pulmonary capillaries. This, and its high solubility, ensure rapid CO_2 diffusion into the alveolar space. The reactions involved are the same as those described previously, but occur in the opposite direction. The binding of O_2 and Hb drives CO_2 from the erythrocyte. This occurs because the carbamino groups of HbO_2 can hold less CO_2 than can Hb. Also HbO_2 is a stronger acid than Hb and releases H^+ that combines with HCO_3^-, which diffuses into the erythrocyte to form H_2CO_3. Catalyzed by carbonic anhydrase, this dissociates into H_2O and CO_2. The latter diffuses across the erythrocyte membrane into plasma and finally into the alveolus, from which it is expired. This continues until the capillary CO_2 tension is equal to that in the alveolus. The rapid transit of CO_2 across the alveolar–capillary membrane makes CO_2 diffusion impairment extremely unlikely. Oxygenated blood then flows back to the left heart with a normal $P\text{CO}_2$ of 5.33 kPa (40 mmHg).

Impaired Carbon Dioxide Elimination

Impaired CO_2 elimination (hypercapnia) can result from reduced $\dot{V}_A$, increased metabolic production or re-breathing expired air, and is detailed in Chapter 14.

REFERENCES

Amis TC, Pascoe JR, Hornof W 1984 Topographic distribution of pulmonary ventilation and perfusion in the horse. American Journal of Veterinary Research 45: 1597–1601

Bisgard GE, Orr JA, Will JA 1975 Hypoxic pulmonary hypertension in the pony. American Journal of Veterinary Research 36: 49–52

Clerbaux T, Serteyn D, Willems E, et al 1986 Determination of the standard oxyhemoglobin dissociation curve in horses. Effects of temperature, pH and diphosphoglycerate. Canadian Journal of Veterinary Research 50: 188–192

Derksen FJ 1991 Applied respiratory physiology. In: Beech J. (editor) Equine Respiratory Disorders. Lea & Febiger, Philadelphia, p.10

Dobson A, Gleed RD, Meyer RE et al 1985 Changes in blood flow distribution in equine lungs induced by anesthesia. Quarterly Journal of Experimental Physiology 70: 283–297

Fenger CK, McKeever KH, Hinchcliff KW et al 2000 Determinants of oxygen delivery and haemoglobin saturation during incremental exercise in horses. American Journal of Veterinary Research 10: 1325–1332

Gallivan GI, McDonell WN, Forrest JB 1989 Comparative ventilations and gas exchange in the horse and cow. Research in Veterinary Science 46: 331–336

Gehr P, Erni H 1980 Morphometric estimation of pulmonary diffusion capacity in two horse lungs. Respiratory Physiology 41: 199–210

Gehr P, Mwangi DK, Ammann A et al 1981 Design of the mammalian respiratory system. V. Scaling morphometric diffusing capacity to body mass: wild and domestic animals. Respiratory Physiology 144: 61–86

Greene HM, Wickler SJ, Anderson TP et al 1999 High-altitude effects on respiratory gases, acid–base balance and pulmonary artery pressures in equids. Equine Veterinary Journal Supplement 30: 71–76

Hedenstierna G, Nyman G, Kvart C et al 1987 Ventilation/perfusion relationships in the standing horse: an inert gas elimination study. Equine Veterinary Journal 19: 514–519

Hlastala MP, Bernard SL, Erickson HH et al 1996 Pulmonary blood flow distribution in standing horses is not dominated by gravity. Journal of Applied Physiology 81: 1051–1061

Jarvis KA, Steffey EP, Tyler WS et al 1992 Pulmonary blood flow distribution in anesthetized ponies. Journal of Applied Physiology 72: 1173–1178

Katz LM, Bayly WM, Hines MT et al 1999 Differences in the ventilatory responses of horses and ponies to exercise of varying intensities. Equine Veterinary Journal Supplement 30: 49–51

Marshall BE, Marshall C 1985 Anesthesia and pulmonary circulation. In: Covino BC, Fozzard HA, Rehder K et al (editors) Effects of Anesthesia. American Physiological Society, Bethesda, pp.121–136

Nyman G, Lindberg R, Weckner D et al 1991 Pulmonary gas exchange correlated to clinical signs and lung pathology in horses with chronic bronchiolitis. Equine Veterinary Journal 23: 253–260

Pelletier N 1987 Effect of submaximal exercise and training on dead space ventilation in the horse. In: Gillespie JR, Robinson NE (editors) Equine Exercise Physiology 2. ICEEP Publications, Davis, pp.225–234.

Robinson NE 1982 Some functional consequences of species differences in lung anatomy. Advances in Veterinary Science and Comparative Medicine 26: 1–33

Robinson NE, Sorenson PR 1978 Collateral flow resistance and time constants in dog and horse lungs. Journal of Applied Physiology 44: 63–68

Sinha AK, Gleed RD, Hakim TS 1996 Pulmonary capillary pressure during exercise in horses. Journal of Applied Physiology 80: 1792–1798

Sorensen PR, Robinson NE 1980 Postural effects on lung volumes and synchronous ventilation in anesthetized horses. Journal of Applied Physiology 48: 97–103

Taylor CR, Maloiy GM, Weibel ER 1981 Design of the mammalian respiratory system. III. Scaling aerobic capacity to body mass: wild and domestic animals. Respiratory Physiology 44: 25–37

Wagner PD, Gillespie JR, Landgren GL 1989 Mechanism of exercise-induced hypoxemia in horses. Journal of Applied Physiology 66: 1227–1233

West JB 1977 Ventilation perfusion relationships. American Review of Respiratory Disease 116: 919–943

5

Airway Secretions and Mucociliary Function

Vincent Gerber and N Edward Robinson

This chapter focuses on the airway mucociliary apparatus that includes mucus secretions, secretory and ciliated cells; other components of the innate airway defense system, such as cough, bronchospasm, and macrophages are discussed in Chapter 6. In this chapter, the terms "mucus (accumulation)" and "(airway) secretions" are used interchangeably to describe the entity of gel-like secretions with all their components. Respiratory mucus has important physiological roles: trapping, inhibition, destruction, and removal by mucociliary clearance of inhaled materials as well as humidification of air and prevention of fluid loss. Increased mucus can be a normal defense reaction enhancing some of these physiological functions. There can also be "too much of a good thing", however. When mucus production and secretion exceed clearance capacity, excessive accumulation is observed in the airways.

The clinician evaluating the lower respiratory tract of horses is frequently confronted with airway mucus accumulation and is then faced with the following questions.

- What degree of mucus accumulation is clinically relevant?
- What are the causes and mechanisms of excess mucus accumulation?
- How can excess mucus accumulation be treated and prevented?

Mucus accumulation is a major pathophysiological component of equine lower airway disease. Compared with airway inflammation and bronchospasm/airway hyper-reactivity, however, it has received much less investigative attention and is therefore less well understood. In the following, we examine the available information on the mucociliary apparatus in the horse with a focus on recurrent airway obstruction (RAO) and inflammatory airway disease (IAD). Comparative aspects are included when relevant to the above questions.

Components of the Mucociliary Apparatus

The mucus blanket overlying the airway epithelium (Figs 5.1 and 5.2) is composed of a liquid sol layer that bathes the cilia, overlain by a more viscous mucus gel layer. Mucus is a mix of water (~95%), electrolytes (1%), lipids (1%), and proteins (2–3%), in particular, high molecular weight O-linked glycoproteins referred to as mucins, as well as variable amounts of epithelial and inflammatory cells (Matthews et al 1963).

Mucus-producing cells

Goblet cells and submucosal glands synthesize, store and release mucins. In the horse, unlike all other investigated species, submucosal glands are very sparse even in the large airways (Widdicombe & Pecson 2002). Goblet cells, in contrast, are found in normal equine airways down to 0.5 mm diameter but not including the terminal airways (Viel 1980).

Alcian blue–periodic acid Schiff (AB-PAS) is the standard histological stain for mucus-storing cells but Giemsa–PAS will also stain mucus-storing cells and provides better overall morphological detail (Fig. 5.2B). For ultrastructural studies, transmission electron microscopic fixation and osmium-staining methods have been used to identify mucus-producing cells based on their morphologically characteristic storage granules and the osmiophilic surfactant film at the air–mucus interface (Fig. 5.1).

Semi-quantitative scoring systems allow grading of mucus cell hyperplasia and metaplasia. Quantitative morphometric methods (Harkema et al 1987) can be used to (1) count the number of goblet cells, (2) measure the volume of stored mucus product, and (3) measure submucosal gland size (the Reid index). Except for investigation of very small airways where a biopsy will suffice, post-mortem or large biopsy samples requiring thoracoscopy (see Chapter 20) are necessary.

Mucins

Mucins, also called mucus glycoproteins, are high molecular weight glycoconjugates (Fig. 5.3). Numerous oligosaccharide side chains are O-glycosidically linked to threonine and serine tandem repeat sequences of the peptide core or apomucin, which represents the primary gene product. Gel-forming mucins contain non-repetitive cysteine-rich regions, believed to allow intra- and inter-molecular cross-linking through disulfide bond formation. Three gel-forming mucin apoproteins are known in

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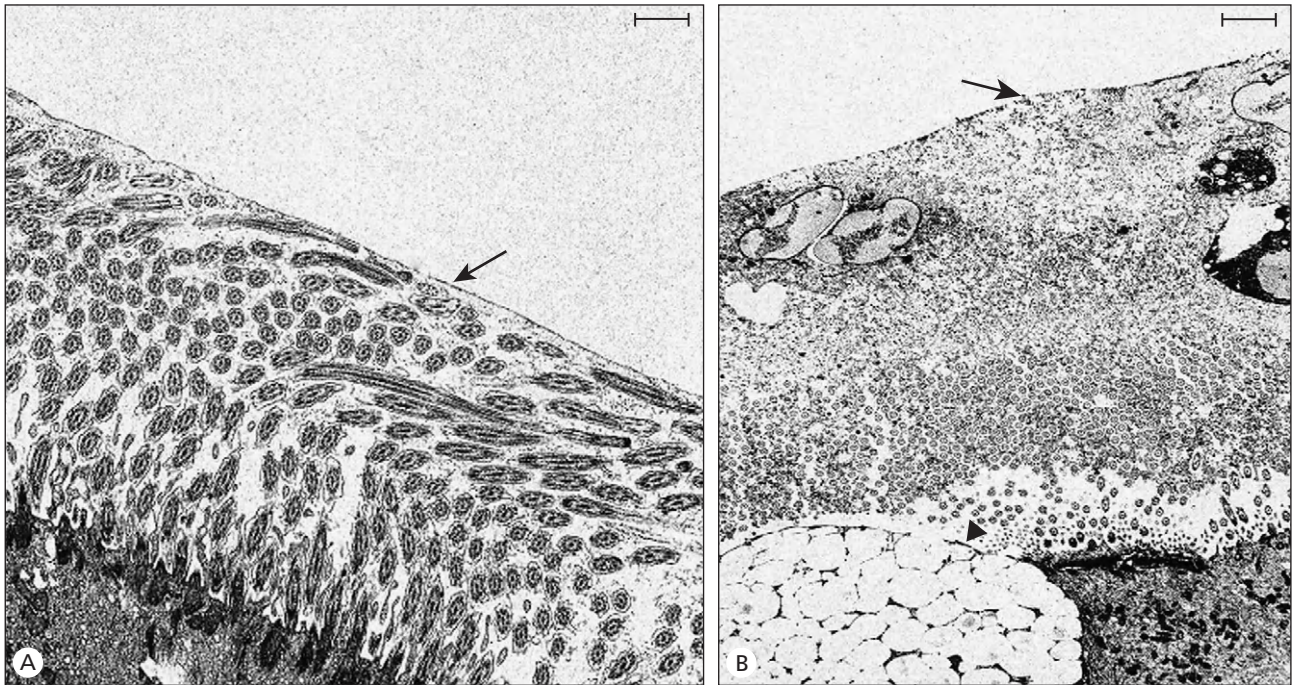
Fig. 5.1. (A,B) Transmission electron microscopic photographs of respiratory epithelium and mucus. The healthy respiratory epithelium (A) shows the sol layer that bathes the cilia and a practically absent mucus gel layer. The inflamed respiratory epithelium (B) has a prominent gel layer with inflammatory cell debris. The left lower corner of B shows the apex of a goblet cell (arrowhead) with characteristic mucus storage granules. On top of the cilia an osmiophilic surfactant film (arrows) is present at the air–liquid interface. (A) Bar = 1 μm; (B) bar = 2 μm. Photomicrographs by Gerber, Kupferschmied & Gehr, Equine Clinic and Department of Anatomy, University of Berne, Switzerland.
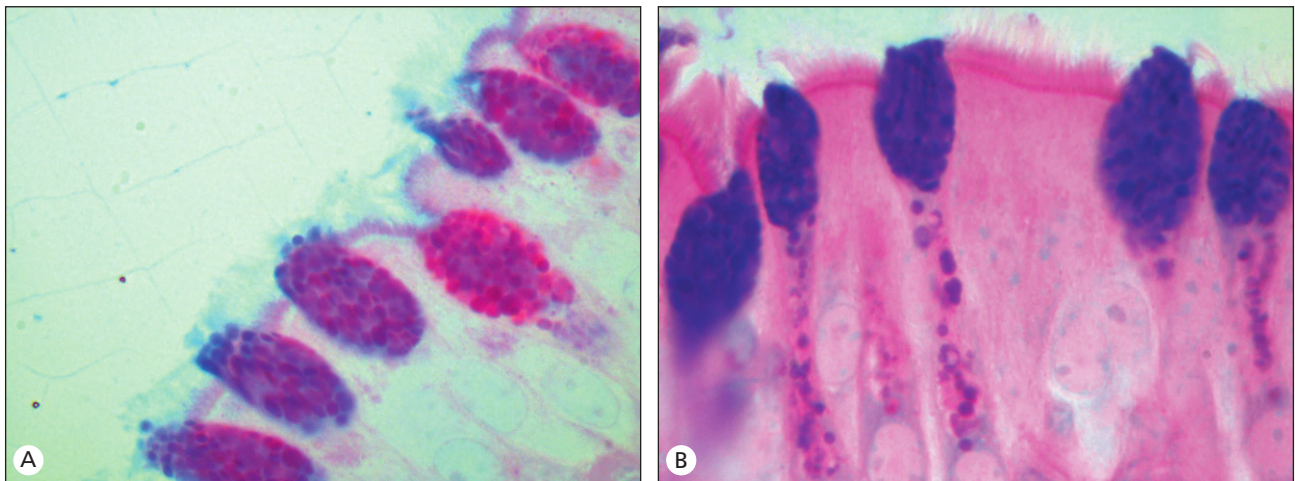
Fig. 5.2. (A,B) Staining for stored mucosubstance (arrows) in goblet cells with AB-PAS (A) and Giemsa-PAS (B) on ultrathin sections from methacrylate-embedded equine airway sections. Photomicrographs by deFeijter-Rupp & Gerber, Pulmonary Laboratory, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.

human and rodent airways: MUC2, MUC5AC and MUC5B (Jeffery & Li 1997).

Two equine homologs of mucin genes *MUC5AC* and *MUC2* have been genetically identified and, based on reactivity with polyclonal antibodies raised against the human mucins, MUC5B has also been described (Walley et al 2001). In healthy horses, eqMUC5AC mRNA can be detected in all airway generations and in the stomach (Fig. 5.4). In contrast, eqMUC2 is expressed in the colon but not in the airways (Gerber et al 2003b).

Central tandem-repetitive domain
• Core protein – apomucin
• Abundant O-glycosylation sites (Ser and Thr)
• Sequence varies widely from mucin to mucin

Flanking regions
• Naked protein – few oligosaccharides
• Unique and non-repetitive sequence (Cys-rich)
• Mucin oligomer formation by disulfide linkages

Flanking regions
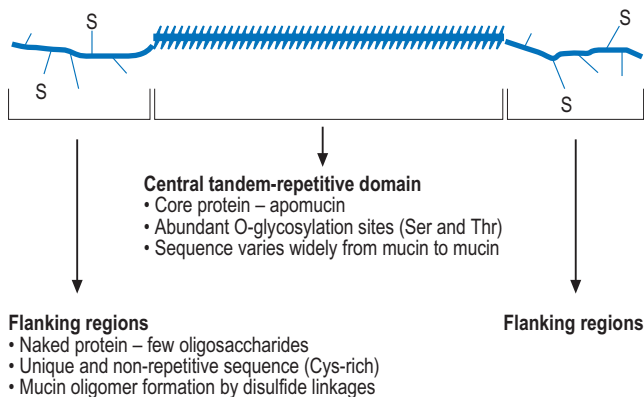
Fig. 5.3. Basic molecular structure of gel-forming mucins. Redrawn from J. Hotchkiss, Laboratory for Experimental and Toxicologic Pathology, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA, with permission.

The oligosaccharide side chains that make up 90% of the molecular weight of equine mucins are composed of fucose, *N*-acetyl-galactosamine, sialic acid (*N*-acetyl-neuraminic acid) and galactose (Jefcoat et al 2001). The composition of these carbohydrate side chains imparts specific binding activity to certain bacterial adhesion molecules and can influence the viscoelasticity of the mucus layer.

The physical properties of mucus – rheological measurements

The mucus physical properties of viscoelasticity, spinnability and adhesivity determine its clearability (see below). Viscosity and viscoelasticity are the only mucus physical properties that have been investigated in the horse. Mucus viscoelasticity is a result of its three-dimensional, cross-linked mucin network structure. Disulfide bonds join mucin subunits into extended macromolecular chains,

which because of their size readily form entanglements among each other. Oligosaccharide side chains form hydrogen bonds with complementary sugar units on neighboring mucins and ionic interactions between negatively charged sugar units and positively charged amino acids extend the macromolecular conformation. Furthermore, inflammation can add an extra network of high molecular weight DNA and actin filaments released from dying leukocytes. Interactions with water, electrolytes, hydrogen ions, lipids, enzymes, and proteins influence these mucin gel-bonds, resulting in the physical properties of mucus.

The viscosity of normal horse mucus measured by microrheometry is in the range observed in healthy humans and dogs (Gerber et al 2000). Rheological measurements are difficult to perform, so clinical researchers have tried to grade endoscopically the viscosity, or rather the apparent stiffness and stickiness of tracheal mucus accumulations. However, apparent viscosity scores do not correlate with measured mucus viscoelasticity. Only the localization of mucus in the trachea can give an indication of its viscoelasticity (Gerber et al 2004b), because dorsally located mucus is more viscous.

Clearance of mucus by ciliary cells

The rate of mucociliary clearance is determined by ciliary amplitude and beat frequency and by the physical properties of mucus, with the latter being most important (Gerber et al 1997). The bronchiolar cilia are less densely packed than those in the bronchi so mucociliary transport is slower in the bronchioles than in the bronchi and trachea. Because gravity affects clearance (Gerber et al 1996), mucociliary transport is faster when the horse's head is down than when it is up. Defects occur in 5% of equine airway epithelial cilia (Galati et al 1991), a frequency comparable to other species but well below the 50% ciliary loss necessary to reduce clearance.
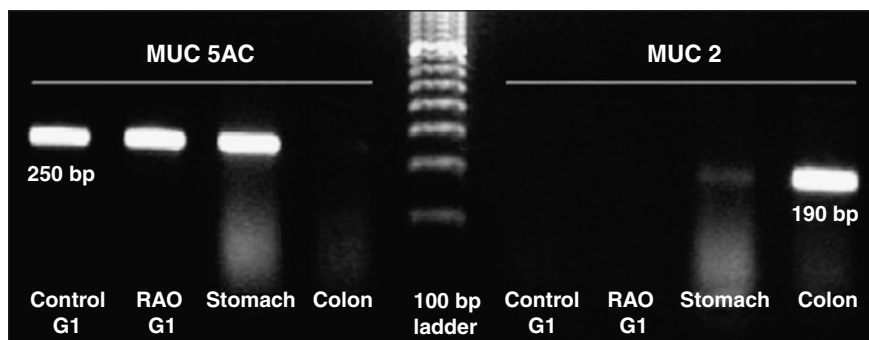
Fig. 5.4. Tissue specificity of MUC5AC and MUC2 mRNA detected by RT-PCR. Samples from airway generation 1 (G1) from control and RAO-affected horses, as well as from stomach and colon tissue. Ethidium bromide-stained PCR products on 3% agarose gel. Reproduced from Gerber et al, 2003, with permission.

Table 5.1. Summary of methods for assessing mucus with their advantages and limitations	
Techniques and methods	**Practicality; advantages and limitations**
Endoscopic scoring of mucus accumulation (see Fig. 5.5; Gerber et al 2004b)	Reliable, clinically applicable technique when using standardized grading scale and repeated observations. Do not use compounded scores that include cytological criteria.
Scoring of mucus viscosity, color and location (Dieckmann 1987, Gerber et al 2004b)	Extremes of localization (dorsal versus ventral) give indication of viscoelasticity and apparent viscosity is associated with severity of disease, but inter- and even intra-observer repeatability is unreliable.
Rheology; microrheometry, bulk viscosimetry and other specialized techniques (see Figs 5.8 and 5.9; Gerber et al 2000)	Research methods; technically demanding, available only in specialized laboratories.
RT-PCR; ELISA/ELLA for measurement of mucin production (see Fig. 5.4; Jefcoat et al 2001, Gerber et al 2003b)	Research methods; can be established with moderate effort. Problems with specificity of ELISAs and ELLAs, especially when carbohydrates are characterized.
Mucus cell histology and morphometry (see Figs 5.1 and 5.2; some in Viel 1980)	Laborious techniques, reliable results. Invasive; thoracoscopy necessary for large biopsy samples
Measurements of mucus clearance rate with endoscopy (Sweeney 1989, Turgut & Sasse 1989, Im Hof et al 1996) or radioactive markers (Willoughby et al 1991)	Mostly for research, but basically clinically applicable techniques; standardization not trivial, repeat measurements necessary
Ciliated cells: *ex vivo* ciliary beat frequency (Gerber et al 1996)	Research method; technically demanding

RT-PCR, reverse transcription polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; ELLA, enzyme-linked lectin assay.

Mucociliary clearance is under autonomic control: β-adrenergic agonists are potent stimulants of clearance, and cholinergic antagonists are potent depressants, while the effect of cholinergic agonist drugs is variable (Houtmeyers et al 1999). In the standing horse, mucociliary clearance can be determined by use of a radioactive marker that is followed up the airway by scintigraphy (Willoughby et al 1991) and by simpler endoscopic methods using surface markers (Sweeney 1989, Turgut & Sasse 1989, Im Hof et al 1996). In healthy horses, the mean tracheal mucus velocity is about 2 cm/min but there are considerable differences between individual horses (Sweeney 1989) as in humans where genetic determination of mucociliary clearance rate has been suggested by twin studies.

Quantifying mucus accumulation

Subjective scoring of mucus accumulation by endoscopy is often employed to quantify mucus accumulation. Various systems with scores from 0 to 3 (Dixon et al 1995a) and 0 to 5 (Dieckmann 1987, Holcombe et al 2001) have been used. In some studies, however, a mucus score can be compounded into a general airway inflammation score by inclusion of cell parameters (Chapman et al 2000), so that it is impossible to extract mucus-specific information. A standardized illustrated grading scale based on Dieckmann (1987) was recently proven to be a reliable clinical and research tool (Fig. 5.5A–C) (Gerber et al 2004b): it showed excellent intra- and inter-observer agreement, moderate horse-related variance and a good correlation with measured volumes of "artificial mucus".

Of all the techniques that have been used in the horse and that are outlined here (Table 5.1), only endoscopic scoring of gross mucus accumulation can presently be regarded as a readily available and reliable clinical tool. It is also the method that has provided the most data on the clinical significance of mucus accumulation.

Epidemiology and Clinical Significance of Mucus Accumulation

Mucus accumulation is a non-specific sign of respiratory disease

Mucus accumulation is generally regarded as a hallmark of both RAO (Robinson et al 1996, Anon 2001) and IAD (Burrell 1985, MacNamara et al 1990, Anon 2002) and is often attributed a causative role in airway obstruction. However, mucus increases not only in RAO, but also in "undifferentiated pulmonary disease", "infectious pulmonary disease", "*Streptococcus zooepidemicus* pulmonary infection", "lungworm infestation", "exercise-induced pulmonary hemorrhage" and "miscellaneous pulmonary
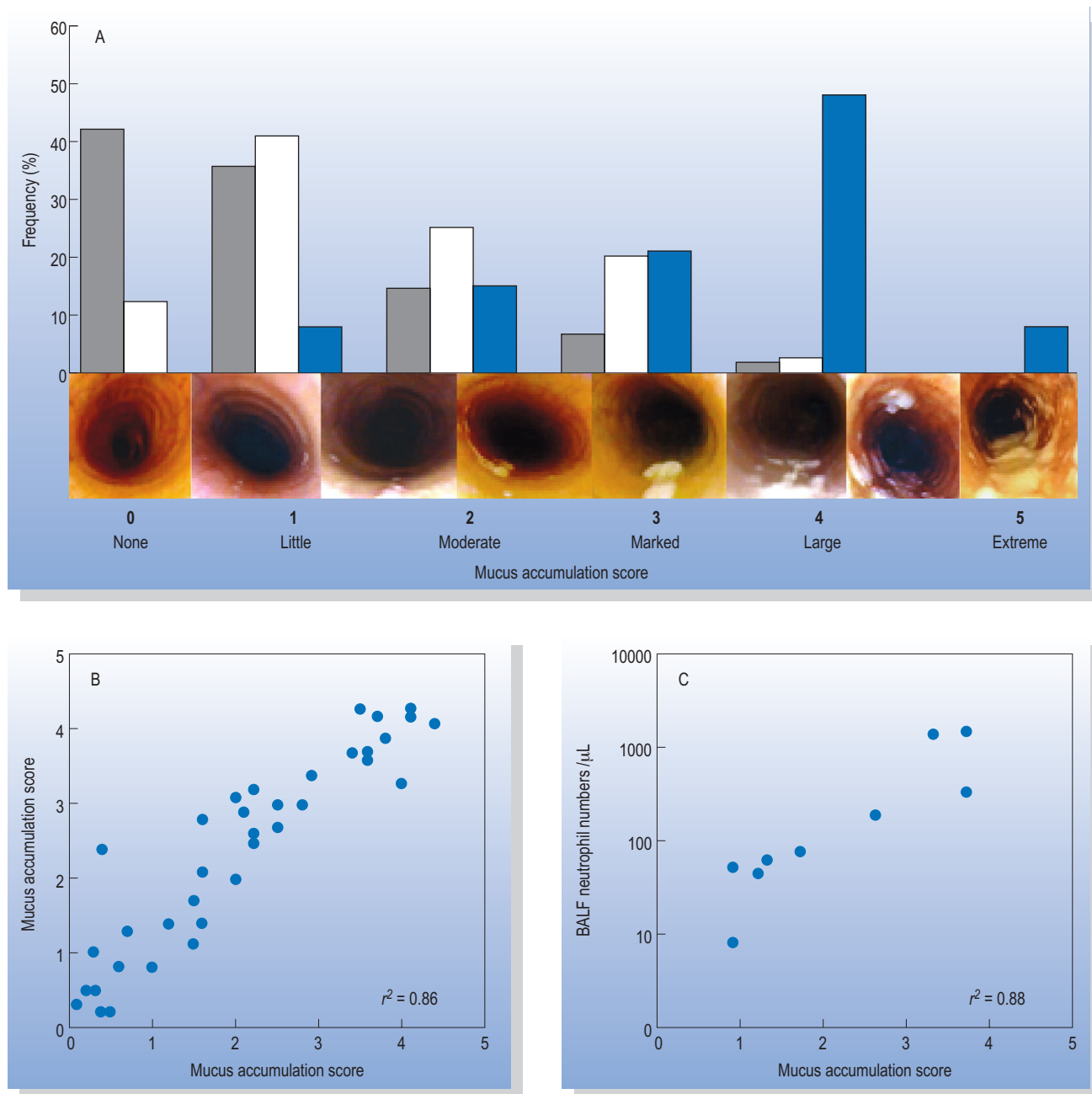
Fig. 5.5. Illustrated scoring scale for mucus accumulation (A) showing the frequency distribution between unselected horses from 16 stables in six geographic regions in Michigan, USA (gray bars, $n = 260$ observations), clinically healthy research control horses (white bars, $n = 44$) and RAO-affected animals (blue bars, $n = 48$). Intra-observer correlation (B) of scoring endoscopic videos once and then again 3 weeks later; (C) illustrates correlation of mucus accumulation scores with neutrophil numbers in bronchoalveolar lavage fluid (BALF). Redrawn in part from Gerber et al, 2003, 2004, from data in Robinson et al, 2004, with permission.

disease" (Dixon et al 1995b). Horses with RAO show the highest score (median score of 3), but this is not significantly different from all the other groups. Throughout all studies, the large variation between individuals of all disease categories is obvious.

Mucus accumulation in RAO is a function of disease status and/or environment

When RAO-affected horses have been at pasture for several weeks, bronchospasm and inflammation wane, but mucus accumulation scores are still increased in comparison to clinically healthy horses (Gerber et al 2004a) (Fig. 5.6). During exacerbations of RAO, endoscopic mucus accumulations further increase in RAO-affected horses. This is in accordance with the results of carbohydrate-specific enzyme-linked lectin assays (ELLAs): RAO-affected horses in remission show increased levels of α-1,2-fucose, which may be associated with MUC5AC (Jefcoat AM, personal communication) and which further increases during acute exacerbations (Jefcoat et al 2001).

In clinically healthy horses, in contrast, short-term exposure to the environment in the stable does not increase mucus accumulation despite a moderate increase in airway neutrophils. As a result of the overall large variation in mucus scores in clinically healthy horses, however, intermediate grades (Grade 2–3) completely overlap between RAO-affected and control animals. Only high mucus grades (Grade >3) separate RAO-affected horses from clinically healthy animals (Gerber et al 2003a, 2004,a,b) (Fig. 5.5). The combination of our studies on endoscopic mucus grades and reports on mucociliary clearance rates from ours and other groups allow for an estimate to be made of the mucus transport and thus its production. In clinically healthy horses, 2–8 ml/h or 120 ml/day of endoscopically visible mucus is transported along the trachea. This

amount is consistent with the estimate of about 10–15 ml/day in adult healthy humans. In RAO-affected animals, the volumes can be extrapolated to 6–14 ml/h at pasture and 15–30 ml/h or 540 ml/day when stabled, which is proportionally less than the estimated 200–300 ml/day in humans with exacerbations of chronic bronchitis. It is important to note, however, that these estimates only account for mucus volumes cleared by mucociliary action, but not by coughing.

Clinical significance of increased mucus accumulations

Increased mucus accumulation can directly cause bronchial obstruction as well as effectively increase resting airway wall thickness. This latter effect amplifies to the fourth power the lumen-narrowing effect of broncho-constriction (Moreno et al 1986). That is, the same amount of mucus will for simple geometrical reasons obstruct the lumen increasingly as the inner diameter of the airway is decreased by contraction of the surrounding airway smooth muscle. The logic of this argument is simple and convincing, and the obstructive effect of excessive secretions seems intuitively clear. However, in human airway diseases, the magnitude of any direct causative effect of excessive airway mucus on morbidity and mortality by causing the patient discomfort and airflow obstruction is controversial and, at best, difficult to assess (Kim 1997). The same is true for horse airway diseases. Increased mucus accumulation scores are associated with decreased lung function (Luft 1987) and following administration of bronchodilators to RAO-affected horses in clinical exacerbation, airway resistance rapidly decreases but still remains greater than in control horses (Broadstone et al 1988, Derksen et al 1992). It has been suggested but not proven that these remaining lung function deficits and the inconsistent effect of bronchodilators on dynamic compliance are the result of mucus accumulation and airway wall thickening (Robinson et al 1996).

When the amount of mucus exceeds the mucociliary clearance capacity, secretions progressively accumulate, and must then be cleared by coughing (King & Rubin 1994). The association of excess mucus accumulation and cough in horses has been shown in several studies (Dixon et al 1995c, Christley et al 2001, Robinson et al 2003).

Clinically healthy racehorses (Holcombe et al 2004) and pleasure horses (Robinson et al 2004) in the USA frequently have several small non-coalescent drops of mucus (Grade 1) in their trachea. This amount is not associated with altered racing performance but larger amounts (Grade 2 or greater: coalescent drops or a stream of mucus) are associated with poorer race placement in standardbreds (MacNamara et al 1990) and thoroughbreds (Holcombe et al 2004). Many asymptomatic pleasure horses (Robinson et al 2004) and well-performing sport
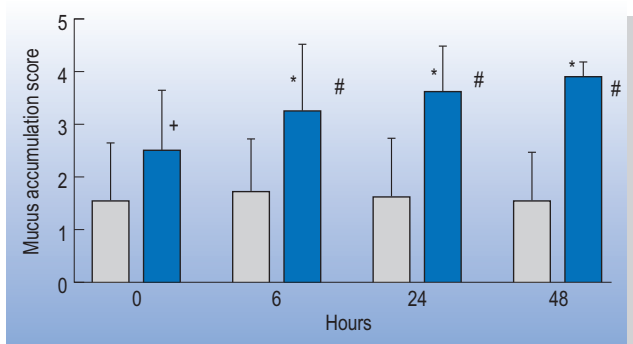
Fig. 5.6. Mucus grades in RAO-affected (blue bars) and control (grey bars) horses before and at 6, 24, and 48 h after stabling. *Significantly different (P < 0.05) from 0 h; # significantly different from control at same time point; [+] trend toward a difference compared to control at that time point (P = 0.054).

horses (Gerber et al 2003a) of various ages have mucus scores >2 (Fig. 5.5), so it appears that mild to moderate degrees of mucus accumulation (and also airway inflammation) are tolerated as long as horses are not asked to perform intense exercise like racing.

Finally, increased mucus accumulations are also associated with bacterial colonization of the airways. When horses are prevented from lowering their head for a prolonged period of time, mucus pooling with excessive bacterial growth occurs in the distal trachea (Raidal et al 1995). This may increase the risk of bacterial pleuropneumonia in immunocompromised animals. Furthermore, inflammation scores, which include mucus accumulation, are highly correlated with positive bacterial cultures from tracheobronchial secretions in IAD of young racehorses (Chapman et al 2000). Molecular interactions between bacteria and mucin glycoproteins may play a role here. However, even in healthy horses *Streptococcus zooepidemicus* and other upper airway commensals are frequently isolated from tracheal secretions (Sweeney et al 1985), and exacerbations associated with bacterial infections are not a clinical problem recognized in RAO-affected horses (Anon 2001).

In summary, increased mucus accumulation is a characteristic, but not a specific feature, of IAD and RAO, and it is associated with:

- lung function deficits
- poor performance in racehorses (mucus accumulation score >1)
- exacerbations in RAO-affected horses (mucus accumulation score >3)
- coughing (mucus accumulation score >1) and bacterial colonization of large airways.

The foregoing discussion clearly shows that these are mere associations, however, and no causative relationships between mucus accumulation and any of these negative effects have been firmly established. The clinician must therefore judge the clinical relevance of an observed excessive mucus accumulation in the light of the overall assessment and situation of the patient. Before discussing therapeutic options, however, it is necessary to shed light on the causes and mechanisms that may lead to mucus accumulation.

Causes and Mechanisms of Airway Mucus Accumulation

In horses with IAD or RAO, mucus accumulation scores correlate well with tracheobronchial secretion neutrophil percentages and bronchoalveolar lavage fluid neutrophil numbers in horses (Gerber et al 2004b) (Fig. 5.5C). Inflammatory cells in large numbers and the inflammation-associated transudation of fluid into the airway contribute to the volume of airway secretions. In addition, neutrophilic inflammation unfavorably alters mucus physical properties and decreases the clearability of the secretions. However, the major link between mucus accumulation and inflammation is through increased production and secretion of mucins.

Mucin production and secretion

Many particulate and gaseous irritants can directly increase airway mucus. Inhaled endotoxin may be of particular importance in horses that are stabled and fed hay (Pirie et al 2001). Furthermore, mucus stasis may encourage bacterial growth in the airways, which in turn may also directly stimulate the production and secretion of mucins. However, most effects of inhaled irritants and allergens are likely mediated by inflammation. Figure 5.7 illustrates some immunological and pathophysiological mechanisms that may be involved in mucin hypersecretion of equine lower airway disease (see also next section).

"Too much of a good thing" aptly describes the situation where the airway mucosa produces and releases so much mucin glycoprotein as to overwhelm the capacity of the mucociliary transport system. Such a hypersecretory state is found in human chronic bronchitis, asthma exacerbation, and cystic fibrosis. Relative amounts, gene expression, and glycosylation variants of the gel-forming mucins, MUC2, MUC5AC, and MUC5B, can be altered signifi-
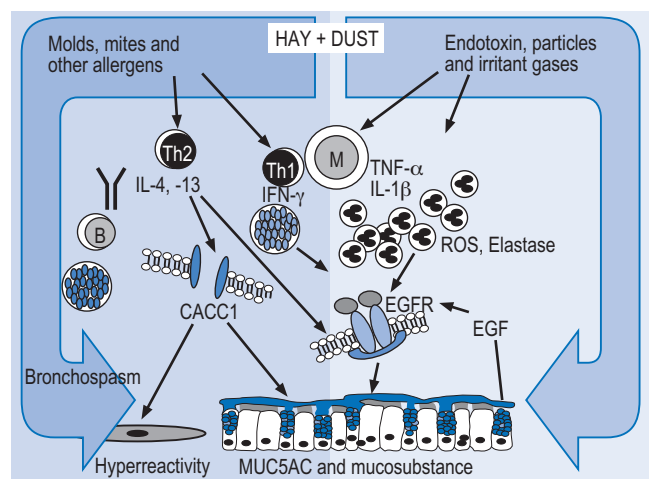
Fig. 5.7. Illustrated view of some immunological and pathophysiological mechanisms that may be involved in equine lower airway disease. Th = T-helper cell; M = macrophage; B = B cell; MC = mast cell; IL = interleukin; IFN = interferon; TNF = tumor necrosis factor; CACC = calcium-activated chloride channel (also, CLCA); ROS = reactive oxygen species; EGF(R) = epidermal growth factor (receptor); MUC = mucin. Conceptual model and figure by V. Gerber; some graphic elements from J.A. Hotchkiss, Laboratory for Experimental and Toxicologic Pathology, Department of Pathobiology and Diagnostic Investigation, Michigan State University, USA, with permission.

cantly, both in rodent models and in human airway diseases. Most studies, however, have shown predominant MUC5AC up-regulation in response to various stimuli and mediators. Presently, MUC5AC is regarded as the main "signature" mucin in experimental and natural models of airway disease.

In the horse, present evidence indicates:

- MUC2 probably does not play a role, because no detectable mRNA levels of this mucin were observed in the airways of healthy and or RAO-affected horses (Fig. 5.4) (Gerber et al 2001).
- MUC5B, which has not yet been studied in equine lower airway disease, is probably not a key mucin, because submucosal glands are sparse in equine airways and no enlargement has been found in RAO-affected animals (Viel 1980, Kaup et al 1990a,b).
- MUC5AC may be the key inducible mucin in equine RAO (Gerber et al 2003b).

These findings are supported by our findings of increased α-1,2-fucose levels – a mucin sugar associated with MUC5AC (Jefcoat AM, personal communication) – in the bronchoalveolar lavage fluid of RAO-affected horses both in exacerbation and during remission (Jefcoat et al 2001). MUC5AC is produced predominantly by goblet cells. Several histopathological and ultrastructural studies describe goblet cell hyperplasia, metaplasia, and hypertrophy in the pulmonary airways of RAO-affected horses (reviewed in Dixon 1992).

Only a few studies are based on quantitative morphometric techniques, however: Viel (1980) found increased goblet cell counts in RAO- and IAD-affected horses, but only in the small airways of <2-mm diameter. The increase correlated with the severity of clinical disease and was most marked in preterminal airways, which contain almost no goblet cells in normal horses. Recent investigations of heaves-affected and control animals exposed to the same environment have found no differences in the amount of stored mucosubstances in bronchi or bronchioles (Bartner et al 2006, Lugo et al 2006). In the bronchioles, mucous cell metaplasia was associated with the severity of inflammation regardless of whether the animal had heaves or not (Lugo et al 2006). In the bronchi, severe inflammation in the heaves-affected animals was associated with emptying of mucous cells; that is, a reduction in the amount of stored mucosubstances, presumably because the mucus had been secreted into the airway lumen.

Such measurements reflect a "moment in time" within the dynamic process of mucin production, storage, and secretion. Current understanding of inflammatory airway pathogenesis indicates that after injury, immediate release of stored mucosubstance is followed by up-regulation of mucin production within hours, and an increase in goblet cell numbers within days (Haschek 1998). These dynamics may explain why at times, total stored mucosubstance is not increased in RAO airways: the higher numbers of goblet cells can be in a "high-throughput state" with increased production and secretion but decreased storage of mucin products.

Immunological context of mucin production

In this section, based mainly on comparative data and not on direct evidence from studies in horses, we present a possible model (Fig. 5.7) of the inflammatory and immunological background of mucin gene induction and secretion in equine lower airway disease.

Many different T helper type 1 and 2 (Th1, Th2) pathways, as well as innate immunity pathways, converge on up-regulation of MUC5AC with subsequent mucus cell metaplasia and hypersecretion. Epidermal growth factor receptor (EGFR) and a chloride channel (calcium-activated, family member 1; CLCA1) as well as the B-cell leukemia-2 (Bcl-2) protein are key signaling molecules involved in these pathways but are only beginning to be investigated in equine lung disease.

Neutrophils, the predominant inflammatory cell type in RAO, produce potent secretagogues that are increased in equine RAO: reactive oxygen species (Art et al 1999), proteases, in particular elastase (Jefcoat AM, personal communication), and leukotriene B$_4$ (Lindberg et al 2002). Neutrophils can up-regulate EGFR expression through tumor necrosis factor-α and subsequently activate EGFR in a ligand-independent fashion through the release of oxidative radicals (Takeyama et al 2000, Shim et al 2001). CLCA1 has been identified as an important downstream effector element of interleukin-13 (IL-13), mediating airway hyperresponsiveness and overproduction of mucus in animal models and, possibly, in asthmatic subjects (Nakanishi et al 2001, Zhou et al 2001, Toda et al 2002). IL-13 as well as Th1-type and innate immunity cytokines can also up-regulate and activate EGFR.

Furthermore, irritants and toxins, in particular endotoxin that is abundant in the stable environment (Pirie 1998), can both directly and indirectly increase and potentiate airway mucin expression and mucous cell hyper- and metaplasia (Gordon et al 1996, Fanucchi et al 1998, Harkema & Wagner 2002).

Less well studied, but just as important as the induction of mucin hypersecretion, is what happens afterwards. There is evidence that the Th2 cytokine IL-13 and up-regulated Bcl-2 can delay apoptosis of goblet cells, leading to persistence of mucus cell metaplasia (Tesfaigzi et al 2000, Shi et al 2002). The persistence of mucus accumulation in RAO may thus be, at least in part, mediated by Bcl-2, but other mechanisms, such as increased nuclear factor-κB, which is involved in mucin gene up-regulation *in vitro* (Basbaum et al 1999), may also play a role. Finally, although neural control of mucus secretions is likely less important in the horse than in species with many

submucosal glands, mediators of "neurogenic inflammation" such as substance P may induce mucus hypersecretion in horses (Sonea et al 1999).

In the horse the first few pieces of this complex puzzle are starting to fall into place:

- Equine CLCA1 and EGFR mRNA levels as well as neutrophil percentages are associated with eqMUC5AC mRNA levels in the airways of horses suffering from RAO as well as of horses with milder degrees of airway inflammation (Gerber V, unpublished results).
- Horses with RAO show an increased percentage of Bcl-2-positive mucus cells compared to their normal counterparts (Bartner et al 2006).
- When RAO horses are removed from conventional stable environments, residual lung function deficits, airway neutrophils and mucus persist and are associated with increased nuclear factor-κB activity of epithelial cells (Bureau et al 2000).

Mucus biophysical properties and their alterations in disease

The available data suggest that alterations in mucus rheology in horses are a function of the intensity and duration of exposure to irritants and allergens as well as the severity of airway disease in the individual (Gerber et al 2000; Figs 5.8 and 5.9).

- When RAO-affected horses are in remission, viscoelasticity and clearability of mucus are similar to that in clinically healthy horses.
- In clinical crisis as a result of stabling and hay-feeding, the viscoelasticity of mucus in RAO-affected horses increases three-fold into the range observed in human patients suffering from cystic fibrosis.

These results are in accordance with an older report indicating increased viscosity of tracheal mucus in RAO-affected horses in crisis, but normal viscosity in horses with RAO after hay was eliminated from the diet (Hajer 1979, PhD thesis, cited in Turgut & Sasse 1989). A recent study found significantly higher values of mucus viscosity in horses with RAO than in horses affected with IAD (Pietra et al 2000). We have also observed that horses with mild to moderate clinical signs of RAO even showed remarkably lower average viscoelasticity than clinically healthy horses (Fig. 5.9) (Gerber et al 1998).

Although rheological values overall do not significantly correlate with bronchoalveolar lavage fluid cytology, the increase of mucus viscoelasticity in RAO coincides with the dramatic influx of neutrophils in the airways of RAO-affected horses. Neutrophils released into the airways degenerate and release breakdown products. Specifically, high molecular weight DNA (Pietra et al 2000) and filamentous (F)-actin (Gerber 2003) have been demon-
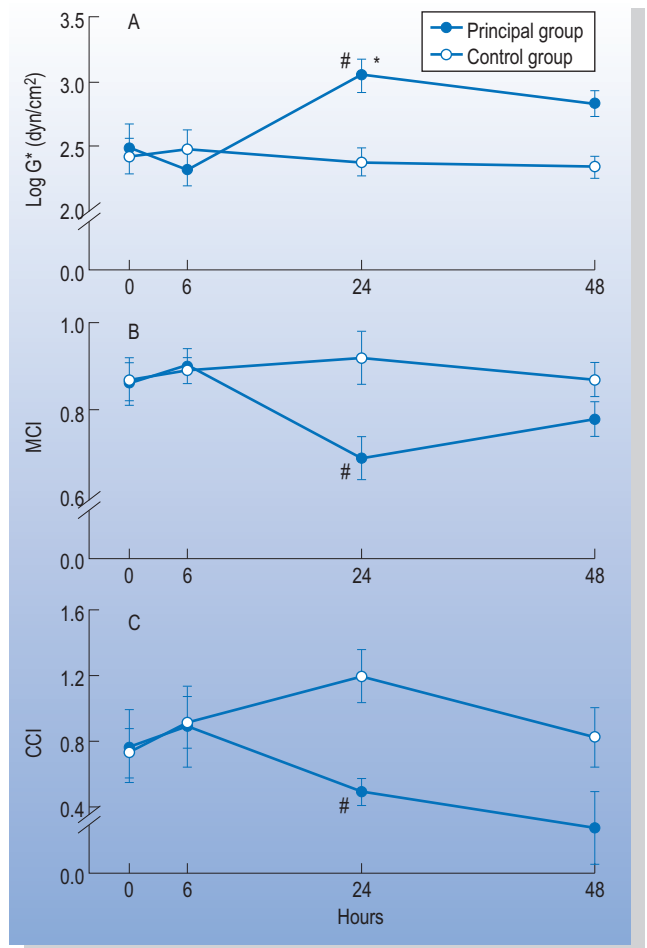
Fig. 5.8. Viscoelasticity and derived clearability of mucus in RAO-affected horses (blue dots) compared with control horses (white dots): (A) viscoelasticity at 10 radian/s on a logarithmic scale (log G*; dyn/cm²), (B) mucociliary clearability index (MCI), and (C) cough clearability index (CCI) before (0; pasture) and after environmental challenge (6, 24 and 48 h; stabling, feeding hay). # Significant difference between groups; * significant difference between time point and baseline in the same group. Redrawn from Gerber et al, 2000, with permission.

strated in equine mucus with unfavorably altered mucus rheological properties.

Changes in the mucin carbohydrate side chains – fucose (α-1,2 linkage), *N*-acetyl-glucosamine, and *N*-acetyl-galactosamine – that we detected in RAO-affected horses in acute disease (Jefcoat et al 2001), may also contribute to the degree of viscoelasticity of the mucus layer.

Rheological factors other than viscoelasticity may negatively influence clearability in RAO-affected horses as well. Adhesivity and wettability, which contribute to the optimal interface properties of mucus, are primarily influenced by its phospholipid content and composition. Exogenous surfactant increases equine tracheal mucus transport velocity *ex vivo* (Gerber 1995) and *in vivo* (Im Hof et al 1996). Surfactant can be observed at the
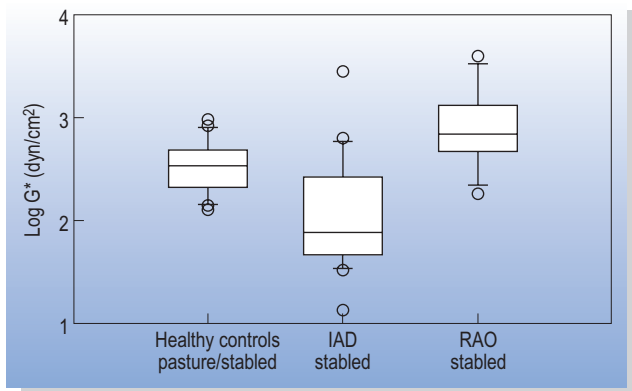
Fig. 5.9. Mucus rheological differences between disease groups: Viscoelasticity at 10 radian/s on a logarithmic scale (log G*; dyn/cm^2) in healthy control horses both at pasture and when stabled, compared to stabled IAD- and RAO-affected horses. All measurements by microrheometry. Figure based on data from Gerber et al, 2000, with permission, and from Gerber, unpublished results.

air–mucus interface (Fig. 5.1) and is present in amounts sufficient to significantly reduce surface tension in the trachea of healthy horses (Im Hof et al 1997), but clinical observation (reduced foam of bronchoalveolar lavage) and biochemical analysis of airway secretions suggest that a deficit and altered composition of surfactant are present in RAO-affected horses (Dorwald et al 1991), and after horses have been subjected to long periods of transport (Hobo et al 2001).

Mucociliary clearance

A variety of airborne pollutants may have deleterious effects on mucociliary clearance in horses, and, similar to smoking cessation in humans (Houtmeyers et al 1999), it may take months to return to normal (Schlesinger 1985). A single allergen challenge reduces mucociliary clearance rate by 28% in asthmatics (Houtmeyers et al 1999, Del Donno et al 2000). Airway inflammation appears to play a significant part in compromising mucociliary clearance, and may similarly contribute to the accumulation of airway secretions in horses with allergic lower airway disease (Dixon 1992).

Investigations of the effectiveness of mucociliary clearance in RAO-affected horses have yielded conflicting results, however. Clearance rate was found in one study to be decreased by between 24 and 90% and markers were less likely to move as a discrete bolus (Coombs & Webbon 1987). Other studies, in contrast, found no difference between RAO-affected and healthy horses (Willoughby et al 1991). No studies have investigated mucociliary clearance rate in IAD.

Excessive mucus loads may simply overwhelm mucociliary clearance. Mucus that is deep, i.e. more than the physiological 5–10 μm depth, is well suited for cough clearance, but unfavorable for mucociliary transport (King & Rubin 1994). Alternatively, clearance can be decreased by changes in the ciliary apparatus or unfavorable physical properties of the mucus gel.

Ultrastructural studies revealed a loss of ciliated cells and abnormal ciliary structure in the large conducting airways of RAO-affected horses (Kaup et al 1990b), with large differences in severity and extent between individuals. Considering the high reserve capacity of the ciliary apparatus (Houtmeyers et al 1999), the clinical significance of these changes is unclear and it seems more likely that a combination of excessive mucus production and rheological changes play the major roles in mucus clearance. In *ex vivo* experiments we have also shown that ciliary beat frequency is less important for clearance rate than mucus quality (Gerber et al 1997). Changes in viscoelasticity that are dependent on environment and on disease-status (Gerber et al 2000) may thus explain the contradictory findings reported in studies of mucociliary clearance in RAO-affected horses (Coombs & Webbon 1987, Turgut & Sasse 1989, Willoughby et al 1991).

The importance of ciliary damage is thus inconclusive in RAO, and fatal ciliary diseases – such as the human syndromes of immotile cilia and ciliary dyskinesia – have not been described in horses. Notwithstanding, ciliary defects are important sequelae of certain equine viral infections. Mucociliary clearance is severely depressed in all horses after influenza infection and takes 4 weeks to return to normal. Herpes virus reduces clearance in half of the infected horses. In contrast, infection with rhinovirus has no detectable effect on mucociliary clearance (Willoughby et al 1992).

Prevention and Therapy of Mucus Accumulation

Some of the therapeutic options presented here specifically target certain components of the mucociliary system, but others act much more broadly. Common to most of them, however, is the lack of good evidence for their effectiveness in decreasing mucus accumulation in equine lower airway diseases. This is mainly because of the absence of studies in the horse, but even in human medicine the value of mucoactive drugs is mostly controversial. Similar to the situation in the horse, the main consensus in human medicine is that mucus accumulation is difficult to treat.

The following section outlines the sparse data on mucoactive treatment in the horse. All of these therapeutic options presently appear to have their risks (e.g. side effects of long-term corticosteroid treatment, botulism with feeding bad haylage), questionable efficacy and benefit (e.g. classical mucolytics) or economic drawbacks (e.g. peptide mucolytics).

Before choosing one of these treatments, the clinician must therefore carefully judge the clinical relevance of the

observed mucus accumulation. In particular, the use of the horse, its stable environment, cough, respiratory distress and the severity of other clinical signs must be taken into account to decide if and what kind of therapy should be instituted.

Prevention of mucus accumulation

An environment as free of respirable irritants and allergens as possible is the mainstay of both prevention and therapy of RAO and likely also of most forms of IAD. Also, frequent and targeted influenza vaccination should prevent the decreased mucociliary function that is a sequel of clinical infection. The severe and prolonged depression of mucociliary clearance after influenza infection (Willoughby et al 1992) underlines the importance of adequate rest periods and good air hygiene after clinically apparent respiratory viral infections.

However, neither the benefit of environmental control nor of vaccinations against influenza for prevention of mucus accumulation has been specifically studied. In contrast, allowing horses to lower their heads during long transport has been shown to decrease tracheal mucus pooling, thereby decreasing bacterial colonization and the risk of ensuing pneumonia.

Environment

RAO-affected horses at pasture have lower mucus scores than when they are exposed to hay, but still more mucus in the airways than clinically healthy horses (Gerber et al 2004a). Thus, the therapeutic effect is useful but incomplete, as mucus accumulation persists for weeks (Jefcoat et al 2001), and, as in ex-smokers, it may return to normal levels only after months or possibly not at all. Thus, it appears that we can only expect a reliable effect of environmental control in RAO exacerbation with mucus scores >3. Even then the effect is mostly limited to a decrease towards mucus scores of 2–3. The effect is even more inconsistent with only moderately increased mucus scores (Holcombe et al 2001).

Corticosteroids

Dexamethasone decreases mucin gene expression and mucous cell hyperplasia *in vitro* and in some rodent models of asthma (Jungsuwadee et al 2004) but not in others (Kibe et al 2003). Based on our present results, which are comparable to those in human asthma patients (Fahy & Boushey 1998), we cannot expect a comparably good effect of corticosteroids on mucus accumulation as on other components of equine lower airway disease such as coughing and bronchospasm. Dexamethasone (0.1 mg/kg, intravenously once daily for 7 days) decreased mucus accumulation by an average of about 1.7 scores in a cross-over study with RAO-affected horses. The effect, however,

was slower and less consistent than the response of clinical signs and bronchospasm (Robinson et al 2003). Even more remarkably, in a double-blind placebo-controlled study (0.1 mg dexamethasone/kg body weight orally for 3 weeks) in clinical patients with IAD and RAO, we observed significant effects on coughing, arterial oxygenation, even on nasal discharge, abnormal lung sounds and performance but no decrease of mucus accumulation scores (Gerber et al 2003c).

Influencing mucociliary clearance

While atropine (0.02 mg/kg intravenous) significantly decreases tracheal clearance rate (Maxson et al 1995), a β_2-agonist (clenbuterol 0.8 µg/kg intravenous) significantly increases tracheal mucus velocity *in vivo* (Turgut & Sasse 1989). In contrast, water vapor-saturated air therapy, exercise and mild dehydration by furosemide have no effect on mucociliary clearance in healthy horses (Sweeney et al 1989). Gravitational force can decrease or accelerate tracheal mucus velocity *in vivo* (Raidal 1996) and *in vitro* (Gerber et al 1996), which has important implications for horses during longer periods of transportation, when they should be allowed to lower their heads and be fed on the ground.

Secretolytics

The chemically closely related molecules bromhexine, ambroxol and dembrexine, alone or in combination with bronchodilators, are extensively used in parts of Europe to treat horses with lower airway disease. None of their purported mechanisms of action, such as increasing the sol layer through alteration in epithelial ion transport, favorably altering mucin glycosylation to decrease viscosity, or increasing surfactant, have been investigated in the horse. Most of the studies reporting clinical benefits like improved endoscopic mucus scores, decreased coughing and improved lung function in IAD and RAO (Pearce et al 1978, Sasse & Deegen 1984, Matthews et al 1988) can be criticized on methodological grounds, e.g. unblinded observers and lack of placebo control groups. However, because we have little to offer as valid therapeutic alternatives, these molecules remain interesting.

Classical mucolytics

The agent *N*-acetyl cysteine (NAC), which breaks down mucin disulfide bonds and has antioxidant effects, is also widely used in Europe to decrease mucus accumulation in horses. Relatively high doses (5–10 mg/kg body weight orally, twice a day for 20 days) decreased mucus apparent viscosity and accumulation scores in RAO-affected patients in a double-blind, placebo-controlled study (Keller et al 2001). However, these decreases were small (about 1–2

scores) and there was no improvement of blood gas values. Furthermore, we have shown that such apparent viscosity scores are unreliable (Gerber et al 2004b).

We have been unable to demonstrate an effect of oral NAC (5 mg/kg body weight) on mucus viscoelasticity (Gerber & King, unpublished data). This could be the result of poor uptake of the orally administered drug because there was no measurable increase in blood levels (Gerber & Marlin, unpublished results). These latter findings call into question the validity of using oral NAC in horses. Inhalation instead of the oral route would improve the availability of NAC in the airways by circumventing the liver first-pass and would avoid the potential side effects induced by damage to the protective gastric mucus. Unfortunately, NAC can induce bronchoconstriction because of its acidity and osmolar effects. Nacystelyn, a novel lysine salt of NAC, is better suited for inhalation and has been successful in human clinical trials, but for equine veterinary use its cost is likely to be prohibitive. Finally, because NAC and similar mucolytics indiscriminately break down mucin disulfide bonds, they may not be beneficial in horses with mild to moderate lower airway disease, whose mucus viscoelasticity can already be abnormally low (Gerber et al 1998) (Fig. 5.9).

Peptide mucolytics

There is good evidence that leukocyte breakdown products, specifically DNA and filamentous (F-)actin released from dying neutrophils, have an unfavorable effect on mucus rheology of RAO horses in exacerbation. DNase (Pietra et al 2000) and Gelsolin (Gerber 2003) decrease mucus viscoelasticity of this abnormal mucus in RAO-affected horses. Unfortunately, these peptide mucolytics, used mainly in human cystic fibrosis, are prohibitively expensive for use in horses.

Conclusions

Clearly, the options for prevention and therapy to avoid and reduce mucus accumulation in the horse are unsatisfactory at present. Economical treatments such as the secretolytics, NAC, hypertonic saline inhalation, and possibly the mucoactive effect of macrolide antibiotics should be further evaluated not only for their effect on the mucociliary apparatus, but specifically for any potential lung function improvement and clinical benefit. Meanwhile, it is crucial to carefully evaluate whether any given mucus accumulation should be treated in the individual horse and to always address the underlying causes.

REFERENCES

Anon 2001 International Workshop on Equine Chronic Airway Disease. Proceedings of Workshop at Michigan State University, June 2000. Equine Veterinary Journal 33: 5–19

Anon 2002 International Workshop on Inflammatory Airway Disease: Defining the Syndrome. Proceedings of Workshop in Boston, October 2002. Equine Veterinary Education 15: 61–63

Art T, Kirschvink N, Smith N et al 1999 Indices of oxidative stress in blood and pulmonary epithelium lining fluid in horses suffering from recurrent airway obstruction. Equine Veterinary Journal 31: 397–401

Bartner LR, Tesfaigzi Y, Robinson NE 2006 Persistent mucus accumulation: A consequence of delayed mucous cell death in heaves-affected horses? American Journal of Physiology. Lung Cell Molecular Physiology: 171: in press

Basbaum C, Lemjabbar H, Longphre M et al 1999 Control of mucin transcription by diverse injury-induced signaling pathways. American Journal of Respiratory and Critical Care Medicine 160: S44–S48

Broadstone RV, Scott JS, Derksen FJ et al 1988 Effects of atropine in ponies with recurrent airway obstruction. Journal of Applied Physiology 65: 2720–2725

Bureau F, Bonizzi G, Kirschvink N et al 2000 Correlation between nuclear factor-kappaB activity in bronchial brushing samples and lung dysfunction in an animal model of asthma. American Journal of Respiratory and Critical Care Medicine 161: 1314–1321

Burrell MH 1985 Endoscopic and virological observations on respiratory disease in a group of young thoroughbred horses in training. Equine Veterinary Journal 17: 99–103

Chapman PS, Green C, Main JP et al 2000 Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. Veterinary Record 146: 91–95

Christley RM, Hodgson DR, Rose RJ et al 2001 Coughing in thoroughbred racehorses: risk factors and tracheal endoscopic and cytological findings. Veterinary Record 148: 99–104

Coombs SL, Webbon PM 1987 Observations on tracheal mucociliary clearance in horses. Tierärztliche Praxis Supplement 2: 5–9

Del Donno M, Bittesnich D, Chetta A et al 2000 The effect of inflammation on mucociliary clearance in asthma: an overview. Chest 118: 1142–1149

Derksen FJ, Robinson NE, Berney CE 1992 Aerosol pirbuterol: bronchodilator activity and side effects in ponies with recurrent airway obstruction (heaves). Equine Veterinary Journal 24: 107–112

Dieckmann MP 1987 Zur Wirksamkeit von Ambroxol-hydrochlorid (Mukovent) bei lungenkranken Pferden-klinische, funktionelle und zytologische Untersuchungen, Inaugural Dissertation (Dr. med. vet.), Tierärztliche Hochschule Hannover, Germany

Dixon PM 1992 Respiratory mucociliary clearance in the horse in health and disease, and its pharmaceutical modification. Veterinary Record 131: 229–235

Dixon PM, Railton DI, McGorum BC 1995a Equine pulmonary disease: a case control study of 300 referred cases. Part 1: Examination techniques, diagnostic criteria and diagnoses. Equine Veterinary Journal 27: 416–421

Dixon PM, Railton DI, McGorum BC 1995b Equine pulmonary disease: a case control study of 300 referred cases. Part 2: details of animals and historical and clinical findings. Equine Veterinary Journal 27: 422–427

Dixon PM, Railton DI, McGorum BC 1995c Equine pulmonary disease: a case control study of 300 referred cases. Part 3: Ancillary diagnostic findings. Equine Veterinary Journal 27: 428–435

Dorwald M, Vanden Bosche G, Gerull A 1991 Zura Surfactant-phospholipidzusammensetzung im Tracheobronchialsekret des Pferdes und ihrer klinischen relevanz fuer die Beurteilung des Lungenstatus bei chronisch lungen-kranken Pferden. Wiener Tierärztliche Wochenschrift 78: 118–126

Fahy JV, Boushey HA 1998 Effect of low-dose beclomethasone dipropionate on asthma control and airway inflammation. European Respiratory Journal 11: 1240–1247

Fanucchi MV, Hotchkiss JA, Harkema JR 1998 Endotoxin potentiates ozone-induced mucous cell metaplasia in rat nasal epithelium. Toxicology and Applied Pharmacology 152: 1–9

Galati P, Roperto F, De Vico G et al 1991 Atypical cilia in the tracheal epithelium of healthy horses. Journal of Comparative Pathology 105: 185–190

Gerber V 1995 Untersuchungen über die muköziliäre Clearance in der Luftröhre des Pferdes [Investigation of mucociliary clearance in equine trachea]. Inaugural Dissertation (Dr. med. vet.), University of Berne, Switzerland

Gerber V 2003 Nature and causes of mucus accumulation in equine lower airway disease. PhD thesis, Michigan State University, USA

Gerber V, Gehr P, Straub R et al 1996 Influence of gravity on tracheal mucus velocity in excised horse tracheas. American Journal of Respiratory and Critical Care Medicine 153: A483

Gerber V, Gehr P, Straub R et al 1997 Mucus quality on horse tracheal epithelium: microscopic grading based on transparency. Respiration Physiology 107: 67–74

Gerber V, King M, Lee M et al 1998 Rheology of mucus in horses with moderate COPD. World Equine Airways Symposium CD-ROM, Lifelearn, Guelph, Canada

Gerber V, King M, Schneider DA et al 2000 Tracheobronchial mucus viscoelasticity during environmental challenge in horses with recurrent airway obstruction. Equine Veterinary Journal 32: 411–417

Gerber V, Robinson NE, Rawson J et al 2001 Equine mucin genes: MUC5AC but not MUC2 is expressed in equine airways. Proceedings of the Annual Veterinary Medical Forum. American College of Veterinary Internal Medicine. CD-ROM, Omnipress, Chicago.

Gerber V, Robinson NE, Luethi S et al 2003a Airway inflammation and mucus in two age groups of asymptomatic well-performing sport horses. Equine Veterinary Journal 35: 491–495

Gerber V, Robinson NE, Venta RJ et al 2003b Mucin genes in horse airways: MUC5AC, but not MUC2, may play a role in recurrent airway obstruction. Equine Veterinary Journal 35: 252–257

Gerber V, Schott HC, Carr E et al 2003c Oral dexamethasone resolves owner complaints but inconsistently improves clinical parameters of equine lower airway disease in a randomized, double-blind placebo-controlled study. Proceedings of the Annual Veterinary Medical Forum. American College of Veterinary Internal Medicine. CD-ROM, Omnipress, Chicago.

Gerber V, Lindberg A, Berney C et al 2004a Airway mucus in recurrent airway obstruction—short-term response to environmental challenge. Journal of Veterinary Internal Medicine 18: 92–97

Gerber V, Straub R, Marti E et al 2004b Endoscopic scoring of mucus: Reproducibility, and relation to mucus viscosity and airway inflammation. Equine Veterinary Journal 36: 576–582

Gordon T, Nadziejko C, Plant M et al 1996 One-month exposure to inhaled endotoxin produces a dose-dependent increase in stored mucosubstances in rat intrapulmonary airways. Experimental Lung Research 22: 509–523

Harkema JR, Wagner JG 2002 Non-allergic models of mucous cell metaplasia and mucus hypersecretion in rat nasal and pulmonary airways. Novartis Foundation Symposium 248: 181–197; discussion 197–200, 277–182

Harkema JR, Plopper CG, Hyde DM et al 1987 Regional differences in quantities of histochemically detectable mucosubstances in nasal, paranasal, and nasopharyngeal epithelium of the bonnet monkey. Journal of Histochemistry and Cytochemistry 35: 279–286

Haschek WM 1998 Respiratory system. In: Haschek WM, Rousseaux CG (editors) Fundamentals of Toxicologic Pathology. Academic Press, San Diego, pp.108–122

Hobo S, Yoshihara T, Oikawa M et al 2001 Surfactant proteins in bronchoalveolar lavage fluid of horses: assay technique and changes following road transport. Veterinary Record 148: 74–80

Holcombe SJ, Jackson C, Gerber V et al 2001 Stabling is associated with airway inflammation in young Arabian horses. Equine Veterinary Journal 33: 244–249

Holcombe SJ, Robinson NE, Derksen FJ et al 2004 Tracheal mucus is associated with poor racing performance in thoroughbred horses. Proceedings of the 50th Annual Convention of the American Association of Equine Practitioners, AAEP, Lexington, USA, pp. 272–273

Houtmeyers E, Gosselink R, Gayan-Ramirez G et al 1999 Regulation of mucociliary clearance in health and disease. European Respiratory Journal 13: 1177–1188

Im Hof V, Gerber V, Gehr P et al 1996 Exogenous surfactant accelerates tracheal mucus velocity in spontaneously breathing non-anaesthetized horses. American Journal of Respiratory and Critical Care Medicine 153: A109

Im Hof V, Gehr P, Gerber V et al 1997 In vivo determination of surface tension in the horse trachea and in vitro model studies. Respiration Physiology 109: 81–93

Jefcoat AM, Hotchkiss JA, Gerber V et al 2001 Persistent mucin glycoprotein alterations in equine recurrent airway obstruction. American Journal of Physiology. Lung Cell Molecular Physiology 281: L704–L712

Jeffery PK, Li D 1997 Airway mucosa: secretory cells, mucus, and mucin genes. European Respiratory Journal 10: 1655–1662

Jungsuwadee P, Dekan G, Stingl G et al 2004 Inhaled dexamethasone differentially attenuates disease relapse and established allergic asthma in mice. Clinical Immunology 110: 13–21

Kaup FJ, Drommer W, Damsch S et al 1990a Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD). II: Pathomorphological changes of the terminal airways and the alveolar region. Equine Veterinary Journal 22: 349–355

Kaup FJ, Drommer W, Deegen E 1990b Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD). I: Alterations of the larger conducting airways. Equine Veterinary Journal 22: 343–348

Keller H, Faulstich M, Elker M et al 2001 Klinische Studie zur Wirksamkeit und Verträglichkeit von N-Acetylcystein bei der Behandlung der COB/COPD des Pferdes [efficacy and

tolerance study of acetylcysteine in the COB/COPD therapy in horses]. Praktischer Tierarzt 82: 108–117

Kibe A, Inoue H, Fukuyama S et al 2003 Differential regulation by glucocorticoid of interleukin-13-induced eosinophilia, hyperresponsiveness, and goblet cell hyperplasia in mouse airways. American Journal of Respiratory and Critical Care Medicine 167: 50–56

Kim WD 1997 Lung mucus: a clinician's view. European Respiratory Journal 10: 1914–1917

King M, Rubin BK 1994 Rheology of airway mucus: relationship with clearance function. Takashima T, Shimura S (editors) Airway Secretion: Physiological Bases for the Control of Mucous Hypersecretion. Marcel Dekker, Inc., New York, pp.283–314

Lindberg A, Nasman-Glaser B, Lindgren JA et al 2002 Evaluation of leukotriene biosynthetic capacity in lung tissues from horses with recurrent airway obstruction. American Journal of Veterinary Research 63: 794–798

Luft J 1987 Der Einfluss koerperlicher Belastung auf die Zytologie des Tracheobronchialsekretes beim Pferd [The effect of exercise of the composition of tracheobronchial secretions in the horse]. Inaugural Dissertation (Dr. med. vet.), Tierärztliche Hochschule Hannover, Germany

Lugo J, Harkema J, Defeijter-Rupp H et al 2006 Airway inflammation is associated with mucous cell metaplasia and increased epithelial stored mucosubstances in horses. The Veterinary Journal (in press)

MacNamara B, Bauer S, Iafe J 1990 Endoscopic evaluation of exercise-induced pulmonary hemorrhage and chronic obstructive pulmonary disease in association with poor performance in racing Standardbreds. Journal of the American Veterinary Medical Association 196: 443–445

Matthews AG, Hackett IJ, Lawton WA 1988 The mucolytic effect of Sputolosin in horses with respiratory disease. Veterinary Record 122: 106–108

Matthews LM, Spector S, Lemm J et al 1963 Studies on pulmonary secretions. American Review of Respiratory Disease 88: 199–204

Maxson AD, Soma LR, May LL et al 1995 Effects of furosemide, exercise, and atropine on tracheal mucus transport rate in horses. American Journal of Veterinary Research 56: 908–912

Moreno RH, Hogg JC, Pare PD 1986 Mechanics of airway narrowing. American Review of Respiratory Disease 133: 1171–1180

Nakanishi A, Morita S, Iwashita H et al 2001 Role of gob-5 in mucus overproduction and airway hyperresponsiveness in asthma. Proceedings of the National Academy of Sciences, USA 98: 5175–5180

Pearce HG, Wyburn RS, Goulden BE 1978 A clinical evaluation of Bisolvon® for the treatment of some equine respiratory diseases. New Zealand Veterinary Journal 26: 28–30

Pietra M, Guglielmini C, Forni M et al 2000 In vitro mucolytic activity of recombinant human deoxyribonuclease on equine tracheobronchial mucus. Veterinary Record 147: 627–629

Pirie RS 1998 In: Role of inhaled endotoxin in the pathogenesis of equine COPD. Dixon PM, Schmallenbach K, Maisi P, et al (editors) Proceedings of Workshop on Allergic Diseases of the Horse. Lipica, Slovenia, p.10

Pirie RS, Dixon PM, Collie DD et al 2001 Pulmonary and systemic effects of inhaled endotoxin in control and heaves horses. Equine Veterinary Journal 33: 311–318

Raidal SL 1996 The incidence and consequences of failure of passive transfer of immunity on a thoroughbred breeding farm. Australian Veterinary Journal 73: 201–206

Raidal SL, Love DN, Bailey GD 1995 Inflammation and increased numbers of bacteria in the lower respiratory tract of horses within 6 to 12 hours of confinement with the head elevated. Australian Veterinary Journal 72: 45–50

Robinson NE, Derksen FJ, Olszewski MA et al 1996 The pathogenesis of chronic obstructive pulmonary disease of horses. British Veterinary Journal 152: 283–306

Robinson NE, Berney C, Eberhart S et al 2003 Cough, mucus, airway obstruction, and inflammation in heaves-affected and control horses. American Journal of Veterinary Research 64: 550–557

Robinson NE, Kamaus W, Holcombe SJ et al 2006 Airway inflammation in Michigan pleasure horses: prevalence and risk factors. Equine Veterinary Journal (in press)

Sasse W, Deegen E 1984 Zur Wirksamkeit von Sputolysin bei Pferden mit chronischen Bronchialerkrankungen [On the efficacy of sputolysin in horses with chronic bronchial diseases]. Tierärztliche Umschau 39: 941–949

Schlesinger RB 1985 Clearance from the respiratory tract. Fundamental and Applied Toxicology 5: 435–450

Shi ZO, Fischer MJ, De Sanctis GT et al 2002 IFN-gamma, but not Fas, mediates reduction of allergen-induced mucous cell metaplasia by inducing apoptosis. Journal of Immunology 168: 4764–4771

Shim JJ, Dabbagh K, Ueki IF et al 2001 IL-13 induces mucin production by stimulating epidermal growth factor receptors and by activating neutrophils. American Journal of Physiology. Lung Cellular and Molecular Physiology 280: L134–L140

Sonea IM, Bowker RM, Robinson NE 1999 Distribution of substance P binding sites in equine airways. Equine Veterinary Journal 31: 238–242

Sweeney CR 1989 Tracheal mucus transport rate in healthy horses. American Journal of Veterinary Research 50: 2135–2137

Sweeney CR, Beech J, Roby KA 1985 Bacterial isolates from tracheobronchial aspirates of healthy horses. American Journal of Veterinary Research 46: 2562–2565

Sweeney CR, Leary HJ 3rd, Ziemer EL et al 1989 Effect of water vapor-saturated air therapy on bronchoalveolar lavage and tracheal mucus transport rate in clinically normal horses. American Journal of Veterinary Research 50: 276–279

Takeyama K, Dabbagh K, Jeong Shim J et al 2000 Oxidative stress causes mucin synthesis via transactivation of epidermal growth factor receptor: role of neutrophils. Journal of Immunology 164: 1546–1552

Tesfaigzi Y, Fischer MJ, Martin AJ et al 2000 Bcl-2 in LPS- and allergen-induced hyperplastic mucous cells in airway epithelia of Brown Norway rats. American Journal of Physiology: Lung Cellular and Molecular Physiology 279: L1210–L1217

Toda M, Tulic MK, Levitt RC et al 2002 A calcium-activated chloride channel (HCLCA1) is strongly related to IL-9 expression and mucus production in bronchial epithelium of patients with asthma. Journal of Allergy and Clinical Immunology 109: 246–250

Turgut K, Sasse HH 1989 Influence of clenbuterol on mucociliary transport in healthy horses and horses with chronic obstructive pulmonary disease. Veterinary Record 125: 526–530

Viel L 1980 Structural-functional correlations of the lung in the normal light horse. MSc thesis, University of Guelph, Canada

Walley EA, Thornton DJ, Corfield AP et al 2001 Characterization of equine respiratory tract mucins. World Equine Airway Symposium, CD-ROM, Lifelearn, Guelph, Canada

Widdicombe JH, Pecson IS 2002 Distribution and numbers of mucous glands in the horse trachea. Equine Veterinary Journal 34: 630–633

Willoughby R, Ecker G, McKee S et al 1992 The effects of equine rhinovirus, influenza virus and herpesvirus infection on tracheal clearance rate in horses. Canadian Journal of Veterinary Research 56: 115–121

Willoughby RA, Ecker GL, McKee SL et al 1991 Use of scintigraphy for the determination of mucociliary clearance rates in normal, sedated, diseased and exercised horses. Canadian Journal of Veterinary Research 55: 315–320

Zhou Y, Dong Q, Louahed J et al 2001 Characterization of a calcium-activated chloride channel as a shared target of Th2 cytokine pathways and its potential involvement in asthma. American Journal of Respiratory Cell and Molecular Biology 25: 486–491

6

Immunology and Immunopathology

D Paul Lunn, Cormac Breathnach and Gisela Soboll

The immune defenses of the equine respiratory tract protect an area of over 2,000 m^2 from the myriad micro-organisms, particulates, and noxious agents inhaled in an estimated 100,000 liters of air on a daily basis (Dixon & McGorum 1997). All this while maintaining surfaces that can allow the free diffusion of respiratory gases to the blood across barriers no more than two cells thick. Although a robust immune response must be mounted against many serious pathogens, the same system must establish and maintain tolerance to many non-threatening but antigenic inhaled materials.

The respiratory immune system shares all the components of the immunological response found throughout the rest of the body, but also has several unique features that are characteristic of the mucosal immune system. An extensive review of equine immune responses was recently published (Lunn & Horohov 2003), and this chapter will focus on the specific specialization of the equine respiratory tract, and mucosal immune responses in particular.

Innate and Adaptive Immunity

Respiratory defense mechanisms are complex, integrating responses that include mechanical barriers, the chemical poisons, neutrophils and macrophages of the innate immune system, and the development of highly specific antibody and lymphocyte responses by the adaptive immune system. The upper and lower airways, and the alveolar space each have different defense mechanisms (Table 6.1) (Pilette et al 2001). Mechanical defenses, capable of removing inhaled particulate matter, predominate in the airways and are reviewed in the previous

Table 6.1. Defence mechanisms of the respiratory tract		
Mechanical	**Innate immunity**	**Adaptive immunity**
Upper respiratory tract (nasopharynx and larynx)		
• Nasal, oropharyngeal and sinus ciliated epithelium and sneezing • Vocal cords • Mucus	• Complement • Proteases • Lactoferrin	• Secretory IgA and IgM in mucus layer (major Ig classes), in lamina propria, and *in transit* through endosomal compartment of repiratory epithelial cells • IgG subclasses (IgGa and IgGb) in mucus layer, and in lamina propria • Full complement of lymphocyte subsets, including inflammatory CD4 cells and cytotoxic lymphocytes in organized (tonsils) and dispersed lymphoid tissues in mucosa
Lower respiratory tract (tracheobronchial tree)		
• Mucociliary clearance • Impaction on bronchial branching and coughing	• Recruited neutrophils • (Alveolar?) Macrophages	• Secretory IgA and IgM as in upper respiratory tract, plus increasing amounts of IgG • Full complement of lymphocyte subsets as in upper respiratory tract, organized as bronchial-associated lymphoid tissue
Lung parenchyma (alveoli and lung interstitium)		
• None	• Surfactant products • Phagocytic cells including resident alveolar macrophages and recruited neutrophils and their products	• Parenchymal lymphocytes and recruited lymphocytes in case of inflammatory response

Adapted from Pilette et al, 2001

Box 6.1. T-helper cells and cytokines

The CD4^{+} T-helper lymphocytes "help" other effector cells to fight off pathogens or respond to antigens. At least two different subsets of T-helper cells, characterized by their cytokine production profile, are responsible for regulating the immune response to infectious agents or to inhaled antigens. The subsets are the T-helper 1 (Th1) subset which stimulates cytotoxic and inflammatory functions and production of the equine IgG subclasses IgGa and IgGb, and the T-helper 2 (Th2) subset which stimulates equine IgG subclasses IgG(T), IgA, and allergic responses mediated by IgE. These two types of T-helper subsets and the cytokines they produce, tend to suppress each other. As a result, in an immune response to a particular pathogen, either the Th1 or Th2 subset will predominate and give rise to either an inflammatory/cytotoxic immune response or different antibody-mediated immune responses. Several factors have been identified which may influence whether a Th1 or Th2 response will predominate and these include the type of antigen-presenting cell, the dose of antigen, and the cytokines present during antigen presentation.

Cytokines associated with different T-helper responses			
Cytokine	*Function*	*Th1*	*Th2*
IL-2	Provides proliferative signal for T cells. Also affects B cells, macrophages and NK cells. High concentrations of IL-2 stimulate cytolytic activity in NK cells and T cells.	X	
IL-12	NK-cell differentiation factor. Augments NK-cell function and stimulates generation of Th-1 cells. Produced by macrophages.	X	
IFN-γ	Produced by NK cells in response to IL-12, and by Th-1 cells. Leads to increased MHC I and II expression, activation of macrophages and cytotoxic lymphocytes, and IgGa and IgGb production.	X	
IL-4	Stimulates growth, maturation and differentiation of B cells.		X
IL-5	Stimulates B-cell proliferation and immunoglobulin synthesis. Also stimulates T-cell proliferation and differentiation as well as eosinophil formation in the bone marrow.		X
IL-6	Promotes maturation and immunoglobulin production by B cells. Stimulates T-cell growth and IL-2 synthesis. Induces the production of acute-phase proteins by hepatocytes. Produced by macrophages, T cells, stromal cells, fibroblasts, and a variety of other cell lines.		X
IL-10	Cytokine synthesis inhibitory factor. Inhibits the production of IL-2 and IFN-γ by Th-1 cells.		X
IL-13	Down-regulates cytokine production by macrophages/monocytes while activating B cells.		X

IL = interleukin; NK = natural killer; IFN-γ = interferon-γ; MHC = major histocompatibility complex

chapter (Chapter 5). In contrast, the alveolar epithelium lacks mucociliary properties and removes particles and microorganisms by use of resident alveolar macrophages. The innate immune response provides a constant defense against everyday encounters with potential invaders, and in the face of greater threats can rapidly recruit polymorphonuclear neutrophils to the airways and supporting tissues. Danger signals leading to this response can come from several sources, but it is interesting to note that even respiratory epithelial cells can initiate inflammatory reactions by release of cytokines, this recruiting phagocytic myeloid cells and lymphocytes, and can serve as effective antigen-presenting cells to initiate adaptive immune responses by lymphoid cells.

The adaptive immune response depends on lymphocytes, which are relatively scarce in the normal airway and alveolar lumen, although they are common in the submucosa throughout the respiratory tract. The full complement of lymphocyte responses is present or readily recruited from the systemic lymphocyte pool, together with the unique adaptations of the mucosal immune system that play a critical response in all aspects of respiratory immunology. One important concept in understanding the adaptive immune system is the importance of the regulatory role of T-helper cell subsets, which modulate immune responses through the secretion of cytokines. A detailed review is available elsewhere (Lunn & Horohov 2003), but Box 6.1 highlights the essential elements of this process.

Mucosal Immunity

The adaptations that maximize absorption and exchange at mucosal surfaces render these tissues especially vulnerable to invasion by pathogens. This challenge is confronted by the mucosal immune system, consisting of organized and dispersed lymphoid tissues that are closely associated with

mucosal epithelial surfaces (Mestecky et al 2003). The mucosal immune system maintains immune surveillance across the largest external surface area of the body, and mucosal immune responses generated in one location are transferred throughout the mucosal immune system by lymphocytes that are programmed to home to regional effector sites. The principal immunoglobulin produced by the mucosal immune system is secretory immuno-globulin A (sIgA), which, in humans, is the most abundant immunoglobulin class in the body. Secretory IgA has unique adaptations that promote transport out onto mucosal surfaces, where it protects the body from bacteria and viruses principally by immune exclusion, i.e. by physically preventing attachment to mucosal surfaces. The importance of mucosal IgA has already been demonstrated in immunity to equine influenza virus (Hannant et al 1989b, 1999, Nelson et al 1998), equine herpesvirus-1 (EHV-1) infection (Breathnach & Allen 2001), and *Streptococcus equi* (Sheoran et al 1997). For many equine diseases, and especially viral respiratory infections, a mucosal immune response may be the most effective type of immune protection. In addition to regulating this mammoth defense strategy, the mucosal system must distinguish food and other antigens against which an immune response would be disastrous.

Distribution of lymphoid tissues in the respiratory tract

Coordination of the mucosal immune response depends on organized mucosa-associated lymphoid tissue (MALT), principal examples of which are the pharyngeal tonsils and the intestinal Peyer's patches. In the gastrointestinal tract MALT is distributed throughout the gut, but in the respiratory tract these tissues are only found in the nasopharynx and oropharynx. MALT consists of lymphoid follicles containing IgA-committed B cells, surrounded by interfollicular T-cell areas with antigen-presenting cells and high endothelial venules, with an overlying follicle-associated epithelium. Naive lymphocytes enter the MALT by extravasation from the high endothelial venules (there are no afferent lymphatics in MALT), and after antigen encounter in the MALT they leave through efferent lymphatics. The follicle-associated epithelium is specialized for antigen sampling, by having reduced secretion of mucus, and by the presence of specialized antigen uptake cells termed microfold or M cells. These M cells are typically closely associated with underlying aggregates of lympho-cytes, often within large basolateral membrane pockets, and play a critical role in mucosal immune surveillance. Adherent macromolecules or particles bound to the apical M-cell membrane undergo endocytosis or phagocytosis and are released at the pocket membrane, where antigen presentation is initiated by dendritic cells resulting in the
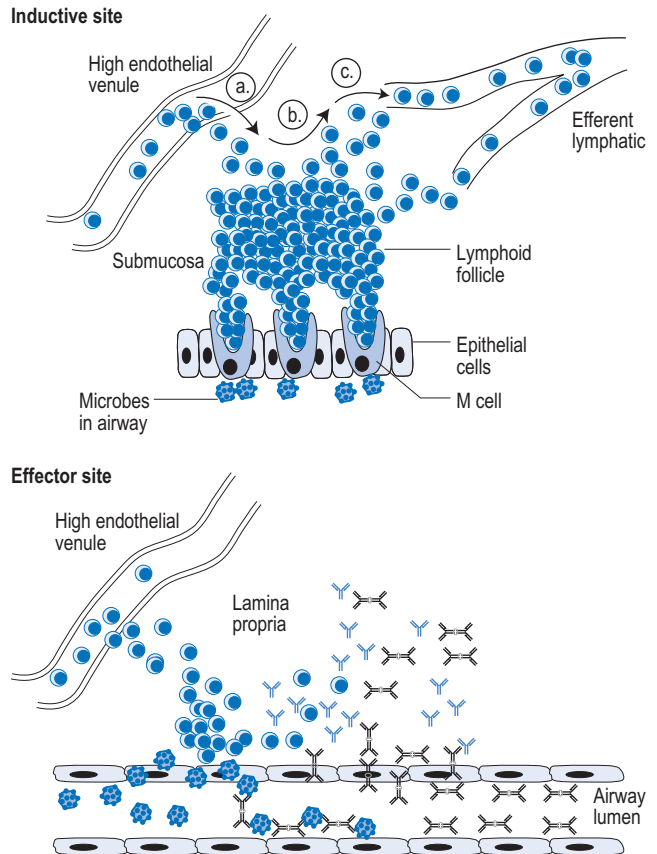
Fig. 6.1. Initiation of mucosal immune responses. Respiratory mucosal immune responses typically originate after antigenic encounter at inductive sites, which are the tonsils of the nasopharynx and oropharynx in the horse. Naive lymphocytes enter the inductive sites from high endothelial venules via the specialized cuboidal endo-thelium of those vessels in response to specific molecular signals. Antigens, such as microbes, are taken up by microfold or M cells which are part of the highly specialized follicle-associated epithelium present at these sites. Antigenic material is transported across the M cell, and antigen presentation to B and T lymphocytes is accomplished by dendritic cells in the underlying tissues. The underlying lymphoid follicle is composed primarily of B lymphocytes, surrounded by T-lymphocyte areas. Antigen-specific B lymphocytes become com-mitted primarily to IgA production at these sites, although some IgG B lymphocytes are also generated. Subsequently the primed lymphocyte populations exit the inductive site via efferent lymphatics, eventually reaching the blood circulation through the thoracic duct. Subsequently these cells traffic to high endothelial venules of effector sites throug-hout the respiratory epithelium, and extravasate to make up the intra-epithelial lymphocyte and lamina propria lymphocyte population, and to give rise to lymphoid aggregates. Subsequent antigen encounter results in terminal differentiation to plasma cells, primarily IgA-producing although some IgG plasma cells are also formed. IgG is largely restricted to tissues, but secretory IgA is transported to the respiratory epithelial surface where it can aggregate infectious organisms.

activation of antigen-specific B cells (Fig. 6.1). Subsequent trafficking and recirculation of memory IgA-positive B cells to the other components of the mucosal immune system (respiratory tract, intestinal tract, etc.) is responsible for the dissemination of local mucosal IgA responses
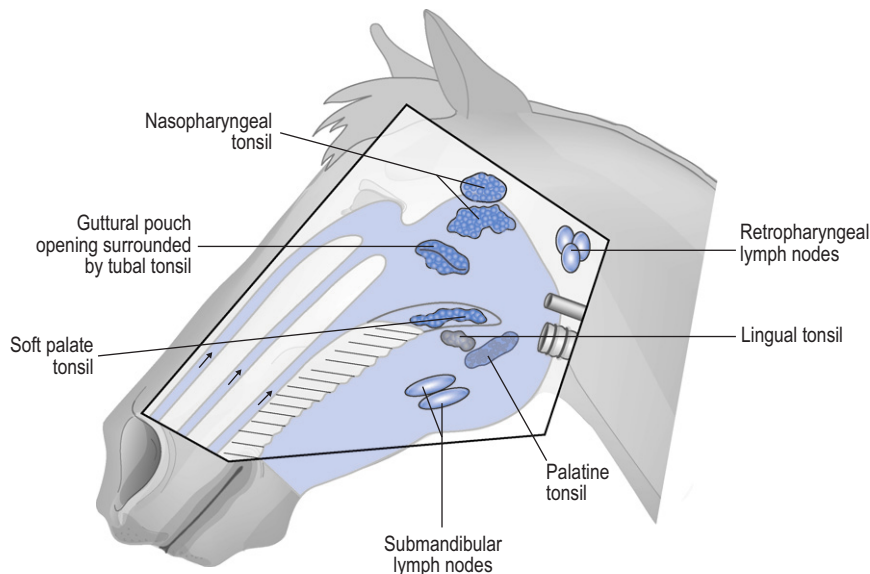
Fig. 6.2. Lymphoid tissues of the equine upper respiratory tract. The nasopharyngeal tonsils are bilateral structures in the dorsolateral nasopharyngeal roof, and are the largest mass of lymphoid tissue in the respiratory tract of horses of all ages. Lymphoid aggregates are common in the adjacent mucosa. Tubal tonsils surround the operculum (opening to the guttural pouch). Palatine tonsils extend longitudinally along each side of the oropharynx at the base of the tongue. Embedded within the base of the tongue lies the lingual tonsil. The tonsil of the soft palate consists of strings of lymphoid nodules extending longitudinally along the center of the soft palate. Regional lymphatic drainage is via the retropharyngeal lymph nodes and to the submandibular lymph nodes.

throughout what is termed the "common mucosal immune system". After the homing of these B cells to effector sites such as the lamina propria of the gut and respiratory tract, and extravasation into the lamina propria from high endothelial venules, further antigen encounter and second signals from antigen-presenting cells and from T-helper cells result in further differentiation into IgA-producing plasma cells. The short half-life of IgA-secreting plasma cells requires a constant generation of precursors in induction sites and flow to effector sites. Antigen sampling and presentation are not restricted to organized MALT, and throughout the mucosal surfaces, including areas such as pseudostratified airway epithelium, dendritic cells play a key role in antigen uptake and presentation, subsequently migrating to local lymph nodes or MALT to initiate immune responses.

In the horse, understanding of the architecture and functions of respiratory lymphoid tissues largely comes from work conducted by Mair et al (1987, 1988a,b). This group described five levels of respiratory tract-associated lymphoid tissue in the horse:

- *Free luminal lymphocytes* exist primarily in the respiratory mucus overlying subepithelial lymphoid aggregates.
- *Intraepithelial lymphocytes* are scattered throughout the respiratory epithelium, but cluster at the base of the epithelia overlying mucosal lymphoid nodules.

- *Isolated lamina propria lymphocytes* are present throughout the subepithelial connective tissue, primarily in areas surrounding lymphoid nodules.
- Densely packed *aggregated lymphocytes* are present close to serous gland ducts in the nasal mucosa, and make up subepithelial lymphoid nodules throughout the bronchi.
- *Nodular lymphoid tissue,* which in the nasopharynx and oropharynx can have an overlying lymphoepithelium specialized for antigen uptake and processing as in the case of tonsillar tissues. Additional nodular lymphoid tissue is typically present at sites where antigen-laden mucus and air currents converge throughout the trachea and bronchi and is called bronchus-associated lymphoid tissue or BALT. Mucosal lymphoid nodules are apparent in foals as early as 9 months of gestational age and develop at specific locations, suggesting that the ontogeny of mucosal lymphoid tissue is genetically regulated (Mair et al 1988a). The number of mucosal lymphoid nodules peaks in young adult horses (≤ 5 years of age), and wanes in older animals (Mair et al 1988a).

Tonsils represent the most complex mucosal nodular lymphoid tissues. Horses possess all of the various tonsillar tissues that are recognized in other species (depicted in Fig. 6.2). Tubal tonsils surround the operculum (opening to the guttural pouch). Palatine tonsils extend longitudinally along each side of the oropharynx at the base of the tongue. Embedded within

Table 6.2. Immunophenotype of respiratory mucosal and lymph node lymphocytes

Source of lymphocytes	CD3	CD4	CD8	MHCII	CD8/CD4
PBMC	71.1	42.9	3.9	63.8	0.09
Retropharyngeal lymph node	40.6	30.8	5.5	73.2	0.18
Hilar lymph node	52.7	36.6	6.1	76.9	0.17
Inguinal lymph node	43.0	28.8	7.1	71.4	0.25
Pharyngeal follicular tissue	35.8	22.9	7.2	62.0	0.31
Nasal mucosa	36.8	30.4	6.1	74.4	0.20

Tissues were dissected and dissociated with hyaluronidase and collagenase prior to purification of mononuclear cells by density-gradient centrifugation and flow cytometric analysis using previously described monoclonal antibodies (Lunn et al 1998). Results of lymphocyte antigen expression by peripheral blood mononuclear cells (PBMCs), regional lymph node lymphocytes, and pharyngeal wall (nasopharyngeal tonsil) and nasal mucosa are shown. All results are means of percentage positive staining; $n = 6$ horses. These results demonstrate that in comparison to PBMCs, lymph nodes and mucosal tissue T-cell numbers (CD3-positive) are reduced, but the proportion of CD8 cells is increased.

the base of the tongue lies the lingual tonsil. The tonsil of the soft palate consists of strings of lymphoid nodules extending longitudinally along the center of the soft palate. Finally, the best studied tonsillar tissue in the horse is the nasopharyngeal tonsil, deemed to represent the largest mass of lymphoid tissue in the respiratory tract of horses of all ages (Mair et al 1988a). Kumar et al (2001) have characterized this tissue and identified a classical follicle-associated epithelium overlying it. This epithelium is heavily folded to form crypts, and also contains M cells. The nasopharyngeal tonsil exists in the dorsal recess of the nasopharynx, and extends ventrally towards the opercula on either side of the nasopharynx. Therefore, it is ideally placed for the sampling of antigens prior to entry to the airways or alimentary tract and may serve as an important target for intranasal vaccines. This tissue appears to be most abundant in young foals, and atrophies with age, though many lymphoid follicles remain throughout the nasopharynx. It is not known whether the other tonsils of the horse have a classical follicle-associated epithelium, and this could significantly affect their role in initiating respiratory immune responses as they may not necessarily all be components of the mucosal immune system.

The nasopharynx is a dynamic immunological site. Within the nasopharyngeal epithelium lie numerous lymphocytes. Immmunohistochemical studies indicate that the majority of these lymphocytes are CD8^{+} T lymphocytes although B lymphocytes are also present (Kumar et al 2001). However, when these tissues are dissociated and studied by flow cytometry, B lymphocytes predominate, although the numbers of CD8^{+} T lymphocytes is considerably greater than in blood or respiratory tract lymph nodes (Table 6.2). The contribution of these lymphocytes to mucosal cellular immune defense of the upper respiratory tract is poorly studied. However, following

intranasal challenge of yearling and 2-year-old horses with EHV-1, virus-specific cytotoxic activity is detectable in several mucosal lymphoid tissues of the upper respiratory tract, as well as the local draining lymph nodes, and is particularly evident in the nasopharyngeal lining (Breathnach et al 2006). This cellular immune response is presumably mediated by CD8^{+} T lymphocytes found in the nasopharyngeal epithelium and underlying lamina propria, and may provide an important contribution to the clearance of infectious virus from the upper respiratory tract.

Immunoglobulins of the Respiratory Tract

Immunoglobulin G (IgG) is composed of two identical light chains and two identical heavy chains that form a disulfide-linked Y-shaped molecule (Fig. 6.3). The multimeric immunoglobulins are IgM (five IgG-like units) and IgA (a dimeric structure: Fig. 6.3). The association of the
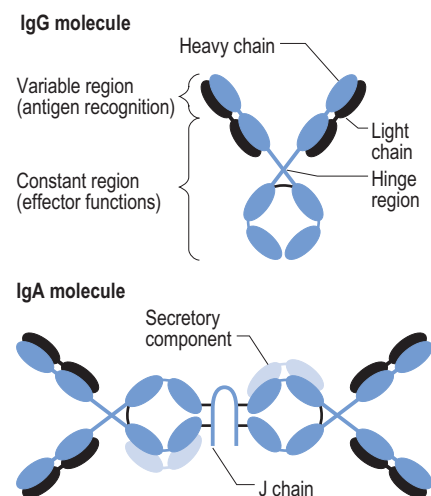
Fig. 6.3. Immunoglobulin molecules; see text for details.

amino ends of a light and a heavy chain forms the antigen-binding region of all antibody molecules, while the carboxyl end of the heavy chain determines the isotype of the molecule (the Fc region). Five different classes or isotypes of antibody molecules have been identified in most species: IgD, IgM, IgG, IgA, and IgE. Additionally, antibody classes can be subdivided into subclasses, each with distinct properties. Analysis of equine genomic DNA has indicated the existence of one IgM, IgD, IgE, and IgA genes, and seven IgG heavy-chain genes (Wagner et al 1998, Wagner 2006). This means that only one subclass or subisotype of each equine IgM, IgD, IgE, and IgA is formed. For equine IgG the situation is more complex. At least four IgG subclasses have been previously identified by monoclonal antibodies and functional studies, and these are called IgGa, IgGb, IgGc, and IgG(T) (Lunn et al 1998). Currently IgGa is known to be endocoded by heavy-chain gene *G1*, IgGb by *G4*, IgGc by *G6*, and IgG(T) by *G3* and *G5*. The product of heavy-chain gene *G2* is currently unknown (Wagner 2006). The products of heavy-chain genes *G2* and *G7* are presently unknown. It is possible that IgG(T), as defined by monoclonal antibodies, each represent two subclasses, as genetic studies suggest each IgG(T) heavy-chain gene is expressed. However, these may represent gene replications with limited implications for immunological function. In the future, equine immunoglobulins are likely to be named by their genetic derivation, but for the present the older names (IgGa etc.) are more useful for interpretation of the literature and explanation of biological effects. What is known about equine immunoglobulins is that IgGa and IgGb have Fc receptors capable of reacting with components of the innate immune system including complement, neutrophils, and macrophages to facilitate antimicrobial immunity, while IgGc and IgG(T) are better adapted to immunity to toxins and parasites (Lunn & Horohov 2003).

The principal immunoglobulin produced by the mucosal immune system is sIgA. Secretory IgA is formed by dimerization of two IgA monomers, which are attached by means of disulfide bonds to a J-chain, also produced by the same plasma cell that secretes the IgA. This confers the advantage of increased valency to sIgA, which can bind up to four of its targets so increasing its agglutinating ability. Secretory IgA protects the body from bacteria and viruses principally by immune exclusion, i.e. by physically preventing attachment to mucosal surfaces. Immunoglobulin A is relatively "non-inflammatory" (i.e. does not fix complement as effectively as IgGa or IgGb), which is consistent with a role in defense by immune exclusion (Mestecky et al 2003). Similarly, although myeloid cells possess Fc receptors for IgA it is not clear that it functions as an efficient opsonin or promotes phagocytosis.

After release of sIgA by plasma cells into the interstitium, it is bound by the polymeric immunoglobulin receptor on the abluminal surface of epithelial cells
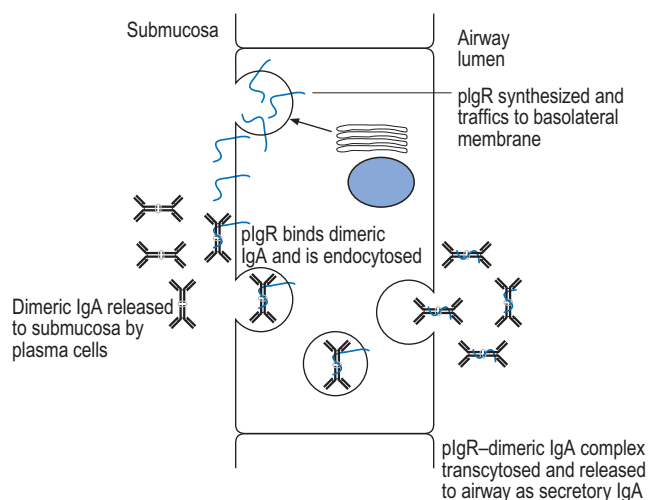
Fig. 6.4. Transcellular transport of IgA. Epithelial cells synthesize the polymeric immunoglobulin receptor (pIgR), which traffics to the basolateral membrane, where it associates with dimeric IgA. During transcytosis the pIgR forms covalent bonds with IgA, and is subsequently cleaved to release secretory component (a fragment of pIgR) combined with the dimeric IgA as secretory IgA onto the respiratory epithelial surface.

(Fig. 6.4). Subsequently the sIgA is transported across the epithelial cell and released at the luminal surface together with the secretory component formed by cleavage of part of the polymeric immunoglobulin receptor. Secretory component can also be found in a free form in mucosal secretions. Secretory component confers resistance to the proteolytic enzymes found in the respiratory and gastroenteric environment, some of which are secreted by pathogens, and prolongs the longevity of sIgA. During its transit through the epithelial cell, sIgA can neutralize intracellular infections encountered in the endosomal compartments of cells (Mazanec et al 1993). In addition, sIgA can bind antigens in the submucosa and literally transport or excrete them to the mucosa by this mechanism. The majority of the IgA in the mucosa is dimeric sIgA, while the bone-marrow-derived IgA in the circulation is predominantly monomeric.

Studies by Mair (1987, 1988a) indicated that IgA-producing plasma cells predominate in the upper airways but IgG-producing cells are more common in the lower respiratory tract (Mair et al 1988b). However, a recent study of the pulmonary humoral immune system found that IgA-producing plasma cells predominated in this location also (Blunden & Gower 1999). Antigen-specific IgA has been demonstrated in nasal mucus directed against a number of pathogens including influenza virus, EHV-1, and *S. equi* (Sheoran et al 1997, Nelson et al 1998, Breathnach et al 2001). These same studies also identified antigen-specific IgG subclasses, in particular IgGa and IgGb, in these locations, although at much lower titers

than were detected in serum. It is likely that some of this IgG represents a transudate from the lamina propria and from serum. However, when tissue samples from the nasal respiratory mucosa are processed to harvest lymphocytes, these mucosal cells will secrete antigen-specific IgGa and IgGb, demonstrating that local immune responses are not restricted to IgA (Soboll et al 2003a,b).

Respiratory Immunity to Infectious Disease

The importance of respiratory mucosal immunity in defense against infectious disease cannot be overemphasized. Our understanding of specific equine respiratory defense against fungal infections is limited, although examples of these relatively infrequent infections are most common in immunocompromised or very young horses. Similarly there is relatively little information available about immunity to parasites of the lung (Klei 2000). However, we have a growing understanding of respiratory tract immunity to both viral and bacterial pathogens.

Respiratory immunity to viruses

Equine antiviral immunity has been extensively reviewed (Slater & Hannant 2000). Both the innate and adaptive immune responses are important in resistance to respiratory viral infection. The earliest immune response is the innate response, which aims to limit viral spread until an effective adaptive immune response can be mounted. Key components of the innate response include complement, mononuclear phagocytic cells, and cytokines. In the respiratory tract the cytokines with direct antiviral action are the type 1 interferons (IFN-α and IFN-β) and tumor necrosis factor-α (TNF-α). The type 1 interferons are rapidly induced by viral invasion and induce cellular enzymes that interfere with viral replication and up-regulate antigen presentation. IFN-α is produced by macrophages and virus-infected cells produce IFN-γ. In contrast, TNF-α is produced by a variety of cells, and can directly kill virus-infected cells by cytolysis or by inducing apoptosis.

The best understood example of the role of the adaptive immune response to respiratory viral pathogens in the horse is equine influenza virus. Natural equine influenza virus infection confers complete clinical immunity for over 6 months and partial immunity for over 1 year (Hannant et al 1988). Serum antibody to influenza virus is frequently undetectable by 12 months post infection. This indicates that factors other than circulating IgG are responsible for protection after natural infection, such as local respiratory tract antibody production (IgA) or cellular immunity.

Studies of host defenses in other species have demonstrated the importance of both local antibody responses in preventing infection and cell-mediated immunity for viral clearance and recovery from infection (Bender & Small

1992). In a murine model, IgA in respiratory secretions specific for the viral hemagglutinin glycoprotein protects against influenza virus infection (Renegar & Small 1991). Intravenously administered IgA is transported physiologically to the respiratory tract and appears in secretions. This transport occurs by diffusion into the lamina propria and by receptor-mediated transport across respiratory epithelial cells as described above. Such transport is a basic characteristic of IgA and is the reason for its predominant role in local immunity as described above. It has subsequently been shown that IgA not only neutralizes virus in respiratory secretions, but can also neutralize intracellular virus, a role previously ascribed only to cellular immunity (Mazanec et al 1992). A critical difference between natural equine influenza virus infection and vaccination is that infection induces high levels of IgA in nasal mucosal secretions whereas killed vaccines induce no IgA antibodies (Hannant et al 1989a, Nelson et al 1998). While the importance of IgA is clear, other components of the immune response are also important for respiratory immunity. Both influenza-specific IgGa and IgGb can be found in respiratory secretions after infection, the source of which includes serum transudates and local production (Soboll et al 2003a).

While viral pathogens that typically produce short-lived infections of the respiratory epithelium, such as influenza virus, are highly susceptible to antibody-mediated protection, more invasive pathogens such as EHV-1 require additional immune responses for their control (Slater & Hannant 2000). Once EHV-1 has invaded the lamina propria of the respiratory tract, it rapidly becomes cell-associated, and is then protected from antibody-mediated immunity (Allen et al 1999). It is known that cytotoxic lymphocytes measured at the systemic level play a critical role in EHV-1 immunity (O'Neill et al 1999). Recent research indicates that these effector cytotoxic lymphocytes can also be found in the respiratory epithelium (Breathnach et al 2006), and it remains possible that these cytotoxic lymphocytes are amongst the most important defense mechanisms for invasive viral pathogens such as EHV-1.

Respiratory immunity to bacteria

For a comprehensive review of equine immunity to bacterial infections, the reader is referred to an excellent review (Giguere & Prescott 2000). Much of what has been said about immunity to viral infection also applies to bacterial infection. Mucosal antibody responses are a critical defense against bacterial invaders such as *S. equi* (Sheoran et al 1997), although results can be more variable in the case of intracellular bacteria such as *Rhodococcus equi* (Prescott et al 1997). The importance of T-helper cells in modulating inflammatory responses to pathogens such as *R. equi* is critical to the outcome of these infections, and one specific defense strategy of these

agents is to down-regulate the production of macrophage-activating IFN-γ by T-helper 1 lymphocytes (Giguere et al 1999). While foals appear susceptible to this strategy, adult horses normally immune to *R. equi* can generate potent IFN-γ responses to infection (Hines et al 2003).

Vaccine-induced Respiratory Immunity

There are several vaccines that can provide partial or complete protection from viral infection of the respiratory tract, although protection against bacterial infections such as *S. equi* is frequently less effective (Lunn & Townsend 2000). However, the principal immunological responses generated and measured consequent to vaccination are systemic, and there are very few examples of respiratory immune responses generated by equine vaccines. Manipulation of the equine mucosal immune response to generate specific IgA responses remains extremely difficult, despite some experimental successes (Hannant et al 1999, Soboll et al 2003b). Generating mucosal IgA responses with killed vaccines is challenging, and the most effective mucosal adjuvants are the bacterial exotoxins of enteric bacterial pathogens such as cholera toxin or the labile toxin of *Escherichia coli* (Foss & Murtaugh 1999). An obvious and major disadvantage of using cholera toxin is that it can produce cholera diarrhea in humans. An alternative approach to mucosal vaccination involves the use of microspheres, which are preferentially taken up by the M cells of the tonsils. This approach has been used with success in the case of experimental *S. equi* intranasal vaccination (Nally et al 2000).

Modified live vaccines have enjoyed the greatest success as mucosal vaccines. An intranasal cold-adapted equine influenza vaccine is amongst the most effective equine vaccine available (Townsend et al 2001). Surprisingly it is very difficult to detect measurable antibody responses to this vaccine (Lunn et al 2001), and nasal IgA responses cannot be detected even after repeated vaccination (Lunn, unpublished data). Nevertheless, it is very probable that the success of this vaccine depends on the establishment of immunological responses at the respiratory mucosal level, although currently these are difficult to measure. Live vector vaccines can also be used for mucosal vaccination, and an attenuated salmonella strain has been used in the horse to generate immune responses to *S. equi* proteins (Sheoran et al 2001).

Immunopathology of the Respiratory Tract

Hypersensitivity and inflammatory disorders

While much of the foreign material that the equine tonsil meets requires a vigorous immunological response, there is an equal or greater number of antigens that require

tolerance if disease is to be avoided. The mechanisms by which tolerance is induced are complex (Weiner 1997), but the consequences of failure may be most clearly illustrated in pharyngeal lymphoid hyperplasia and recurrent airway obstruction (RAO).

Pharyngeal lymphoid hyperplasia

Pharyngitis in horses, also called lymphoid follicular hyperplasia, has been extensively reviewed (Pascoe 1996) and is discussed elsewhere in this text. The condition involves both the nasopharyngeal tonsil, and the extensive lymphoid tissue in the roof and walls of the pharynx. The condition occurs from 2 to 3 months of age, reaching its highest prevalence in 2-year-old horses in training and then declining considerably by 5 years of age. The pathology and epidemiology of equine pharyngeal lymphoid hyperplasia is very similar to that of tonsillitis and adenoiditis in humans (Richardson 1999). In both species both the etiology and the appropriate treatments are uncertain. Inflammation of the respiratory tract in young horses is common, and the role of nasopharyngeal lymphoid tissue in the etiopathogenesis is unknown. Although pharyngeal lymphoid hyperplasia and inflammatory airway disease (IAD) are associated with stabling and exposure to organic dust, no direct link has been demonstrated between the two (Holcombe 2000). Inflammation of the equine tonsil may have additional ramifications in that it is likely involved in lymphadenopathy of the retropharyngeal lymph nodes, a condition that may play a role in dorsal displacement of the soft palate (Holcombe et al 1999, Holcombe 2000). The identification of M cells in the equine nasopharyngeal tonsil may help explain the common involvement of pharyngeal lymphoid tissue in infectious inflammatory disease. The antigen sampling adaptations of the M cell also make it an attractive site for pathogen involvement, and bacteria and viruses that exploit M-cell transport can rapidly infect the mucosa and spread systemically (Neutra et al 1996). The exuberant inflammatory response that characterizes pharyngeal lymphoid hyperplasia has at least a partial infectious etiology. The age-related improvement in this condition probably reflects a combination of maturation of immune responses and age-related changes in management of the horse.

Recurrent airway obstruction

The horse is commonly affected by two forms of chronic respiratory disease, RAO in middle-aged horses and IAD in young performance horses (Robinson 2001), that are very probably caused at least in part by immunopathological or hypersensitivity responses. Because moldy hay can exacerbate RAO, it has been described as a hypersensitivity reaction to molds such as *Aspergillus fumigatus* and *Faenia rectivirgula*. In RAO-susceptible horses, exposure to hay dust leads to invasion of the lungs and airways by

neutrophils within 4–6 hours and concurrent airway obstruction as a result of bronchospasm, inflammation, and increased mucus viscosity, which principally affects the bronchioles. In contrast, IAD commonly affects young horses in training, and has been associated with bacterial and viral infections, although in many horses no infectious etiology is identified and allergic and environmental factors are implicated. The condition is typically associated with neutrophilic airway inflammation, although in some cases eosinophils or mast cell numbers are increased.

Both RAO and IAD have been extensively studied, and are discussed in detail in Chapters 41 and 42 respectively. The immunological basis of RAO remains poorly elucidated (Marti et al 2003). While IgE levels are increased in bronchoalveolar lavage fluid of RAO-affected horses, consistent with a type-1 hypersensitivity, the immediate onset of airway obstruction typical of a type-1 reaction to allergen exposure is not observed. In addition, intra-dermal tests with various allergen extracts correlate poorly with the clinical diagnosis (Jose-Cunilleras et al 2001). Results of initial studies on the type of immune response associated with RAO have been inconsistent. A number of studies have found cytokine profiles consistent with T-helper 2 responses (Lavoie et al 2001, Beadle et al 2002, Cordeau et al 2004), while another study found T-helper 1 responses were more characteristic of at least chronic RAO (Ainsworth et al 2003). A full understanding of the immunological basis of equine chronic airway diseases requires more investigation, and our lack of understanding of this critically important disease complicates therapeutic decisions.

Immunodeficiency

Foals are born essentially with no IgA, although IgA is passively transferred in colostrum (Wilson et al 2001), and antigen-specific IgA can subsequently be found in nasal secretions (Galan et al 1986). Onset of endogenous production of foal IgA appears to occur in the first weeks of life, and IgA is one of the earliest immunoglobulins to be synthesized by foals, although adult levels are not achieved for several months (Holznagel et al 2003). In terms of cellular responses, there is evidence that alveolar macrophages recovered from bronchoalveolar lavages may be low in number up to 2 weeks of age and have impaired chemotactic function (Liu et al 1987). However, other components of the foal immune system are in place at birth, even if these responses remain immature and antigen-naive.

It has been postulated that foals are susceptible to an age-related immunodeficiency that leads to common respiratory infections (Prescott 1993). Several studies have reported an increased susceptibility to bacterial respiratory tract infection during the first 2–5 months of life (Hoffman et al 1993), and the occurrence of *R. equi* pneu-

monia between 2 and 4 months of age, when maternally derived antibody is waning and cellular immunity has yet to become fully developed, also supports this theory. Possible explanations for increased susceptibility to infection in foals in the first year of life could also include low concentrations of IgA or other immunoglobulin classes (Holznagel et al 2003). While lack of development of adult immunocompetence may be an important factor in juvenile respiratory infection, it is critical to remember that the foal is also antigenically naive, and development of a full complement of immune responses requires exposure through infection or vaccination.

In humans, IgA deficiency is the most common immune defect after AIDS, affecting as many as 1 in 400 people in some societies (Mestecky et al 2003). However, this condition is not reported in the horse. Certainly IgA deficiency occurs in horses but to date it has only been reported in combination with other immunoglobulin class and cellular deficits (Freestone et al 1987, Boy et al 1992, MacLeay et al 1997, Flaminio et al 2000). Acquired equine mucosal immunodeficiency, which may result from immunosuppression caused by leukoproliferative diseases, can result in bacterial or fungal respiratory tract disease (McClure 2000). Exercise may also cause changes in equine immune responses, and may result in an increased susceptibility to influenza virus infection (Folsom et al 2001). In elite human athletes, both transient and chronic respiratory mucosal immunodeficiency is described after exercise (Gleeson & Pyne 2000).

The impact of cellular immunodeficiency on respiratory tract immunity is best understood by considering foals affected by severe combined immunodeficiency (SCID; McClure et al 1993). Passive transfer of antibodies can protect these foals from common bacterial infections, but does not prevent the development of bronchopneumonia, which is often caused by adenovirus which affects two-thirds of all SCID foals (Perryman et al 1978). Other common pulmonary pathogens are *Pneumocystis carinii* and *R. equi*. This respiratory adenoviral infection frequently extends to the gastrointestinal and urogenital systems, and causes pancreatic disease leading to loss of endocrine and exocrine tissue and possibly contributing to the impaired growth and weight loss observed in SCID foals (Perryman 2000).

Concluding Remarks

The respiratory tract of the horse is superbly adapted to athletic function, and is specialized for stressful physiological conditions and for the maximal possible exchange of respiratory gases. The performance extremes of which the horse is capable also maximize the challenges to the respiratory immune system, and any perturbation of respiratory function is immediately manifest as decreased performance. It is perhaps remarkable that

the equine immune system performs so well under these exacting challenges. Nevertheless, in the case of many disease conditions, such as RAO, or viral infections such as EHV-1, our understanding of respiratory immunity remains rudimentary, and the potential for further refinements of our therapeutic and prophylactic treatments is considerable.

REFERENCES

Ainsworth DM, Grunig G, Matychak MB et al 2003 Recurrent airway obstruction (RAO) in horses is characterized by IFN-gamma and IL-8 production in bronchoalveolar lavage cells. Veterinary Immunology and Immunopathology 96: 83–91

Allen GP, Kydd JH, Slater JD et al 1999 Advances in understanding of the pathogenesis, epidemiology, and immunological control of equid herpesvirus abortion. In: Wernery U, Wade JF, Mumford JA, Kaaden O-R (editors) Equine Infectious Diseases VIII. Proceedings of the Eighth International Conference, Dubai 23–26 March 1998. R & W Publications, Newmarket, pp.129–146

Beadle RE, Horohov DW, Gaunt SD 2002 Interleukin-4 and interferon-gamma gene expression in summer pasture-associated obstructive pulmonary disease affected horses. Equine Veterinary Journal 34: 389–394

Bender BS, Small PAJ 1992 Influenza: pathogenesis and host defense. Seminars in Respiratory Infection 7: 38–45

Blunden AS, Gower SM 1999 A histological and immuno-histochemical study of the humoral immune system of the lungs in young Thoroughbred horses. Journal of Comparative Pathology 120: 347–356

Boy MG, Zhang C, Antczak DF et al 1992 Unusual selective immunoglobulin deficiency in an arabian foal. Journal of Veterinary Internal Medicine 6: 201–205

Breathnach CC, Yeargan MR, Sheoran AS et al 2001 The mucosal humoral immune response of the horse to infective challenge and vaccination with equine herpesvirus-1 antigens. Equine Veterinary Journal 33: 651–657

Breathnach CC, Yeargan MR, Timoney JF et al 2006 Detection of equine herpesvirus-specific effector and memory cytotoxic immunity in the equine upper respiratory tract. Veterinary Immunology and Immunopathology (in press)

Cordeau ME, Joubert P, Dewachi O et al 2004 IL-4, IL-5 and IFN-g mRNA expression in pulmonary lymphocytes in equine heaves. Veterinary Immunology and Immunopathology 97: 87–96

Dixon PM, McGorum BC 1997 Clinical immunology of the equine respiratory tract. In: Rantanen NW, Hauser ML (editors) Dubai International Equine Symposium: The Diagnosis and Treatment of Respiratory Disease. Matthew R. Rantanen Design, San Diego, pp.69–87

Flaminio MJ, LaCombe V, Kohn CW et al 2000 Common variable immunodeficiency in a horse. Journal of the American Veterinary Medical Association 221: 1296–1302

Folsom RW, Littlefield-Chabaud MA, French DD et al 2001 Exercise alters the immune response to equine influenza virus and increases susceptibility to infection. Equine Veterinary Journal 33: 664–669

Foss DL, Murtaugh MP 1999 Role of macrophage cytokines in mucosal adjuvanticity. Advances in Veterinary Medicine: Veterinary Vaccines and Diagnostics 41: 83–104

Freestone JF, Hietala S, Moulton J et al 1987 Acquired immunodeficiency in a seven-year-old horse. Journal of the American Veterinary Medical Association 190: 689–691

Galan JE, Timoney JF, Lengeman FW 1986 Passive transfer of mucosal antibody to Streptococcus equi in the foal. Infection and Immunity 54: 202–206

Giguere S, Prescott JF 2000 Equine immunity to bacteria. Veterinary Clinics of North America; Equine Practice 16: 29–47

Giguere S, Wilkie BN, Prescott JF 1999 Modulation of cytokine response of pneumonic foals by virulent Rhodococcus equi. Infection and Immunity 67: 5041–5047

Gleeson M, Pyne DB 2000 Special feature for the Olympics: effects of exercise on the immune system: exercise effects on mucosal immunity. Immunology and Cell Biology 78: 536–544

Hannant D, Mumford JA, Jessett DM 1988 Duration of circulating antibody and immunity following infection with equine influenza virus. Veterinary Record 122: 125–128

Hannant D, Jessett DM, O'Neill T et al 1989 Antibody isotype responses in the serum and respiratory tract to primary and secondary infections with equine influenza virus (H3N8). Veterinary Microbiology 19: 293–303

Hannant D, Easeman R, Mumford JA 1999 Equine mucosal immune system: intranasal vaccination with inactivated equine influenza virus protects from infection, In: Wernery U, Wade JF, Mumford JA, Kaaden O-R. (editors) Equine Infectious Diseases VIII. Proceedings of the Eighth International Conference, Dubai 23–26 March 1998. R & W Publications, Newmarket, pp.50–56

Hines SA, Stone DM, Hines MT et al 2003 Clearance of virulent but not avirulent Rhodococcus equi from the lungs of adult horses is associated with intracytoplasmic gamma interferon production by CD4+ and CD8+ T lymphocytes. Clinical and Diagnostic Laboratory Immunology 10: 208–215

Hoffman AM, Viel L, Prescott JF 1993 Microbiologic changes during antimicrobial treatment and rate of relapse of distal respiratory tract infections in foals. American Journal of Veterinary Research 54: 1608–1614

Holcombe SJ 2000 Stabling, airway inflammation, and dorsal displacement of the soft palate in young horses. In: Proceedings of the 46th annual convention of the American Association of Equine Practitioners, San Antonio, TX, pp.254–255

Holcombe SJ, Derksen FJ, Stick JA et al 1999 Pathophysiology of dorsal displacement of the soft palate in horses. Equine Veterinary Journal Supplement 30: 45–48

Holznagel DL, Hussey S, Mihalyi J et al 2003 Onset of immunoglobulin production in foals. Equine Veterinary Journal 35: 620–622

Jose-Cunilleras E, Kohn CW, Hillier A et al 2001 Intradermal testing in healthy horses and horses with chronic obstructive pulmonary disease, recurrent urticaria, or allergic dermatitis. Journal of the American Veterinary Medical Association 219: 1115–1121

Klei TR 2000 Equine immunity to parasites. Veterinary Clinics of North America; Equine Practice 16: 69–78

Kumar P, Timoney JF, Sheoran AS 2001 M cells and associated lymphoid tissue of the equine nasopharyngeal tonsil. Equine Veterinary Journal 33: 224–230

Lavoie JP, Maghni K, Desnoyers M et al 2001 Neutrophilic airway inflammation in horses with heaves is characterized by a Th2-type cytokine profile. American Journal of Respiratory and Critical Care Medicine 164: 1410–1413

Liu IKM, Walsh EM, Bernoco M et al 1987 Bronchoalveolar lavage in the newborn foal. Journal of Reproduction and Fertility Supplement 35: 587–592

Lunn DP, Townsend HGG 2000 Equine vaccination. Veterinary Clinics of North America; Equine Practice 16: 199–226

Lunn DP, Horohov DW 2003 Immunology. In: Reed S, Bayly W, Sellon DC (editors) Equine Internal Medicine. WB Saunders, Philadelphia, pp.1–58

Lunn DP, Holmes MA, Antczak DF et al 1998 Report of the Second Equine Leucocyte Antigen Workshop, Squaw Valley, CA, July 1995. Veterinary Immunology and Immunopathology 62: 101–143

Lunn DP, Hussey S, Sebring R et al 2001 Safety, efficacy, and immunogenicity of a modified-live equine influenza virus vaccine in horses after induction of exercise-induced immunosuppression. Journal of American Veterinary Medical Association 218: 900–906

MacLeay JM, Ames TR, Hayden DW et al 1997 Acquired B lymphocyte deficiency and chronic enterocolitis in a 3-year-old quarter horse. Veterinary Immunology and Immunopathology 57: 49–57

Mair TS, Batten EH, Stokes CR et al 1987 The histological features of the equine respiratory tract. Journal of Comparative Pathology 97: 575–586

Mair TS, Batten EH, Stokes CR et al 1988a The distribution of mucosal lymphoid nodules in the equine respiratory tract. Journal of Comparative Pathology 99: 159–168

Mair TS, Stokes CR, Bourne FJ 1988b Immunohistochemical study of the local humoral immune system of the equine respiratory mucosa. Research in Veterinary Science 45: 160–165

Marti E, Horohov DW, Antczak DF et al 2003 Advances in equine immunology: Havemeyer workshop reports from Santa Fe, New Mexico, and Hortobagy, Hungary. Veterinary Immunology and Immunopathology 91: 233–243

Mazanec MB, Kaetzel CS, Lamm ME et al 1992 Intracellular neutralization of virus by immunoglobulin A antibodies. Proceedings of the National Academy of Sciences, USA 89: 6901–6905

Mazanec MB, Nedrud JG, Kaetzel CS et al 1993 A three-tiered view of the role of IgA in mucosal defense. Immunology Today 14: 430–435

McClure JT 2000 Leukoproliferative disorders in horses. Veterinary Clinics of North America; Equine Practice 16: 165–182

McClure JT, Lunn DP, McGuirk SM 1993 Combined immunodeficiency in 3 foals. Equine Veterinary Education 5: 14–18

Mestecky J, Blumberg RS, Kiyona H et al 2003 The mucosal immune system. In: Paul WE (editor) Fundamental Immunology. Lippincott-Raven, Philadelphia, pp.965–1020

Nally JE, Artiushin S, Sheoran AS et al 2000 Induction of mucosal and systemic antibody specific for SeMF3 of *Streptococcus equi* by intranasal vaccination using a sucrose acetate isobutyrate based delivery system. Vaccine 19: 492–497

Nelson KM, Schram BR, McGregor MW et al 1998 Local and systemic isotype-specific antibody responses to equine influenza virus infection versus conventional vaccination. Vaccine 16: 1306–1313

Neutra MR, Pringault E, Kraehenbuhl JP 1996 Antigen sampling across epithelial barriers and induction of mucosal immune responses. Annual Review of Immunology 14: 275–300

O'Neill T, Kydd JH, Allen GP et al 1999 Determination of equid herpesvirus 1-specific, CD8+, cytotoxic T lymphocyte precursor frequencies in ponies. Veterinary Immunology and Immunopathology 70: 43–54

Pascoe JR 1996 Pharyngitis. In: Smith BP (editor) Large Animal Internal Medicine. Mosby, St. Louis, pp.607–609

Perryman LE 2000 Primary immunodeficiencies of horses. Veterinary Clinics of North America; Equine Practice 16: 105–116

Perryman LE, McGuire TC, Crawford TB 1978 Maintenance of foals with combined immunodeficiency: causes and control of secondary infections. American Journal of Veterinary Research 39: 1043–1047

Pilette C, Ouadrhiri Y, Godding V et al 2001 Lung mucosal immunity: immunoglobulin-A revisited. European Respiratory Journal 18: 571–588

Prescott JF 1993 Immunodeficiency and serious pneumonia in foals: the plot thickens. Equine Veterinary Journal 25: 88–89

Prescott JF, Nicholson VM, Patterson MC et al 1997 Use of *Rhodococcus equi* virulence-associated protein for immunization of foals against *R. equi* pneumonia. American Journal of Veterinary Research 58: 356–359

Renegar KB, Small PAJ 1991 Immunoglobulin A mediation of murine nasal anti-influenza virus immunity. Journal of Virology 65: 2146–2148

Richardson MA 1999 Sore throat, tonsillitis, and adenoiditis. Medical Clinics of North America 83: 75–83

Robinson NE 2001 International Workshop on Equine Chronic Airway Disease. Michigan State University 16–18 June 2000. Equine Veterinary Journal 33: 5–19

Sheoran AS, Sponseller BT, Holmes MA et al 1997 Serum and mucosal antibody isotype responses to M-like protein (SeM) of *Streptococcus equi* in convalescent and vaccinated horses. Veterinary Immunology and Immunopathology 59: 239–251

Sheoran AS, Timoney JF, Tinge SA et al 2001 Intranasal immunogenicity of a Delta cya Delta crp-pabA mutant of *Salmonella enterica* serotype typhimurium for the horse. Vaccine 19: 3787–3795

Slater J, Hannant D 2000 Equine immunity to viruses. Veterinary Clinics of North America; Equine Practice 16: 49–68

Soboll G, Horohov DW, Aldridge BM et al 2003a Regional antibody and cellular immune responses to equine influenza virus infection, and particle mediated DNA vaccination. Veterinary Immunology and Immunopathology 94: 47–62

Soboll G, Nelson KM, Leuthner ES et al 2003b Mucosal co-administration of cholera toxin and influenza virus hemagglutinin DNA in ponies generates a local IgA response. Vaccine 21: 3081–3092

Townsend HG, Penner SJ, Watts TC et al 2001 Efficacy of a cold-adapted, intranasal, equine influenza vaccine: challenge trials. Equine Veterinary Journal 33: 637–643

Wagner B 2006 Immunoglobulins and immunoglobulin genes of the horse. Developmental and Comparative Immunology 30: 155–164

Wagner B, Overesch G, Sheoran AS et al 1998 Organization of the equine immunoglobulin heavy chain constant region genes. III. Alignment of c mu, c gamma, c epsilon and c alpha genes. Immunobiology 199: 105–118

Weiner HL 1997 Oral tolerance: immune mechanisms and treatment of autoimmune diseases. Immunology Today 18: 335–343

Wilson WD, Mihalyi JE, Hussey S et al 2001 Passive transfer of maternal immunoglobulin isotype antibodies against tetanus and influenza and their effect on the response of foals to vaccination. Equine Veterinary Journal 33: 644–650

7 Pharmacology and Therapeutics of Pulmonary Medications

Bonnie R Rush and Elizabeth G Davis

A wide range of therapeutic preparations is routinely used successfully in clinical practice for treatment of pulmonary diseases in horses. This is because the lower respiratory tract is readily accessible for diagnostic testing and relatively easy to manipulate pharmacologically. In addition, most veterinary clinicians have a good understanding of the pathophysiology of equine pulmonary diseases, and are able to make an accurate diagnosis in cases of clinical respiratory disease. This chapter discusses the pharmacological agents most commonly used in horses including antimicrobials, immunostimulants, anti-inflammatory agents, and bronchodilators.

Antimicrobial Therapy

The most common organisms associated with pneumonia in horses are opportunistic bacteria originating from the resident microflora of the upper respiratory tract. These bacteria are not capable of primary invasion and require diminished pulmonary defense mechanisms to establish infection. *Streptococcus equi* var. *zooepidemicus* is the most common opportunistic pathogen of the equine lung, although *Actinobacillus equuli*, *Bordetella bronchiseptica*, *Escherichia coli*, *Pasteurella* spp., and *Pseudomonas aeruginosa* are frequently isolated. *Strep. equi* var. *equi*, the causative agent of strangles, is a primary bacterial pathogen of the upper respiratory tract, and is capable of mucosal invasion without predisposing factors. *Rhodococcus equi* is a primary pathogen of the lower respiratory tract of foals less than 5 months of age, which produces pulmonary consolidation and abscessation. *Rhodococcus equi* pneumonia is not found in adult horses with a functional immune system.

Bacterial culture and sensitivity of transtracheal aspirate samples directs appropriate antimicrobial therapy in bacterial pneumonia. However, pending results of *in vitro* testing, antimicrobial therapy is based on severity of clinical signs, knowledge of common pathogens, and results of Gram stain of tracheal secretions (Chaffin & Carter 1993). Because streptococci are the most common isolates, penicillin (22,000 IU/kg) is often a component of treatment for uncomplicated, Gram-positive bacterial pneumonia. If a polymicrobial infection is suspected, gentamicin sulfate (6.6 mg/kg, q 24 h) or enrofloxacin [7.5 mg/kg, intravenous (IV) or orally (PO), q 24 h] can be added to augment penicillin's predominantly Gram-positive spectrum. Gentamicin and enrofloxacin are rarely indicated as the only antibiotic therapy given the limited Gram-positive spectrum, and the regularity of Gram-positive bacterial isolates obtained from horses with pneumonia. Trimethoprim–sulfonamide (15 mg/kg, PO, q 12 h) has the advantages of ease of administration, Gram-positive and Gram-negative spectrum for common opportunistic pathogens, and inexpensive cost. Ceftiofur [5 mg/kg, intramuscularly (IM), q 12 h] has an appropriate antimicrobial spectrum for common opportunistic pathogens, and is often successful as the only therapy for uncomplicated pneumonia. Antimicrobial therapy for mixed infections or extensive pulmonary compromise may require more sophisticated antimicrobial therapy, as directed by *in vitro* sensitivity testing.

Polymicrobial and mixed anaerobic–aerobic infections are common in horses with pleuropneumonia (Chaffin & Carter 1993, Racklyeft & Love 2000). Of horses with pleuropneumonia, 50–90% have more than one organism isolated from transtracheal aspirates (Sweeney et al 1991). Aerobic bacteria are isolated from more than 90% of the cases of pleuropneumonia, and the most common organisms are *Strep. zooepidemicus*, *E. coli*, *Actinobacillus* spp., *Klebsiella* spp., *Enterobacter* spp., *Staphylococcus aureus*, and *Pasteurella* spp. Anaerobic bacteria are isolated from 40–70% of horses with pleuropneumonia, and *Bacteroides* spp., *Clostridium* spp., *Peptostreptococcus* spp., and *Fusobacterium* spp., are the most commonly isolated anaerobic bacteria. Although fetid breath and malodorous pleural fluid are reliable indicators of the presence of an anaerobic infection, the absence of odor does not preclude an anaerobic pulmonary infection. The etiology of pleural infection in horses is usually bacterial, although fungal, *Mycoplasma felis*, and nocardial agents have been isolated from pleural effusion (Chaffin & Carter 1993).

Medical therapy for bacterial pneumonia and pleuropneumonia requires broad-spectrum antimicrobial therapy, anti-inflammatory drugs, and supportive care (Chaffin et al 1994). The combination of penicillin, gentamicin, and metronidazole is often used for initial therapy in horses with pleuropneumonia. *Streptococcus zooepidemicus* isolates are usually sensitive to β-lactam drugs such as penicillin or sodium ampicillin, and an aminoglycoside drug such as

gentamicin sulfate provides Gram-negative spectrum for organisms such as *E. coli*, *Actinobacillus* spp., and *Pasteurella* spp.. Although penicillin is effective against many anaerobic bacterial infections, *Bacteroides* spp. are penicillin resistant as a result of β-lactamase production. Therefore, metronidazole (15 mg/kg, PO, q 8 h) is administered initially to provide more complete anaerobic bacterial coverage until the results of anaerobic culture are negative and clinical signs of anaerobic infection (gas echoes, putrid breath) have resolved. The antimicrobial regimen may require adjustment based on the results of bacterial culture and sensitivity. Alternative antimicrobial agents for successful treatment of pleuropneumonia include enrofloxacin, ceftiofur, amikacin, trimethoprim–sulfadiazine, doxycycline, chloramphenicol, and rifampin. Intravenous antibiotics are preferable in the initial stages of treatment (14–28 days) to ensure adequate drug concentrations. Oral antimicrobial therapy can be instituted as the horse becomes more stable and production of pleural fluid subsides.

Rhodococcus equi

Rhodococcus equi is a Gram-positive, facultative intracellular pathogen that is one of the most important causes of pneumonia in foals between 5 weeks and 4 months of age. Routine evaluation of *R. equi* isolates to identify susceptibility to antimicrobial agents is misleading. *In vitro*, *R. equi* appears sensitive to a wide variety of antimicrobial agents. However, the organism exists intracellularly and within granulomatous masses in the patient; therefore, most antimicrobial agents are ineffective *in vivo*. The combination of erythromycin (25 mg/kg, q 6 h, PO; esters or salts) and rifampin (5–10 mg/kg, PO, q 12 h) has historically been the treatment of choice for *R. equi* infections in foals. These antimicrobials may be bacteriostatic but their activity is synergistic, and the combination has markedly improved survival of foals with *R. equi* pneumonia. The duration of antimicrobial therapy using this combination of medications typically ranges from 4 to 9 weeks. Rifampin is lipid soluble (able to penetrate abscess material) and is concentrated in phagocytic cells. Erythromycin is concentrated in granulocytes and alveolar macrophages; however, its antimicrobial activity is somewhat inhibited by intracellular pH. Adverse reactions are relatively common in foals treated with the erythromycin/rifampin combination (Stratton-Phelps et al 2000). Diarrhea, idiosyncratic hyperthermia, tachypnea, anorexia, bruxism, and salivation can occur as a consequence of erythromycin administration, and antimicrobial resistance of *R. equi* to erythromycin/rifampin has been reported.

Azithromycin is a newer generation macrolide with greater bioavailability than erythromycin, and achieves higher drug concentrations in phagocytic cells and tissues. Azithromycin produces fewer gastrointestinal side effects in human patients. Azithromycin is administered orally (10 mg/kg) once daily until clinical signs stabilize, followed by every other day administration until resolution of disease. The principal advantage of azithromycin–rifampin over erythromycin–rifampin is the convenience of once-per-day dosing.

Clarithromycin is an alternative macrolide with the most favorable minimum inhibitory concentration against *R. equi* isolates obtained from pneumonic foals (90% of isolates are inhibited at 0.12, 0.25, and 1.0 μg/ml; clarithromycin, erythromycin, and azithromycin respectively) (Jacks et al 2003). In foals with *R. equi* pneumonia, the combination of clarithromycin (7.5 mg/kg, PO, q 12 h) and rifampin is superior to erythromycin–rifampin and azithromycin–rifampin, particularly in foals with severe disease (Giguere et al 2004). Foals treated with clarithromycin–rifampin have improved short-term and long-term survival rates and fewer febrile days than foals treated with erythromycin–rifampin and azithromycin–rifampin. Reported adverse effects of clarithromycin–rifampin have been limited to diarrhea in a small number of treated foals.

The survival rate of *R. equi* pneumonia is approximately 70–90% with appropriate therapy. The case fatality rate without therapy (or with inappropriate antimicrobial therapy) is approximately 80%. Cases with dyspnea and severe pulmonary lesions visible on thoracic radiographs carry a poor prognosis; treatment success with this group of foals has improved with the use of clarithromycin. Parameters for successful cessation of therapy include resolution of clinical signs, normalization of fibrinogen concentration, and radiographic or ultrasonographic evidence of resolution of pulmonary consolidation and abscessation.

Life-threatening, antibiotic-induced enterocolitis, as a result of *Clostridium difficile*, has been reported in the dams of nursing foals treated with erythromycin (Baverud 1998). The authors have observed peracute colitis in hospital-housed mares with foals receiving azithromycin and clarithromycin. This is likely the result of the mare's coprophagic behavior that leads to ingestion of sufficient active macrolide to perturb the intestinal flora. The dams of foals being treated for *R. equi* should be closely observed for signs of fever, toxemia, anorexia, depression, and diarrhea during the treatment period.

Bordetella bronchoseptica

Bordetella bronchoseptica pneumonia in foals represents a unique treatment challenge. The organism appears to be overlooked as a significant respiratory pathogen in foals, but is frequently isolated and may be associated with herd outbreaks of pneumonia. *B. bronchoseptica* is a potent β-lactamase producer that may result in bacterial overgrowth as a result of the death of competing, penicillin-susceptible organisms. Foals with *Bordetella* spp.

pneumonia may show an initially favorable response to administration of a β-lactam antibiotic, followed by deterioration in clinical status because of *B. bronchoseptica* overgrowth. In addition, because the organism lives anchored to the epithelial surface of the airway, many foals with *B. bronchoseptica* pneumonia require prolonged antimicrobial administration for complete resolution of disease as a result of extensive epithelial injury by bacterial toxins and inaccessibility of the respiratory epithelium by antimicrobial agents. Aminoglycosides (gentamicin and amikacin) are the antibiotics of choice for treatment of *B. bronchoseptica*; however, erythromycin and tetracycline may also be effective. Administration of gentamicin via nebulization provides direct delivery of antibiotic to the respiratory epithelial surface, improving accessibility of the drug to the organism (McKenzie & Murray 2000, 2004).

Pneumocystis carinii

Pneumocystis carinii is a ubiquitous, unicellular eukaryote that causes opportunistic pneumonia in foals between 6 and 12 weeks of age. Pneumocystis pneumonia is a rarely recognized respiratory disorder, and has been associated with combined immunodeficiency syndrome, corticosteroid administration, CD4^{+} lymphopenia, and *R. equi* infection. Treatment of pneumocystis pneumonia with trimethoprim–sulfamethoxazole (25 mg/kg, PO, q 12 h) has had variable success in foals. In some instances of treatment failure, the disease may have been too advanced to respond to appropriate antimicrobial therapy or death may have resulted from a concurrent infection (i.e. *R. equi*). Human patients are treated with potentiated sulfa antimicrobials or pentamidine isethionate, an antiparasitic drug. Pentamidine is administered intravenously for active cases and via inhalation as prophylaxis for at-risk HIV patients (CD4^{+} cells <200 cells/μl). Dapsone (3 mg/kg, PO, q 24 h), a sulfone antimicrobial, also provides effective prophylaxis for at-risk patients, and may be useful as an adjunctive treatment to traditional administration of trimethoprim–sulfamethoxazole (Clark-Price et al 2004).

Fungal pneumonia

Fungal pneumonia is relatively uncommon in horses and is typically associated with large colon disease with mucosal disruption and profound neutropenia (colitis, colon torsion) (Sweeney & Habecker 1999). Successful treatment is rarely documented, which may reflect rare ante-mortem diagnosis and/or poor response to therapy. Specific antifungal therapy for the treatment of mycotic pneumonia is dependent on the isolate. Amphotericin B is an appropriate therapeutic choice for aspergillosis, but may be cost prohibitive for many horses. Recommended dosage regimens include the following schedule: 0.3, 0.45, and 0.6 mg/kg on days 1, 2, and 3 respectively, followed by every other day administration of 0.6 mg/kg until a cumulative dose of 6.75 mg/kg amphotericin B has been administered. Amphotericin B is mixed in 1 liter of 5% dextrose and administered over 1 h via an intravenous catheter. Side effects include polyuria/polydipsia (fourth week of treatment), intermittent fever (first 2 weeks), and lethargy (after every treatment). Urinalysis and serum biochemical profile should be obtained weekly to detect evidence of renal or hepatic dysfunction. Ketoconazole (30 mg/kg, intragastric, q 12 h) and oral iodides (20 g/450 kg, q 24 h) are less expensive, but in general, are less likely to resolve fungal pneumonia. Successful treatment has been described with ketoconazole initially, followed by aerosolized enilconazole (1.2 mg/kg in saline via ultrasonic nebulization, q 12 h) for long-term treatment in a horse with *Scopulariopsis* spp. pneumonia. The horse responded favorably to ketoconazole therapy, and the follow-up treatment with aerosolized enilconazole was an effective, economic, and safe alternative therapy (Nappert et al 1996). Itraconazole may be an appropriate antifungal therapy for horses, based on the sensitivity pattern of pulmonary isolates. However, bioavailability appears to be poor in the tablet form; the liquid formulation may be sufficient to provide therapeutic efficacy. Alternatively, the oral bioavailability of fluconazole (loading dose 14 mg/kg PO, followed by 5 mg/kg PO) is sufficient to maintain the plasma concentrations above the mean inhibitory concentration reported for fungal pathogens in horses (Latimer et al 2001). Although fluconazole is well absorbed in horses, it is ineffective against many classes of fungal agents, including aspergillosis, that may result in fungal pulmonary disease. A second-generation triazole agent, voriconazole, is superior to amphotericin B for treating invasive aspergillosis in humans. Recent data have demonstrated that voriconazole is well absorbed in horses and should be considered as a therapeutic agent for the effective management of equine patients suffering from fungal pulmonary disease (Clode et al 2006).

Immunostimulant Preparations

In weanlings and yearlings with opportunistic pulmonary infections, clinical signs may fail to resolve completely despite appropriate antimicrobial therapy; alternatively clinical signs may consistently recur upon withdrawal of antimicrobial therapy. Such horses with chronic, unresponsive pulmonary infections may have secondary immunosuppression or may be immunotolerant to the pathogen, and may benefit from immunomodulatory therapy. The indications for immunomodulatory therapy in horses are relatively specific, and these compounds are not intended to treat a broad spectrum of conditions.

Immunostimulant therapy is not recommended for horses with acute infection and/or clinical signs of depression, anorexia, and fever.

The mechanism of action of non-specific immunostimulation is induction of macrophages to produce pro-inflammatory cytokines that drive a T helper type 1 (Th1)-based immune response. Immunostimulant therapy may not be effective in patients with acute, fulminating infections because the immune response is maximally stimulated by the pathogen. Horses with primary immunodeficiency syndromes, such as severe combined immunodeficiency syndrome of Arabian foals, are incapable of responding to immunostimulant therapy. Immunostimulant therapy is indicated in horses with chronic bacterial or viral respiratory infections because of immunosuppression or immunotolerance to the organism. In addition, prophylactic administration of immunostimulant preparations prior to stressful events such as weaning or long-distance transportation may decrease morbidity and mortality associated with acute infection. The benchmark of non-specific immunomodulation is protection against a 50% lethal bacterial challenge in laboratory animals.

Most veterinary immunostimulants are derived from bacterial, viral or plant sources. Bacterial DNA appears to be responsible for the immunostimulatory effects of bacterial extracts (Klinman et al 1996). DNA sequences consisting of alternating unmethylated cytosine and guanine (connected by phosphate) in a repetitive pattern occur in non-coding sequences of bacterial DNA and are termed CpG oligodeoxynucleotides or CpG motifs. These CpG sequences (motifs) are highly expressed in the bacterial genome and are detected by the innate immune system via defense pattern recognition receptors, which trigger a "danger signal", stimulating non-specific immune responses. Repetitive CpG sequences induce a strong Th1-biased innate and acquired immune response in murine, bovine, and human immune cells. Purified CpG oligodeoxynucleotides demonstrate promise as vaccine adjuvant (Sato et al 1996), and may eventually be an important component of antineoplastic and atopic (hyposensitization) therapies (Whitmore et al 2001).

Propionibacterium acnes

The immunostimulant activity of non-viable *Propionibacterium acnes* (formerly known as *Corynebacterium parvum*) has been recognized for more than 30 years, and the first report of its use in horses was published more than a decade ago (Evans et al 1988). The DNA sequence of *P. acnes* contains repetitive CpG sequences, which may be responsible for its immunostimulatory activity. In laboratory animals, administration of *P. acnes* stimulates macrophage function, natural killer cytotoxicity, and cytokine production [interleukin-1, interferon-γ (IFN-γ)],

and provides prophylactic protection against lethal bacterial and viral challenge (Cox 1988). Stimulation of systemic immunity can be documented in laboratory animals for several days after parenteral administration; however, prolonged immunostimulant activity is not demonstrated.

In equine medicine, *P. acnes* (EqStim®, Neogen Inc.) is labeled for treatment of chronic respiratory disease and is recommended for cases that are unresponsive or transiently responsive to conventional antibiotic treatment (Klimczak 1988). In addition, it is recommended for prophylactic administration before stressful events that may impair pulmonary defense mechanisms, including weaning and long-distance transport. *Propionibacterium acnes* is labeled for intravenous administration every 2–3 days for three treatments, and is recommended to be used as an adjunct to antibiotic therapy. Clinical signs of naturally occurring, infectious respiratory disease (cough, fever, nasal discharge) improve within 14 days of treatment in 96% of horses treated with *P. acnes* compared to 35% of horses treated with conventional therapy (Vail et al 1990). Administration of *P. acnes* prior to long distance transport reduces the incidence of infectious respiratory disease during the 7-day period after shipment (Nestved 1996).

Administration of *P. acnes* to healthy, yearling horses using the recommended dosage regimen increases the number of CD4$^+$ lymphocytes, and enhances lymphokine-activated killing activity and non-opsonized phagocytic activity (Flaminio et al 1998). Total white blood cell count, neutrophil count and serum fibrinogen concentrations are not affected by *P. acnes* administration but lymphocyte numbers in bronchoalveolar lavage fluid decrease, probably as a result of the migration of pulmonary lymphocytes into adjacent lymphoid tissues or the resolution of subclinical infection. In addition, circulating mononuclear cell gene expression of IFN-γ and natural killer-lysin (antimicrobial peptide) is increased in healthy, adult horses 1 week after initiation of therapy, consistent with induction of a Th1 response (Davis et al 2003). The duration of increased cytokine/peptide expression is 7 days or less. Fever, anorexia, and lethargy may occur 12–24 h after administration of the first or second injection, presumably as a result of increased interleukin-1 production. Therefore, administration is not recommended immediately before an athletic event. Subsequent injections usually elicit milder reactions.

Mycobacterial preparations

Mycobacterial preparations are potent stimulators of non-specific immunity and act as adjuvants when administered with antigen. The bacillus Calmette–Guérin (BCG) vaccine was developed from a strain of *Mycobacterium bovis* that had been attenuated through serial passage in culture. Live BCG, whole-inactivated BCG, and mycobacterial cell wall

fractions have been used as non-specific immunostimulant agents and vaccine adjuvants for decades. The mechanism of action is macrophage activation and subsequent release of interleukin-1, tumor necrosis factor, and colony-stimulating factors. Whole, inactivated BCG preparations induce tuberculin sensitivity; therefore, deproteinized mycobacterial cell wall products (muramyl dipeptide and lipoarabinomannan) have been developed to prevent induction of tuberculin positivity in treated animals. Purified muramyl dipeptides are the smallest subunits of the mycobacterial cell wall that retain immunostimulant activity.

Mycobacterial cell wall products are used in horses to treat infectious respiratory disease (Equimune®) and sarcoid skin tumors (Regressin®). Purified mycobacterial cell wall extract is labeled for single-dose, intravenous treatment of equine herpesvirus infection. Administration of purified mycobacterial cell wall extract improves the clinical recovery of horses with respiratory disease resulting from stress, transportation, or bacterial and/or viral infections (Cormack et al 1991). Eighty-three per cent of horses treated with mycobacterial cell wall extract are clinically normal within 7 days after administration of a single, intravenous (1.5 ml) dose, whereas only 36% of horses receiving a placebo injection are without clinical signs.

Adverse pulmonary reactions that are suspected to be the result of multiple intravenous administrations of purified mycobacterial cell wall extract include multifocal granulomatous pneumonitis, bronchiolitis, and progressive pulmonary fibrosis (De Diego et al 1997) accompanied by cough, fever, tachypnea, lethargy, and leukocytosis. Diffuse interstitial infiltration is visible radiographically and cytological examination of bronchoalveolar lavage fluid reveals increased total cell counts and marked lymphocytic inflammation. A marked local reaction (20–40 cm in diameter) is elicited by intradermal injection of mycobacterial cell wall extract to affected horses. A similar adverse pulmonary reaction (interstitial pneumonitis with disseminated pulmonary granulomas) occurs in approximately 1% of human patients after intravesical BCG therapy (Viel & Kenney 1993).

Parapoxvirus ovis

Inactivated, purified parapoxvirus ovis (BaypamuneN™) is a virus-based immunostimulant recommended for prophylaxis, metaphylaxis, and treatment of infectious diseases and prevention of stress-induced diseases in dogs, cats, cattle, pigs, and horses. Metaphylaxis is defined as administration at the time of pathogen exposure. Parapoxvirus ovis is the etiologic agent of contagious ecthyma or "orf" in sheep. The immunostimulant properties of poxvirus were first noted after routine smallpox vaccination (Buttner et al 1991, Mayr et al 1997). Some human vaccine recipients experienced spontaneous tumor regression and resolution of chronic viral and bacterial infections. Poxvirus-mediated immunostimulation is independent of viral replication and the immunostimulating components are located within the viral envelope. In mice, intraperitoneal administration of inactivated parapoxvirus ovis increases natural killer cytotoxicity 10–16 h after treatment. In addition, parapoxvirus administration stimulates macrophage activation and interferon production, and protects mice against experimental lethal viral infection (Buttner et al 1991, Mayr et al 1997). The commercial product has demonstrated efficacy against viral and bacterial diseases in livestock and companion animal species (Buttner et al 1991, Ziebell et al 1997).

In horses, inactivated parapoxvirus ovis has predominately been used for prophylaxis and treatment of viral respiratory disease. The recommended dosage schedule is two to four doses at 48-h intervals, and the immunostimulant activity is suspected to occur within hours of administration. The maximum duration of immunostimulatory activity after parapoxvirus ovis administration is 8 days. Intramuscular administration of inactivated parapoxvirus ovis reduces the severity of respiratory disease caused by equine herpesvirus-1 and -4. Prophylactic administration of inactivated parapoxvirus ovis 4 and 6 days before weaning reduces the incidence of respiratory disease in foals (7.9%) compared to placebo-treated foals (24%) (Bottcher 1994). Administration of the product after weaning (three doses) reduces the clinical signs of viral respiratory disease in foals subjected to long distance transport and commingling (natural viral exposure) (Ziebell et al 1997). The incidence of disease in newborn foals is reduced by administration of parapoxvirus ovis immediately after birth and at 24 or 48 h of life (Bottcher 1994). Unlike its efficacy with equine respiratory disease, intralesional administration of inactivated parapoxvirus ovis is no more effective for treatment of sarcoid skin tumors than intralesional placebo (Studer et al 1997).

Recently, Lunn et al evaluated Baypamune N™ for prophylactic administration in horses subjected to a transport stress, followed by natural challenge with influenza virus (Lunn 2004). Immunostimulant administration did not notably reduce clinical or virological signs of infection in this study; however, antibody responses were significantly higher in treated horses. While clinical disease was not affected in this model, the results indicate the potential value of this immunostimulant therapy for improving immune responses in immunosuppressed horses.

Interferon-α

Interferon-α is an endogenous immunostimulant with antiviral, immunomodulatory, and antiproliferative activity. Endogenous interferon production is induced by viral

infection, and is an early, non-specific antiviral defense mechanism. Interferon-α induces an antiviral state in target host cells by stimulating production of more than 20 enzyme systems that inhibit viral protein synthesis and degrade viral RNA. In mice, administration of IFN-α stimulates peripheral T lymphocytes to produce IFN-γ and activate the Th1 cell response, promoting natural killer cell cytotoxicity, macrophage activation, and cytokine production.

Oral administration of IFN-α reduces pulmonary inflammation in racehorses with chronic inflammatory airway disease (IAD) (Rush Moore et al 1996). Low-dose (50–150 IU, PO, q 24 h for 5 days) natural, human IFN-α (Interferon Sciences, NJ) reduces exudate in the respiratory tract, lowers total cell counts in bronchoalveolar lavage fluid, and converts the differential cell count to a non-inflammatory cytologic profile. Interferon-α does not improve the clinical signs of disease or bronchoalveolar lavage cytology in horses with IAD characterized by eosinophilic or mast cell inflammation. The etiology of IAD is unknown and the mechanism of therapeutic benefit of IFN-α is undetermined. Low-dose, oral interferon is capable of modulating the immune system in humans; however, little to no antiviral or antiproliferative activity is observed at this dose.

The effects of orally administered IFN-α do not require small intestinal absorption or peripheral circulation of IFN-α. Oral administration appears to activate unique natural defense systems originating in oropharynx-associated lymphoid tissue that involves cellular communication and amplification of the biologic response (Bocci 1991). Lymphocytes exposed to IFN-α can transfer enhanced biologic effects to naive lymphocytes by a process that requires direct cell-to-cell contact, does not involve a soluble mediator, and does not require the continued presence of IFN-α. Cellular transfer of the antiviral state to naive cells permits low to undetectable concentrations of IFN-α to produce potent activity, and possibly represents a major mechanism for amplification and dissemination of endogenous IFN-α activity.

Interferon administration is not beneficial in the treatment of acute, fulminant viral respiratory infection in horses (Seahorn et al 1990). Oral administration of low-dose (0.22–2.2 IU/kg, q 24 h) recombinant IFN-α2a does not diminish the severity of clinical disease or the duration of virus shedding in horses with experimental equine herpesvirus-1 infection. Treatment failure in this model is attributed to the lack of antiviral activity at this dose and the overwhelming nature of the viral infection.

Treatment failure in human patients can occur after prolonged administration because of the production of anti-IFN-α antibody or down-regulation of IFN-α receptors. The conformational structure of recombinant IFN-α is more likely to induce neutralizing antibody production than natural IFN. The appearance of neutralizing antibodies to recombinant IFN-α correlates with treatment failure in human cancer patients (Antonelli et al 1991), and anti-IFN-α antibody has been identified in calves following treatment with the recombinant product (Roney et al 1985).

Miscellaneous Immunomodulatory Preparations

Acemannan is a water-soluble, polydispersed beta-linked mannan polymer extracted from the pulp of the *Aloe vera* plant. It is predominantly used for antineoplastic activity, and is labeled for treatment of fibrosarcomas in dogs and cats. The product is administered intralesionally and intraperitoneally, and a combination of these routes of administration is recommended for treatment of fibrosarcoma.

Acemannan stimulates macrophage activation and release of interferon, interleukin-1, tumor necrosis factor, and prostaglandin E$_2$ (MacEwen & Helfand 1993). In addition, acemannan enhances macrophage phagocytosis, T-lymphocyte activity, and non-specific cytotoxicity (Kahlon et al 1991). It is an effective adjuvant, directly inhibits *in vitro* viral replication, and has synergistic antiviral activity *in vivo* with azidothymidine and aciclovir.

Acemannan has been recommended for extralabel use in the treatment of equine respiratory disease and sarcoid skin tumor (Van Kampen 1997). The combination of direct antiviral activity and immunostimulant activity may be particularly beneficial in horses with respiratory disease. Serious adverse effects have been reported after intravenous administration including hypotension, tachycardia, and syncope (Van Kampen 1997). Adverse effects associated with intravenous administration are attributed to the large molecular weight of acemannan, because marked hypotension has been observed after rapid intravenous administration of other large molecular weight compounds. Approximately 30% of horses develop a transient reaction after intralesional administration including tachypnea, coughing, and sweating (Van Kampen 1997).

Levamisole phosphate is a synthetic anthelmintic labeled for treatment of nematode infection in cattle. In addition to its anthelmintic effects, it is reported to restore or stimulate host defenses impaired by aging, stress or immaturity of the immune system. It appears to have little or no effect on humoral or cell-mediated immunity in healthy animals. The effects of levamisole are more apparent in immunocompromised individuals, because it stimulates depressed cell-mediated immunity and neutrophil mobility, phosphodiesterase activity, adherence, and chemotaxis (Saperstein et al 1983).

In humans, levamisole enhances lymphoproliferative responses in postoperative patients and reduces viremia in patients with chronic hepatitis B infection (Abdalla et al 1995, Krastev et al 1999). Levamisole improves cell-mediated immune responses and lymphocyte cytotoxicity

in children suffering from severe protein-calorie malnutrition and chronic respiratory infection, and is reported to be an effective adjunct treatment for chronic bronchitis (Prakash et al 1998). Controlled investigation of the immunostimulatory effects of levamisole in healthy or immunocompromised horses has not been reported. However, a favorable clinical response has been reported in horses with heaves.

Extract of **Echinacea angustifolia** is marketed over the counter as an immunostimulant nutriceutical for humans. There is some evidence that *Echinacea* extract is a hematinic and immunostimulant in the horse after 42 days of oral administration (O'Neill et al 2002).

Anti-inflammatory Therapy for Non-infectious Pulmonary Disease

Indications for anti-inflammatory therapy for pulmonary disease in horses include recurrent airway obstruction (RAO, heaves) and some forms of IAD. Corticosteroid preparations improve pulmonary function and reduce pulmonary inflammation in horses with heaves. Because aerosolized (surface-active) corticosteroids are poorly distributed in the lungs of horses with diffuse severe airway obstruction, systemic corticosteroids are indicated in horses with marked respiratory difficulty. In heaves-affected horses in remission or with moderate to mild clinical signs, aerosolized corticosteroids are indicated for maintenance therapy or as a first-line treatment. Aerosolized corticosteroids are no more effective than systemic corticosteroids but they do reduce the total therapeutic dose and limit drug exposure to other tissues, which provides a higher level of safety. Systemic corticosteroids have the advantages of ease of administration and inexpensive cost. In horses with IAD, corticosteroid administration is indicated in cases with eosinophilic and mast cell inflammation, identified via cytologic evaluation of bronchoalveolar lavage.

Systemic corticosteroids

The therapeutic benefit of systemic corticosteroids begins within hours of administration but may not be detected clinically for 24–72 h in heaves-affected horses (Cornelisse et al 2004). Increasing the dose of corticosteroid during the response period does not provide additional improvement in therapeutic efficacy in human asthmatics or heaves-affected horses. Rather, the addition of a bronchodilator optimizes control of clinical signs during that critical stage of treatment. The therapeutic effects of steroids are not dose-dependent beyond the recommended dosage range; however, the magnitude of adrenal suppression and adverse effects are dose-dependent. For these reasons, conservative dosing regimens are recommended.

Triamcinolone is one of the most potent systemic corticosteroid preparations. Triamcinolone acetonide (0.09 mg/kg, IM, single dose) relieves airway obstruction for up to 4 weeks, however, adrenal suppression is also evident for 4 weeks following administration (LaPointe et al 1993). Repeated administration of triamcinolone has produced iatrogenic Cushingoid syndrome, adrenal insufficiency, and laminitis. Because of the risk of adverse side effects, administration of triamcinolone for the treatment of heaves is limited to salvage efforts.

Dexamethasone improves lung function within hours of administration and the maximal response is obtained by day 7 (Rush et al 1998a, Robinson et al 2003, Cornelisse et al 2004). Intravenous administration (0.1 mg/kg body weight) produces measurable therapeutic benefit within 2 h, whereas oral administration (0.164 mg/kg body weight) improves pulmonary function within 6 h. Following cessation of 1 week of treatment, some therapeutic benefit can be detected 7 days later. Administration of dexamethasone produces marked suppression of endogenous cortisol production (but not adrenal responsiveness), which persists for a few days after discontinuation of drug (Rush et al 1998b). A long-acting intramuscular form of dexamethasone (dexamethasone 21-isonicotinate; Voren®, Boehringer-Ingelheim; 0.04 mg/kg, q 3 days) reduces airway obstruction by day 3 and maximal effect is achieved by day 7 (Robinson et al 2002). The dosage and frequency of administration of potent corticosteroid preparations should be reduced gradually to doses sufficient to maintain disease remission.

Prednisolone (0.5–1 mg/kg q 24 h PO) is widely used in Europe as an anti-inflammatory agent in horses affected by heaves, summer pasture-associated obstructive pulmonary disease (SPAOPD), IAD and eosinophilic pulmonary diseases (Mair 1996, Durham 2001). It is used primarily to aid resolution of pulmonary inflammation, in conjunction with managemental changes. At a dose rate of 1 mg/kg q 24 h it may only partially attenuate signs of airway obstruction in horses with heaves, but adverse effects such as laminitis appear to be uncommon.

Oral prednisone (1.0–2.2 mg/kg, PO, q 24 h) is frequently used as an anti-inflammatory agent in horses because of its ease of administration, minimal cost, and perceived safety relative to laminitis. Despite widespread use, there are no supportive pharmacokinetic or clinical response data to support its use (Traub-Dargatz et al 1992, Jackson et al 2000, Peroni et al 2002, Robinson et al 2002). Clinical improvement in heaves-affected horses treated with oral prednisone has not been documented using objective parameters (Traub-Dargatz et al 1992, Robinson et al 2002). Pretreatment of heaves-susceptible horses with prednisone fails to prevent the onset of airway obstruction when horses are stabled in an allergen-challenged environment, and coupling prednisone with environmental management provides no additional benefit
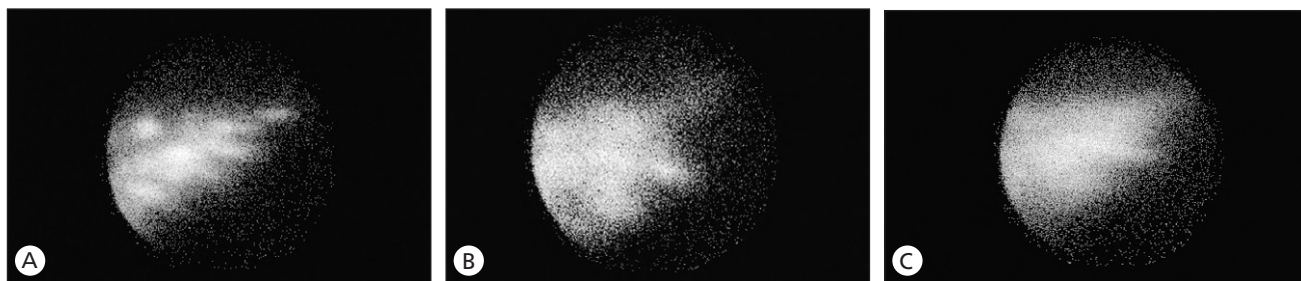
Fig. 7.1. A series of ventilation scans of the caudodorsal lung fields of a heaves-affected horse obtained during an episode of airway obstruction before treatment (A), after a 7-day treatment period with beclomethasone 500 μg, q 12 h (B), and after a 14-day treatment period with beclomethasone 500 μg, q 12 h (C). Prior to treatment (A) the maximal change in pleural pressure was 35 cmH$_2$O (normal <10 cmH$_2$O) and the ventilation scan revealed a patchy distribution of radioaerosol in the peripheral lung with excessive deposition in the central airways. After a 7-day treatment period (B), the maximal change in pleural pressure is nearly normal (12 cmH$_2$O) and the horse appears clinically normal; however, the ventilation scan demonstrates persistent areas of poor ventilation. After 14 days of treatment with beclomethasone (C), the maximal change in pleural pressure is normal (8 cmH$_2$O) and the ventilation scan demonstrates uniform distribution of radionuclide.

in pulmonary function over environmental management alone (Jackson et al 2000). The lack of efficacy of prednisone likely results from its poor oral bioavailability; serum prednisolone (active metabolite) concentrations are minimum to undetectable after administration of oral prednisone (Peroni et al 2002).

Aerosolized corticosteroids

Inhaled corticosteroids are effective in horses with mild to moderate airway obstruction with clinical signs ranging from exercise intolerance to horses with moderate respiratory difficulty. Aerosolized drugs reduce the total therapeutic dose and allow direct delivery of the drug to the lower respiratory tract, but are generally more expensive. There are three aerosolized corticosteroid preparations available in metered-dose inhaler formulation for administration to horses using aerosol delivery devices (see below): beclomethasone dipropionate, fluticasone propionate, and flunisolide. The relative potency of these surface-active corticosteroids is triamcinolone = flunisolide < beclomethasone < fluticasone. Using the affinity of dexamethasone as the standard (equal to 1), the relative glucocorticoid receptor affinity of common corticosteroids is flunisolide 1.9, triamcinolone 2.0, beclomethasone 13.5, and fluticasone propionate 18.0 (Barnes et al 1998).

Fluticasone propionate is an androstane, carbothioate corticosteroid, and is the most potent, most lipophilic, and most expensive aerosolized corticosteroid preparation. Fluticasone also has the longest pulmonary residence time (lipophilicity), and is not metabolized by the lung. It is absorbed into systemic circulation and undergoes extensive first-pass hepatic metabolism (99%) to inactive metabolites. As a result of this extensive first-pass metabolism and its low oral bioavailability (<2%), fluticasone propionate has the most favorable therapeutic index. The terminal half-life of fluticasone propionate after inhalation therapy is approximately 6 h.

Fluticasone (2000 μg, q 12 h, administered by means of the Equine AeroMask™) is very effective in the treatment of heaves. Administration of fluticasone to horses during an episode of airway obstruction resolves clinical signs, reduces pulmonary neutrophilia, normalizes pulmonary function, reduces interleukin-4 expression, and reduces responsiveness to histamine challenge (Viel 1999, Giguere et al 2002). In normal horses, (inhaled) fluticasone propionate reduces serum cortisol concentrations by 40% after 1 day of therapy and 65% after 7 days. Serum cortisol concentrations return to pretreatment values within 1–2 days after discontinuation of drug.

Beclomethasone dipropionate is the most widely dispensed aerosolized corticosteroid for treatment of non-infectious respiratory disease in humans. In heaves-affected horses, beclomethasone reduces pulmonary inflammation, improves parameters of pulmonary function, and improves pulmonary ventilation imaging (Rush et al 1998a; Fig. 7.1). There is no immediate therapeutic effect; clinical signs do not begin to improve until 24 h after administration (Rush et al 2000). Horses may appear clinically normal at rest after a 7-day treatment period with beclomethasone; however, ventilation scanning reveals that regions of poor pulmonary ventilation persist beyond the resolution of clinical signs and may require a 14-day treatment period (Fig. 7.1). Administration of beclomethasone (3750 μg, q 12 h) using the Equine AeroMask™, improves parameters of pulmonary function and arterial oxygen tension for a 2-week treatment period (Ammann et al 1998). Clinical signs of airway obstruction and decreased pulmonary function return within days after discontinuation of beclomethasone. Short-term administration of inhaled

beclomethasone without minimizing environmental allergen exposure is not expected to provide prolonged anti-inflammatory benefit for horses with RAO.

Endogenous cortisol production is suppressed by approximately 35–50% of baseline values within 24 h of administration of high-dose inhaled beclomethasone (>1000 µg, q 12 h), and further reduces by 80–90% after a 7-day treatment period. Serum cortisol concentrations recover within days after discontinuation of beclomethasone, and adrenocorticotropic hormone responsiveness is not affected by a 7-day treatment period (Rush et al 1998b). The threshold for adrenal suppression in normal and heaves-affected horses is approximately 500 µg of inhaled beclomethasone administered twice daily (Ammann et al 1998). The clinical response to this minimally adreno-suppressive dose of beclomethasone is equivalent to the efficacy of doses in excess of 1000 µg twice daily (Rush et al 2000).

Flunisolide is the least potent of the synthetic, topically active corticosteroids. The primary advantage of flunisolide is cost. It is the least lipophilic (shortest pulmonary residence time), has relatively high oral bioavailability (21%), and is extensively absorbed from the respiratory tract (Barnes et al 1998). Flunisolide is similar to triamcinolone in terms of potency, lipophilicity, and clinical efficacy. Much higher dosages are required to achieve therapeutic effects similar to fluticasone or beclomethasone, and adverse effects (adrenal suppression) occur more frequently in human patients with flunisolide. Despite its limitations, the therapeutic index of flunisolide is superior to systemically administered corticosteroids. In human patients with steroid-dependent asthma initiation of flunisolide therapy as a replacement of oral prednisone allows adrenal recovery and superior asthma control. The safety and efficacy of aerosolized flunisolide has not been evaluated in heaves-affected horses.

Horses in apparent remission from heaves may improve exercise tolerance and performance by being maintained on low-dose, long-term, aerosolized corticosteroids. The systemic effects of injectable corticosteroids preclude their use for long-term, daily administration for maintenance therapy. In human asthmatics, the dose timing of low-dose maintenance therapy has a pivotal effect on the safety profile and consequently the risk/benefit ratio of inhaled corticosteroids (Barnes et al 1998). Maximum adrenal suppression occurs with administration of aerosolized corticosteroids in the early morning hours, whereas endogenous cortisol production is least disrupted by afternoon administration. The longer the terminal elimination half-life of the drug, the earlier in the afternoon it should be administered. The adrenosuppressive effects of flunisolide ($t_{1/2}$ = 1.5 h) are minimized when a single daily dose is administered at 19.00 (7 P.M.), whereas the optimum time for administration of fluticasone ($t_{1/2}$ = 6 h) is 16.00 (4 P.M.). The safety and efficacy of once daily, long-term aerosolized corticosteroids have not been objectively investigated in horses with heaves or IAD; however, clinical use of these drugs in this manner is widespread.

Mast cell stabilizers (cromones)

The mast cell-stabilizing drugs, sodium cromoglycate and nedocromil sodium, have a delayed onset of action and are indicated for the prophylaxis of airway obstruction. Their structures are dissimilar and their mechanism of action is unclear. These drugs are not recommended for the stabilization of clinical signs during an episode of heaves. In human asthmatics, aerosolized corticosteroids provide superior asthma control compared to aerosolized cromones, and in fact, mast cell stabilizers do not reduce pulmonary inflammation in patients with asthma. Nonetheless, the addition of nedocromil sodium to high-dose corticosteroid therapy improves asthma control in selected patients (Barnes et al 1998).

Mast-cell-stabilizing agents provide some benefit as maintenance therapy for heaves-affected horses during periods of remission. The administration of disodium cromoglycate (80 mg via nebulization) 20–30 min prior to allergen challenge delays the induction of the clinical signs of airway obstruction (Thomson & McPherson 1981). Administration of 80 mg for 4 consecutive days (once daily) delayed the induction of airway obstruction for approximately 3 weeks. Another study demonstrated a minimal benefit with doses up to 500 mg daily for 2 days prior to allergen exposure (Soma et al 1987). It is important to recognize that the role of mast cells in the pathophysiology of RAO in horses is presently unclear and, for that reason, cromones should not be considered an alternative to corticosteroid therapy in horses with heaves.

Mast cell stabilizers may be indicated for the treatment of a subset of horses with IAD demonstrating evidence of metachromatic (mast cells are 2–5% of total cell count) or eosinophilic (>10% of total cell count) inflammation in bronchoalveolar lavage fluid. This form of chronic IAD may represent a local pulmonary hypersensitivity reaction, and arguably may represent an early form of RAO. Nebulization of sodium cromoglycate (80–200 µg) will improve the clinical signs of respiratory disease and will stabilize mast cell histamine release in young racehorses with mast cell pulmonary inflammation (Hare et al 1994).

Leukotriene receptor antagonists

Leukotriene receptor antagonists (montelukast, zafirlukast) were designed to provide oral, once daily, anti-inflammatory therapy for the treatment of asthma. These preparations are effective for approximately 70% of human asthmatics, and are effective for most cats with asthma.

Montelukast (0.11 mg/kg body weight, PO, q 24 h), a cysteinyl leukotriene receptor antagonist, is ineffective for the treatment of heaves in horses. The lack of efficacy may be the result of its poor bioavailability, as the active metabolite is nearly undetectable (1/28th of therapeutic drug concentrations) after oral administration (Kolm et al 2003). In contrast, the leukotriene D_4 receptor antagonist, L-708,738 (2.5 mg/kg PO, q 12 h), is bioavailable after oral dosing and sustains plasma drug concentrations that exceed *in vitro* efficacy values. However, airway function does not improve after 14 days of administration, suggesting that cysteinyl leukotrienes may not be pivotal mediators of airway inflammation in heaves (Lavoie et al 2002).

Bronchodilator Therapy

Medical treatment for horses with heaves should consist of a combination of bronchodilators and corticosteroids. Corticosteroids reduce pulmonary inflammation and bronchodilators provide symptomatic relief of airway obstruction. Corticosteroid therapy does not relieve clinical signs of airway obstruction for several hours; therefore, bronchodilator therapy is indicated to provide immediate relief of airway obstruction until clinical signs of disease abate. Unlike aerosolized corticosteroids, aerosolized bronchodilators typically remain effective regardless of the severity of disease. Aerosolized bronchodilator therapy is more rapid and powerful than oral administration of bronchodilators, and is the preferred route of administration for horses during crises. It is inappropriate to treat heaves with bronchodilators as the sole therapy; bronchodilators do not reduce the underlying inflammatory process and tolerance develops rapidly to β_2-adrenergic agents (12 days) when administered as sole therapy to horses with heaves. Corticosteroid therapy prevents and/or reverts tolerance to β_2-adrenergic drugs by preventing down-regulation of β_2 receptors and inducing formation of new β_2 receptors on pulmonary cells (Abraham et al 2002).

Anticholinergic bronchodilators

The parasympathetic division is the dominant portion of the pulmonary autonomic nervous system in all mammals. Muscarinic receptors are abundant in airway smooth muscle and stimulation of M_3 receptors results in smooth muscle contraction and bronchoconstriction. Vagally mediated cholinergic stimulation (M_3) is the primary mechanism of bronchospasm in horses with RAO. Parasympathetic innervation can be demonstrated throughout the tracheobronchial tree of the horse, but smooth muscle contraction evoked by stimulation of cholinergic nerves is more pronounced in the trachea than in the smaller bronchi; as expected, parasympathetic blockade of the M_3 receptor with a muscarinic antagonist has the greatest effect in large, central airways.

Atropine is the prototypic, non-selective (M_1, M_2, M_3) muscarinic antagonist, derived from the *Atropa belladonna* plant. By blocking the M_3 receptor it decreases the release of intracellular calcium from the sarcoplasmic reticulum and thereby causes smooth muscle relaxation. Atropine (5–7 mg/450-kg horse, IV) provides rapid and powerful bronchodilation in horses with heaves; however, the abbreviated duration of action (0.5–2.0 h) and adverse effects of systemic administration (ileus, central nervous system toxicity, tachycardia, increased viscosity of mucus secretion, impaired mucociliary clearance) limit its use to a single rescue dose for horses with severe airway obstruction. Atropine is not suitable for routine administration in horses with RAO.

Ipratropium bromide is a synthetic, anticholinergic compound that produces bronchodilation, inhibits cough and protects against stimuli that cause bronchoconstriction. Like atropine, ipratropium bromide is a non-selective muscarinic antagonist. As a result of its quaternary ammonium structure, absorption of ipratropium from the respiratory and gastrointestinal tract is negligible. Consequently, ipratropium produces minimal systemic effects, and does not inhibit gastrointestinal motility, dry respiratory secretions, or affect mucociliary clearance.

Ipratropium bromide can be administered to horses via an ultrasonic nebulizer (2–3 μg/kg), dry powder inhaler (200 μg/100 kg or 2.4 mg/horse), or metered dose inhaler coupled to the Equine AeroMask or Equinehaler (180–360 μg/500 kg horse) (Robinson et al 1993, Hoffman 1997, Duvivier et al 1999). The onset of bronchodilation in heaves-affected horses is approximately 15–30 min and the effect lasts approximately 4–6 h. Administration of ipratropium (and atropine) produces a more significant improvement in pulmonary resistance than in dynamic compliance. This finding is consistent with the bronchodilatory effect of ipratropium on larger, more central airways. Administration of ipratropium before exercise in horses with RAO does not improve exercise performance, which may reflect the bronchodilation normally associated with the sympathetic drive of exercise.

Oxitropium bromide and tiotropium bromide are quaternary scopolamine-derivative anticholinergic agents with a prolonged duration of effect (>12 h) in human patients. Oxitropium is ten times more potent than atropine and has minimal absorption from the respiratory and gastrointestinal tract. Tiotropium is a non-selective muscarinic antagonist; however, it dissociates slowly from M_3 receptors, which is the mechanism for the prolonged (12–24 h) duration of activity. Tiotropium is approximately ten-fold more potent than ipratropium. These newer generation anticholinergic agents may prove attractive for treatment of heaves if the duration of action proves similar to that in humans.
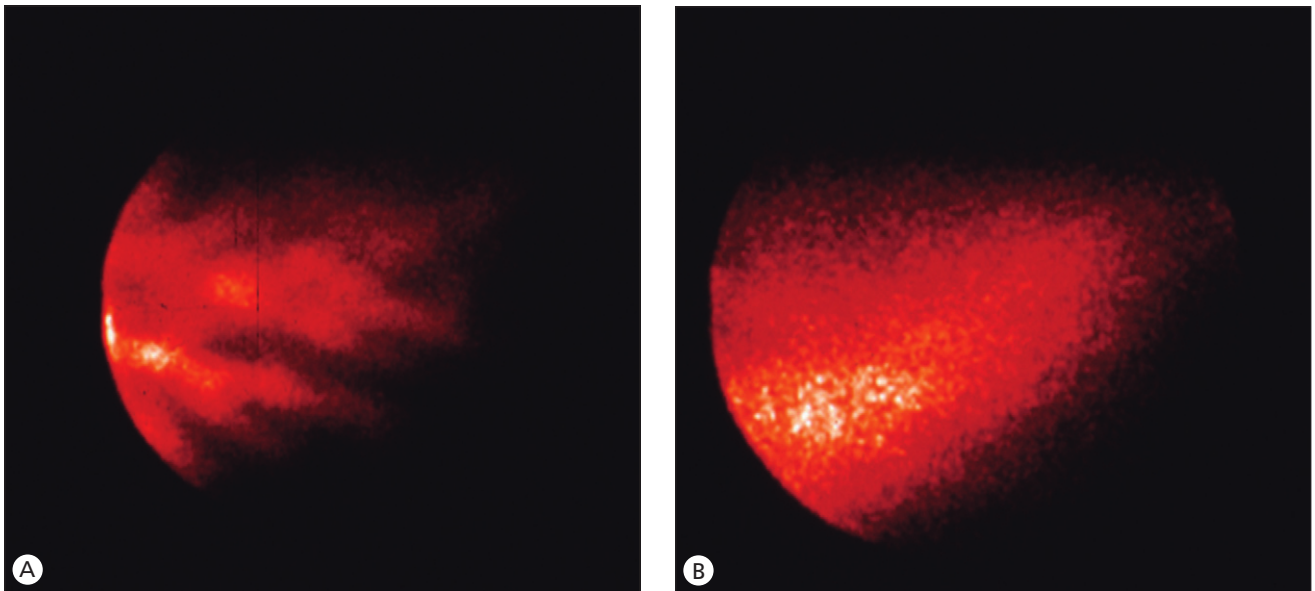
Fig. 7.2. Ventilation scan of the caudodorsal lung fields of a heaves-affected horse. (A) The restricted distribution of aerosolized radionuclide during a crisis and (B) the same pulmonary region of this horse with normal (improved) distribution after administration of 360 μg albuterol via an Equine Aerosol Delivery Device. Reproduced from Rush et al 1999 with permission.

β_2-Adrenergic agonists

Sympathetic innervation of the respiratory tract is sparse; however, non-innervated β_2-adrenergic receptors are distributed throughout the equine pulmonary system associated with respiratory smooth muscle, epithelium and submucosal glands. The β-adrenergic agonists stimulate non-innervated β_2-adrenergic receptors that are coupled to G_s proteins, activation of which increases intracellular cyclic adenosine monophosphate (cAMP), resulting in relaxation of bronchial smooth muscle by a variety of mechanisms. Non-selective β-agonists are not used for bronchodilation in clinical practice because of adverse effects of β_1 stimulation (tachycardia, sweating and excitement).

Short-acting β_2-agonists

There are three basic indications for administration of short-acting β_2-adrenergic bronchodilators for horses: (1) emergency therapy in horses with marked airway obstruction, (2) before exercise to relieve mild to moderate airway obstruction, (3) before administration of aerosol corticosteroid preparations to improve pulmonary distribution of these surface-active agents. Figure 7.2 is a ventilation scan of the caudodorsal lung fields of a heaves-affected horse, demonstrating the distribution of aerosolized radionuclide before and after administration of albuterol.

Albuterol sulfate (360–900 μg) and pirbuterol acetate (600 μg) are potent, short-acting β_2-adrenergic agents that rapidly produce bronchodilation (5 min) in horses (Derksen et al 1992, 1996, 1999, Tesarowski et al 1994, Rush et al 1999). The duration of effective bronchodilation is approximately 1–3 h. Albuterol and pirbuterol improve parameters of pulmonary function by approximately 70% in horses with airway obstruction. Aerosolized short-acting β_2-adrenergic bronchodilators produce minimal systemic effects and are safe for use in horses. Albuterol can be repeatedly dosed, every 15 min for up to 2 h, to relieve severe bronchoconstriction in horses with heaves through sequential bronchodilation. Systemic administration of β_2-adrenergic agents requires ten times the dose to achieve the same level of bronchodilation and is associated with sweating, trembling, muscle tremors, and excitement.

Most albuterol sulfate preparations contain an equimolar, racemic mixture of (R)- and (S)-stereoisomers. (R)-Albuterol has both bronchodilating and bronchoprotective activities (Derksen et al 1992). (S)-Albuterol does not activate β_2 receptors and does not modify the activation of β_2 receptors by (R)-albuterol. (S)-Albuterol is metabolized more slowly than (R)-albuterol and is retained preferentially in the airways. Until recently, (S)-albuterol was considered biologically inert; however, it has now been demonstrated to intensify allergic bronchospasm and appears to have the potential to induce paradoxical reactions in some asthmatic patients. We have observed two heaves-affected horses that appear to bronchoconstrict after administration of albuterol. In horses, (S)-albuterol does not have bronchodilatory activity but does stimulate acetylcholine release via activation of a prejunctional β_2 receptor (Zhang et al 1998). Levalbuterol (homochiral

(R)-albuterol) has been marketed recently and reportedly dramatically reduces the incidence of the side effects associated with racemic albuterol in some patients.

Long-acting β_2-agonists

Salmeterol xinafoate is a long-acting β_2-adrenergic agent that is a chemical analog of albuterol. The receptor-binding site of salmeterol is structurally similar to that of albuterol, although salmeterol has an elongated (aliphatic) side chain thought to bind to an exosite proximal to the region of the β_2-adrenoceptor protein. Exosite binding allows salmeterol to contact the β_2 receptor repeatedly while the drug remains anchored adjacent to the receptor site, allowing for an extended duration of action. In addition, salmeterol has higher lipophilicity, β_2 affinity, β_2 selectivity and potency (ten-fold) than albuterol. Lipophilicity may be the most important determinant of duration of action by influencing the amount of drug entering the cell membrane in the vicinity of the β_2 receptor. The β_2/β_1 activity ratio of salmeterol is $50,000 : 1$ compared with $650 : 1$ for albuterol; isoproterenol is the standard $(1 : 1)$ for the β receptor activity ratio. Enhanced β_2 selectivity provides a greater margin of safety by reducing the frequency of β_1 effects (anxiety, tachycardia, and sweating) at therapeutic doses. In humans, twice daily administration of salmeterol provides superior control of bronchoconstriction compared to regular (four times daily) administration or as-needed administration of albuterol. In addition to bronchodilator activity, salmeterol appears to have some anti-inflammatory properties, such as inhibition of leukotriene and histamine release from mast cells and the reduction of eosinophil activity. Salmeterol (210 µg via Equine AeroMask) provides relief of the clinical signs of airway obstruction for 8–12 h in heaves-affected horses (Henrikson & Rush 2001). Salmeterol has been recommended for maintenance therapy and pre-exercise administration to horses with mild to moderate airway obstruction (Hoffman 1997). Unfortunately, production of salmeterol in a metered dose inhaler formulation has been discontinued; the most popular current formulation for salmeterol is a dry-powder inhalant device for human asthmatics (paired with fluticasone), which has been difficult to adapt for inhalant delivery for horses.

The combination of albuterol sulfate and ipratropium bromide is available commercially in a metered-dose inhaler device for humans. In human patients with chronic obstructive pulmonary disease this anticholinergic/β_2-agonist combination provides more complete bronchodilation than either drug alone. Albuterol predominantly relaxes small (peripheral) airway smooth muscle whereas the ipratropium has a greater effect on the relaxation of larger (more central) airways. Combination anticholinergic/β_2-agonist therapy provides broad-spectrum relief of bronchoconstriction. Short-acting β_2-agonists provide rapid relief from bronchoconstriction whereas the response to ipratropium is delayed. Conversely, the relief provided by ipratropium will last longer (4–6 h) than the short-acting bronchodilator activity of albuterol.

Clenbuterol hydrochloride (0.8–3.2 µg/kg, PO, q 12 h; Ventipulmin®, Boehringer Ingelheim Vetmedica) is the systemic alternative to the aerosolized, long-acting β_2-agonist bronchodilators for management of horses with mild to moderate disease. The $\beta_2 : \beta_1$ selectivity of clenbuterol is similar to that of albuterol, and the oral bioavailability is approximately 80–90% in humans. In addition to bronchodilator activity, clenbuterol is a mucokinetic agent which acts through increased ciliary beat frequency to speed mucociliary clearance. Controlled investigations to document the bronchodilator response to clenbuterol in horses with heaves have produced inconsistent results (Traub-Dargatz et al 1992, Erichsen et al 1994, Sasse 1984). The disparity in results is likely related to differing dosage schedules, routes of administration, and disease severity. In general, clenbuterol administration produces moderate improvement in objective parameters of pulmonary function, which may be difficult to appreciate or detect by clinical observation. The label recommendations outline an incremental dosage schedule based on the response to therapy. In a large clinical trial using incremental dosing and subjective evaluation of airway obstruction, 25% of horses responded favorably to the lowest recommended dose (0.8 µg/kg, PO, q 24 h) (Erichsen et al 1994), whereas the remaining 75% of horses required higher doses to obtain observable therapeutic benefit. Recent *in vivo* and *in vitro* studies (Laan et al 2004, 2005) indicate that clenbuterol was effective at inhibiting pro-inflammatory cytokine production by equine alveolar macrophages following exposure to lipopolysaccharide, hay dust suspension and *Aspergillus fumigatus* extract. While this anti-inflammatory effect may be of clinical use when treating horses with airway diseases such as heaves, SPAOPD and IAD, clenbuterol should only be used in conjunction with improvements in air hygiene.

The equine industry has been concerned about the abuse potential of clenbuterol among performance horse populations because of the repartitioning properties (it favors formation of muscle over deposition of fat) of this β_2-agonist in some species. Depending on discipline and jurisdiction, identification of clenbuterol in post-event samples can lead to sanctions. Consequently, there has been a great deal of interest in the pharmacokinetics, pharmacodynamics, and limits of detection of clenbuterol in horses. Chronic administration of clenbuterol at the higher doses (2.4 µg/kg, PO, q 12 h) does demonstrate repartitioning effects in horses (Kearns et al 2001) but does not enhance exercise performance. In fact, clenbuterol administration diminishes (rather than enhances) aerobic capacity (Beekley et al 2003) and performance (Kearns &

McKeever 2002), and negatively alters cardiac function in horses (Sleeper et al 2002). Regardless of these negative findings relative to performance, detection of clenbuterol remains a priority in most drug testing situations. Clenbuterol accumulates in liver, lung, heart, and kidney relative to plasma concentrations, which contributes to prolonged and inconsistent elimination (Soma et al 2004). Nonetheless, current technology is able to quantify clenbuterol in plasma and urine for a prolonged period of time (up to 30 days) after discontinuation of drug administration. Serum samples are more consistent and reliable for detection compared to urine samples. Surprisingly, it is possible to detect clenbuterol in mane and tail hairs for 360 days after a 10-day administration period (Schlupp et al 2004).

Although higher doses of clenbuterol may be required to produce notable clinical effects in heaves-affected horses, adverse effects may also be observed. The most common adverse effects reflect β_1 stimulation and include sweating, trembling, tachycardia, and excitement. Clenbuterol should not be administered to near-term gestational mares because β_2 stimulation relaxes uterine smooth muscle, which may delay the onset of labor. In fact, intravenous clenbuterol has been used to delay parturition (primarily in cattle) and facilitate uterine repair.

Terbutaline was previously considered to be an acceptable systemic bronchodilator in horses; however, its bioavailability is negligible in horses, and clinical efficacy has not been demonstrated (Torneke et al 2000). Aminophylline and theophylline (phosphodiesterase inhibitors) are alternative systemic bronchodilators for horses with airway obstruction, although the therapeutic index is relatively narrow and the magnitude of bronchodilation is less than that of the β_2-agonists. The phosphodiesterase inhibitors delay fatigue of the muscles of respiration, and may be valuable in horses with severe airway obstruction with impending respiratory failure due to fatigue.

There is growing concern in human medicine that the overuse of the β_2-agonists for asthma is associated with increased morbidity and even mortality. Regular use of β_2-agonists as monotherapy is associated with deterioration in asthma control, increased non-specific and allergen-induced airway responsiveness, increased eosinophilic pulmonary infiltration and progressive deterioration in lung function. Poor control of asthma symptoms triggers a cycle of intensifying need for symptomatic relief and more frequent administration of β_2-agonists. The mechanism of β_2 tolerance (loss of bronchodilator response) and deterioration in pulmonary function appears to be down-regulation or desensitization of β_2 receptors. Down-regulation of β_2-agonists occurs via receptor phosphory-lation, internalization, and destruction. Failure to respond to β_2 stimulation has been documented in asthmatic human patients after 3 weeks of regular (four times daily)

administration of albuterol as monotherapy. Down-regulation of β_2 receptors has been documented in horses after 12 days of administration of clenbuterol (0.8 µg/kg, IV, q 12 h) (Abraham et al 2002). Administration of corticosteroids (dexamethasone 0.1 mg/kg IV) accelerates recovery from down-regulation in horses treated with clenbuterol alone and prevents clenbuterol-induced desensitization when administered concurrently. Given that β_2-agonists have little anti-inflammatory activity, it is difficult to justify their use as monotherapy for a disease characterized by airway inflammation.

Aerosol Delivery Devices

Several devices have been designed for convenient administration of aerosolized drugs in a metered-dose inhaler (MDI) preparation to horses. The advantages of an MDI system include rapid administration, consistent ex-valve dose delivery, minimal risk of pulmonary contamination with environmental microorganisms, ease of cleaning/maintaining equipment, and no requirement for electricity.

The Equine AeroMask™ (Canadian Monaghan, Ontario, Canada) is used for administration of aerosolized drugs via hand-held MDI devices or nebulization solution. This system allows the clinician to administer any medication available for human asthma therapy to horses with heaves. The MDI is actuated into a spacer device with a one-way inspiratory valve. The mask must fit snugly around the muzzle to ensure adequate negative inspiratory pressure to facilitate drug delivery. Drug delivery to the lower respiratory tract using the Equine AeroMask™ with an MDI is approximately 14% of actuated drug when using an hydrofluoroalkane (HFA) propellant. Inhalant medication is uniformly distributed throughout all pulmonary fields.

The Equine Haler™ (Equine Healthcare APS, Hillerod, Denmark) is a spacer device that fits over the entire left nare of the horse, and is designed for administration of aerosolized drug using any hand-held MDI device. The mean particle size generated using the Equine Haler™ is 2.1 µm with a range of 1.1–4.7 µm [fluticasone/chloro-fluorocarbon (CFC)-free propellant]. Drug deposition in the lower respiratory tract is approximately 8% of the actuated dose with diffuse pulmonary drug delivery that is adequately distributed to the periphery of the lung. Unlike the AeroMask™, the Equine Haler™ can accommodate any size of horse without concerns for ensuring an airtight seal with the mask. Poor pulmonary drug delivery can occur if the administrator does not pay particular attention to aligning the MDI with the spacer and the spacer apparatus with the nasal passages of the horse during actuation. Pulmonary drug delivery can be improved by closing the opposite nostril during administration.

Miscellaneous Pulmonary Therapeutic Agents

Expectorant therapy

Expectorant therapy is intended to increase the volume and decrease the viscosity of bronchial secretions so that they can be more easily cleared by the mucociliary system and by coughing. It is discussed in Chapter 5.

Antitussive agents

Cough is an important pulmonary defense mechanism assisting the clearance of secretions and debris from the lower respiratory tract. Cough suppressants are indicated for patients with persistent, fatiguing, non-productive cough. Opiate agonists (dextromethorphan, hydrocodone, butorphanol) are potent antitussive agents. The mechanism of action is direct suppression of the cough reflex at the cough center of the medulla. Antitussive agents are generally used for inflammatory, non-infectious tracheal and pulmonary diseases and during invasive diagnostic techniques such as endoscopic examination and bronchoalveolar lavage. Opiate agonists may increase the viscosity of respiratory secretions, and produce sedation and constipation.

Respiratory stimulants

Doxapram hydrochloride (Dopram®; 0.3 mg/kg, rapid IV bolus) is a respiratory stimulant that is indicated to promote ventilation in horses with reduced central respiratory drive, such as hypoxic–ischemic encephalopathy, sedation, general anesthesia, and head trauma. In the neonatal foal with a poor ventilatory drive (and limited venous access), doxapram can be administered topically under the tongue. Doxapram stimulates both central respiratory centers and peripheral carotid chemoreceptors (Aguilera-Tejero et al 1997). The net response is chemically induced hyperventilation, characterized by an increase in tidal volume and respiratory frequency within 1 min of administration (Wernette et al 1986). The duration of hyperpnea is less than 5 min. In addition to the respiratory stimulant effects, doxapram increases cardiac output and blood pressure and these effects are more prolonged (120 min) (Wernette et al 1986, Sams et al 1992). Continuous or repeated dosing may allow steady-state conditions, prolonging the duration of clinical effects (Sams et al 1992).

The respiratory stimulant lobeline (0.20 mg/kg, rapid IV bolus) is used in clinical practice in Europe to markedly increase inspiratory and expiratory airflow rates as an aid during auscultation (Marlin et al 2000). In addition, lobeline is used as a research tool to facilitate investigation of upper and lower airway function. The respira-
tory stimulant activity of this plant-derived alkaloid (from *Lobelia inflate*) has been recognized for more than 200 years, and has been used in Europe for more than 100 years to aid in auscultation and assessment of upper airway obstruction. The mechanism of hyperpnea is stimulation of peripheral chemoreceptors located in the carotid and aortic bodies. The duration of respiratory stimulation after rapid intravenous bolus administration is approximately 90–160 seconds (Marlin et al 2000). The response is dose-dependent, highly reproducible, and without apparent adverse effects. Respiratory alkalosis (pH 7.77) and hypocapnia ($P_a\text{CO}_2$ 20 mmHg) occur transiently, followed by a period of apnea (40–55 seconds) after hyperpnea subsides.

REFERENCES

Abdalla EE, Adam IJ, Blair GE et al 1995 The immuno-modulatory effect of levamisole is influenced the postoperative changes and type of lymphocyte stimulant. Cancer Immunology Immunotherapy 41: 193–198

Abraham G, Brodde OE, Ungemach FR 2002 Regulation of equine lymphocyte beta-adrenoceptors under the influence of clenbuterol and dexamethasone. Equine Veterinary Journal 34: 587–593

Aguilera-Tejero E, Pascoe JR, Smith BL et al 1997 The effect of doxapram-induced hyperventilation on respiratory mechanics in horses. Research in Veterinary Science 62: 143–146

Ammann V, Vrins A, Lavoie J 1998 Effects of inhaled beclomethasone dipropionate on respiratory function in horses with chronic obstructive pulmonary disease (COPD). Equine Veterinary Journal 30: 152–157

Antonelli G, Currenti M, Turriziani O et al 1991 Neutralizing antibodies to interferon-alpha: relative frequency in patients treated with different interferon preparations. Journal of Infectious Disease 163: 882–885

Barnes P, Pedersen S, Busse W 1998 Efficacy and safety of inhaled corticosteroids. American Journal of Respiratory and Critical Care Medicine 157: 1–53

Baverud V, Franklin A, Gunnarsson A et al 1998 *Clostridium difficile* associated with acute colitis in mares when their foals are treated with erythromycin and rifampin for *Rhodococcus equi* pneumonia. Equine Veterinary Journal 30: 482–488

Beekley MD, Ideus JM, Brechue WF et al 2003 Chronic clenbuterol administration alters myosin heavy chain composition in standardbred mares. Equine Veterinary Journal 165: 234–239

Bocci V 1991 Absorption of cytokines via oropharyngeal-associated lymphoid tissues. Clinical Pharmacokinetics 21: 411–417

Bottcher E 1994 Clinical trials and field experience with parapoxvirus-based immunostimulants in companion animals. In: Immunomodulator Symposium of the World Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Disease. Perugia, Italy 28–33

Buttner M, Czerny CP, Mayr A 1991 A role of poxvirus structural proteins in the induction of peritoneal NK cell activity. Natural Immunity and Cell Growth Regulation 10: 161–166

Chaffin MK, Carter GK 1993 Equine bacterial pleuro-pneumonia Part 1 Epidemiology, pathophysiology, and bacterial isolates. Compendium on Continuing Education for the Practicing Veterinarian 15: 1642–1650

Chaffin MK, Carter GK, Byars TD 1994 Equine bacterial pleuropneumonia Part III Treatment, sequelae, and prognosis. Compendium on Continuing Education for the Practicing Veterinarian 16: 1585–1595

Clark-Price SC, Cox JH, Bartoe JT et al 2004 Use of dapsone in the treatment of *Pneumocystis carinii* pneumonia in a foal. Journal of the American Veterinary Medical Association 224: 407–410

Clode AB, Davis JL, Salmon J et al 2006 Evaluation of concentration of voriconazole in aqueous humor after topical and oral administration in horses. Journal of the American Journal of Veterinary Research 67: 296–301

Cormack SS, Alkemade AJ, Rogan D 1991 Clinical study evaluating a purified mycobacterial cell wall extract for the treatment of equine respiratory disease. Equine Practice 13: 18–22

Cornelisse CJ, Robinson NE, Berney CE et al 2004 Efficacy of oral and intravenous dexamethasone in horses with recurrent airway obstruction. Equine Veterinary Journal 36: 426–430

Cox WI 1988 Examining the immunologic and hematopoietic properties of an immunostimulant. Veterinary Medicine 6: 424–428

Davis EG, Rush BR, Blecha F 2003 Increases in cytokine and antimicrobial peptide gene expression in horses by immunomodulation with *Propionibacterium acnes*. Veterinary Therapy 4: 5–11

De Diego A, Rogado MC, Prieto M et al 1997 Disseminated pulmonary granulomas after intravesical Bacillus Calmette–Guérin immunotherapy. Respiration 64: 304–306

Derksen F, Olszewski M, Robinson N 1999 Aerosolized albuterol sulfate used as a bronchodilator in horses with recurrent airway obstruction. American Journal of Veterinary Research 60: 689–693

Derksen F, Olszewski M, Robinson N 1996 Use of a hand-held, metered-dose aerosol delivery device to administer pirbuterol acetate to horses with heaves. Equine Veterinary Journal 28: 306–310

Derksen F, Robinson N, Berney C 1992 Aerosol pirbuterol: bronchodilator activity and side effects in ponies with recurrent airway obstruction (heaves). Equine Veterinary Journal 24: 107–112

Durham A 2001 Update on therapeutics for obstructive pulmonary diseases in horses. In Practice 23: 474–481

Duvivier D, Bayly W, Votion D et al 1999 Effects of inhaled dry powder ipratropium bromide on recovery from exercise of horses with COPD. Equine Veterinary Journal 31: 20–24

Erichsen DF, Aviad AD, Schultz RH et al 1994 Clinical efficacy and safety of clenbuterol HCl when administered to effect in horses with chronic obstructive pulmonary disease (COPD). Equine Veterinary Journal 26: 331–336

Evans DJ, Rollins JB, Huff GK et al 1988 Inactivated *Propionibacterium acnes* as adjunct to conventional therapy in the treatment of equine respiratory diseases. Equine Practice 10: 17–21

Flaminio MJBF, Rush BR, Shuman W 1998 Immunologic function in horses after non-specific immunostimulant administration. Veterinary Immunology and Immuno-pathology 63: 303–315

Giguere S, Viel L, Lee E, MacKay RJ et al 2002 Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate. Veterinary Immunology and Immunopathology 85: 147–158

Giguere S, Jacks S, Roberts G D et al 2004 Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with *Rhodococcus equi* pneumonia. Journal of Veterinary Internal Medicine 18: 568–573

Hare J, Viel L, O'Byrne P 1994 Effect of sodium cromoglycate on light racehorses with elevated metachromatic cell numbers on bronchoalveolar lavage. Journal of Veterinary Pharmacology and Therapeutics 17: 237–242

Henrikson SL, Rush BR 2001 Efficacy of salmeterol xinafoate in horses with recurrent airway obstruction. American Journal of Veterinary Research 218: 1961–1965

Hoffman A 1997 Inhaled medications and bronchodilator use in the horse. Veterinary Clinics of North America; Equine Practice 13: 519–530

Jacks S, Giguere S, Nguyen A 2003 In vitro susceptibilities of *Rhodococcus equi* and other common equine pathogens to azithromycin, clarithromycin and 20 other anti-microbials. Antimicrobial Agents and Chemotherapy 47: 1742–1745

Jackson CA, Berney C, Jefcoat AM et al 2000 Environment and prednisone interactions in the treatment of recurrent airway obstruction (heaves). Equine Veterinary Journal 32: 432–438

Kahlon JB, Kemp MC, Yawei N 1991 In vitro evaluation of the synergistic antiviral effects of acemannan in combination with azidothymidine and acyclovir. Molecular Biotherapy 3: 314–223

Kearns CF, McKeever KH, Malinowski K 2001 Chronic administration of therapeutic levels of clenbuterol acts as a repartitioning agent. Journal of Applied Physiology 91: 2064–2070

Kearns CF, McKeever KH 2002 Clenbuterol diminishes aerobic performance in horses. Medicine and Science in Sports and Exercise 34: 1976–1985

Klimczak C 1988 Immunostimulant quickly aids weanling ERDC cases. Equine Veterinary Science 12: 68–69

Klinman DM, Yi A-K, Beaucag SL et al 1996 CpG motifs present in bacterial DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon gamma. Immunology 93: 2879–2883

Kolm G, Zappe H, Schmid R et al 2003 Efficacy of montelukast in the treatment of chronic obstructive pulmonary disease in five horses. Veterinary Record 152: 804–806

Krastev Z, Jelev D, Antonov K 1999 Chronic HBV infection. Immunomodulation with levamisole in viremic HBeAg positive or anti-HBe positive patients. Hepatogastro-enterology 46: 3184–3188

Laan TTJM, Bull S, Fink-Gemmels J 2004 Cytokine modulation by beta-2-receptor agonists. American College of Veterinary Internal Medicine. 22nd Annual Forum, Minneapolis, USA June 9–12, 2004

Laan TTJM, Bull S, Pirie S et al 2005 Evaluation of cytokine production by equine alveolar macrophages exposed to lipopolysaccharide, *Aspergillus fumigatus*, and a suspension of hay dust. American Journal of Veterinary Research 66: 1584–1589

LaPointe JM, Lavoie JP, Vrins AA 1993 Effects of triamcinolone acetonide on pulmonary function and bronchoalveolar lavage cytologic features in horses with chronic obstructive pulmonary disease. American Journal of Veterinary Research 54: 1310–1316

Latimer FG, Colitz CM, Campbell NB et al 2001 Pharmaco-kinetics of fluconazole following intravenous and oral administration and body fluid concentrations of flucona-zole following repeated oral dosing in horses. American Journal of Veterinary Research 62: 1606–1611

Lavoie JP, Leguillette R, Pasloske K et al 2002 Comparison of effects of dexamethasone and the leukotriene D4 receptor antagonist L-708,738 on lung function and airway cytologic findings in horses with recurrent airway obstruction. American Journal of Veterinary Research 63: 579–585

Lunn DP, Rush BR 2004 Immunomodulation: Principles and Mechanisms. Proceedings of the 50th Annual Convention of American Association of Equine Practitioners 447–453

MacEwen EG, Helfand SC 1993 Recent advances in the biologic therapy of cancer. Compendium on Continuing Education for the Practicing Veterinarian 15: 909–916

Mair TS 1996 Obstructive pulmonary disease in 18 horses at summer pasture. Veterinary Record 138: 89–91

Marlin DJ, Roberts CA, Schroter RC et al 2000 Respiratory responses of mature horses to intravenous lobeline bolus. Equine Veterinary Journal 32: 200–207

Mayr A, Ahne W, Vilsmeier B 1997 Evaluation of *in vitro* and *ex vivo–in vitro* experiments on the efficacy of inducers of nonspecific immunity prepared from poxviruses. Tierarztlich Umschau 52: 3–11

McKenzie HC 3rd, Murray MJ 2000 Concentrations of gentamicin in serum and bronchial lavage fluid after intravenous and aerosol administration of gentamicin to horses. American Journal of Veterinary Research 61: 1185–1190

McKenzie HC 3rd, Murray MJ 2004 Concentrations of gentamicin in serum and bronchial lavage fluid after once-daily aerosol administration to horses for seven days. American Journal of Veterinary Research 65: 173–178

Nappert G, VanDyck T, Papich M et al 1996 Successful treatment of a fever associated with consistent pulmonary isolation of *Scopulariopsis* sp. in a mare. Equine Veterinary Journal 28: 421–424

Nestved A 1996 Evaluation of an immunostimulant in preventing shipping related respiratory disease. Journal of Equine Veterinary Science 16: 78–82

O'Neill W, McKee S, Clarke AF 2002 Immunological and haematinic consequences of feeding a standardised Echinacea (*Echinacea angustifolia*) extract to healthy horses. Equine Veterinary Journal 34: 222–227

Peroni DL, Stanley S, Kollias-Baker C et al 2002 Prednisone per os is likely to have limited efficacy in horses. Equine Veterinary Journal 34: 283–287

Prakash MS, Rao VM, Reddy V 1998 Effect of levamisole on the immune status of malnourished children. Journal of Tropical Pediatrics 44: 165–166

Racklyeft DJ, Love DN 2000 Bacterial infection of the lower respiratory tract in 34 horses. Australian Veterinary Journal 78: 549–559

Robinson N, Derksen F, Berney C et al 1993 The airway response of horses with recurrent airway obstruction (heaves) to aerosol administration of ipratropium bromide. Equine Veterinary Journal 25: 299–303

Robinson NE, Jackson C, Jefcoat A et al 2002 Efficacy of three corticosteroids for the treatment of heaves. Equine Veterinary Journal 34: 17–22

Robinson NE, Berney C, Eberhart S et al 2003 Coughing, mucus accumulation, airway obstruction, and airway inflammation in control horses and horses affected with recurrent airway obstruction. American Journal of Veterinary Research 64: 550–557

Roney CS, Rossi CR, Smith PC 1985 Effect of human leukocyte A interferon on prevention of infectious bovine rhinotracheitis infection of cattle. American Journal of Veterinary Research 46: 1251–1255

Rush BR, Raub ES, Rhoads WS et al 1998a Pulmonary function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively. American Journal of Veterinary Research 59: 1039–1043

Rush BR, Worster AA, Flaminio MJ et al 1998b Alteration in adrenocortical function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively. American Journal of Veterinary Research 59: 1044–1047

Rush B, Hoskinson J, Davis E et al 1999 Pulmonary distribution of radioaerosol in horses with heaves after inhalation of single-dose albuterol sulfate. American Journal of Veterinary Research 60: 764–769

Rush BR, Raub ES, Thomsen MM et al 2000 Pulmonary function and adrenal gland suppression with incremental doses of aerosolized beclomethasone dipropionate in horses with recurrent airway obstruction. Journal of the American Veterinary Medical Association 217: 359–364

Rush Moore BR, Krakowka S, Cummins JJ et al 1996 Changes in airway inflammatory cell populations in Standardbred racehorses after interferon-alpha administration. Veterinary Immunology and Immunopathology 49: 347–358

Sams RA, Detra RL, Muir WW 3rd 1992 Pharmacokinetics and metabolism of intravenous doxapram in horses. Equine Veterinary Journal Supplement 11: 45–51

Saperstein G, Mohanty SB, Rockemann DD et al 1983 Effect of levamisole on induced bovine viral diarrhea. Journal of the American Veterinary Medical Association 183: 425–426

Sasse HHL 1984 Ein Vergleich des Effektes nach einmaliger parenteraler Gabe von Ventipulmin oder Euphyllin auf die Lungerfunktionsprugung bei an C.O.P.D. erkrankten Pferden. Sasse Tierarztl Umschau 39: 656–662

Sato Y, Roman M, Tighe H et al 1996 Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. Science 273: 352–354

Schlupp A, Anielski P, Thieme D et al 2004 The beta-agonist clenbuterol in mane and tail hair of horses. Equine Veterinary Journal 36: 102–103

Seahorn TL, Carter GK, Marens JG 1990 Effects of human-alpha interferon on experimentally induced equine herpesvirus-1 infection in horses. American Journal of Veterinary Research 51: 2006–2010

Sleeper MM, Kearns CF, McKeever KH 2002 Chronic clenbuterol administration negatively alters cardiac function. Medicine and Science in Sports and Exercise 34: 634–650

Soma L, Beech J, Gerber N 1987 Effects of cromolyn in horses with chronic obstructive pulmonary disease. Veterinary Research Communications 11: 339–344

Soma LR, Uboh CE, Guan F et al 2004 Tissue distribution of clenbuterol in the horse. Journal of Veterinary Pharmacology and Therapeutics 27: 91–98

Stratton-Phelps M, Wilson WD, Gardner IA 2000 Risk of adverse effects in pneumonic foals treated with erythromycin versus other antibiotics: 143 cases (1986–1996). Journal of the American Veterinary Medical Association 217: 68–73

Studer U, Marti E, Stornetta D et al 1997 [The therapy of equine sarcoid with a non-specific immunostimulator: the epidemiology and spontaneous regression of sarcoids]. Schweizer Archiv fur Tierheilkunde 139: 385–391

Sweeney CR, Habecker PL 1999 Pulmonary aspergillosis in horses: 29 cases (1974–1997). Journal of the American Veterinary Medical Association 214: 808–811

Sweeney CR, Holcombe SJ, Barningham SC et al 1991 Aerobic and anaerobic bacterial isolates from horses with pneumonia or pleuropneumonia and antimicrobial susceptibility patterns of the aerobes. Journal of the American Veterinary Medical Association 198: 839–842

Tesarowski D, Viel L, McDonell W 1994 The rapid and effective administration of a beta$_2$-agonist to horses with heaves using a compact inhalation device and metered-dose inhalers. Canadian Veterinary Journal 35: 170–173

Thomson J, McPherson E 1981 Prophylactic effects of sodium cromoglycate on chronic obstructive pulmonary disease in the horse. Equine Veterinary Journal 13: 243–251

Torneke MK, Ingvast-Larsson JC, Johansson JM et al 2000 Pharmacokinetics and pharmacodynamics of terbutaline in healthy horses. American Journal of Veterinary Research 61: 761–765

Traub-Dargatz JL, McKinnon AO, Thrall MA et al 1992 Evaluation of clinical signs of disease, bronchoalveolar and tracheal wash analysis, and arterial blood gas tensions in 13 horses with chronic obstructive pulmonary disease treated with prednisone, methyl sulfonmethane, and clenbuterol hydrochloride. American Journal of Veterinary Research 53: 1908–1916

Vail CD, Nestved AJ, Martins JB et al 1990 Adjunct treatment of equine respiratory disease complex (ERDC) with the *Propionibacterium acnes*, immunostimulant, EqStim. Journal of Equine Veterinary Science 10: 399–403

Van Kampen KR 1997 Immunotherapy and cytokines. Seminars in Veterinary Medicine and Surgery (Small Animal) 12: 186–192

Viel L, Kenney D 1993 Suspected adverse pulmonary reactions to Equimune I.V. in four horses presented to the Ontario Veterinary College. 12th Veterinary Respiratory Symposium Kennett Square, PA, USA

Viel L 1999 Therapeutic efficacy of inhaled fluticasone propionate in horses with chronic obstructive pulmonary disease. Proceedings of the 45th Annual Convention of the American Association of Equine Practitioners 306–307

Wernette KM, Hubbell JA, Muir WW 3rd et al 1986 Doxapram: cardiopulmonary effects in the horse. American Journal of Veterinary Research 47: 1360–1362

Whitmore MM, Li S, Falo L Jr et al 2001 Systemic administration of LPD prepared with CpG oligonucleotides inhibits the growth of established pulmonary metastases by stimulating innate and acquired antitumor immune responses. Cancer Immunology Immunotherapy 50: 503–514

Zhang X, Zhu F, Olszewski M, Robinson N 1998 Effects of enantiomers of beta 2-agonists on ACh release and smooth muscle contraction in the trachea. American Journal of Physiology 274: 32–38

Ziebell KL, Kretzdorn D, Auer S et al 1997 The use of Baypamun N in crowding associated with infectious respiratory disease: efficacy of Baypamun N (freeze dried product) in 4 to 10 month old horses. Journal of Veterinary Medicine B 44: 529–534

Clinical Examination of the Respiratory Tract

Bruce C McGorum and Padraic M Dixon

The widespread availability of ancillary diagnostic aids such as endoscopy and ultrasonography has undoubtedly greatly improved our ability to detect and diagnose disorders of the equine respiratory tract. However, it may have inadvertently led to an over-reliance on these techniques, with many clinicians performing only a cursory clinical examination of the equine respiratory tract. The aim of this chapter is to review the considerable body of valuable diagnostic information that can be obtained simply by performing a detailed clinical examination.

Signalment and History

Signalment and a thorough case history provide essential diagnostic information, because factors such as age, environment, diet, exercise activity, vaccination history, previous and current medications, and transportation history can be important risk factors for particular respiratory diseases.

Clinical Examination of the Upper Respiratory Tract

Nostrils and nasal cavity

The equine muzzle is covered with hair and is very mobile and tactile. The nasal area is normally dry, except for a few drops of serous fluid, which consists mainly of tears. The nares should be assessed for symmetry; asymmetry indicates the presence of disorders such as wry nose, facial paralysis, or injuries. The equine nostrils are semilunar in outline during normal breathing but during deep breathing they dilate and become circular. Horses with dyspnea will fully dilate their nostrils during inspiration, and in horses with severe dyspnea, the nostrils may remain permanently dilated at rest. In contrast, horses with nostril paralysis may have unilateral or bilateral inability to dilate the nostrils and consequently may have asymmetry of the muzzle. The ability of the nostrils to dilate can be assessed by observing the nostril movements during the breath cycle and following temporary occlusion of the nares, which normally induces transient bilateral symmetrical nostril dilatation. Nostril paralysis is of major significance to the equine athlete because the horse is an obligate nasal breather and also because of the great mobility of its nostrils compared to most other domestic species. Identifi-

cation of nostril paralysis should prompt a thorough neurological examination.

The alar fold, which attaches to the nostril and ventral concha, projects laterally into the dorsal part of the nostril and divides the nostril into a dorsal and ventral passage. The dorsal passage leads into the nasal diverticulum (false nostril), whilst the ventral passage leads into the nasal cavity. During dilatation of the true nostril, the lumen of the false nostril is reduced in size. The false nostrils are about 6–8 cm deep and are lined with epithelium, most of which contains dark, hair-covered pigmented skin. Particles of black sebaceous secretions normally lie on their surface. Epidermal cysts are readily palpated as soft, oval-shaped fluctuant swellings on the lateral aspect of the nasal diverticulum.

Because of the mobility of the horse's nostrils it is possible, unlike in other domesticated species, to readily examine a reasonable area of nasal mucosa and septum, the alar folds and the rostral aspect of the nasal conchae. The nasolacrimal orifice is readily identifiable in the ventrolateral aspect of the nostril, at the boundary between the pink nasal mucosa and the pigmented nostril epithelium. The more caudal aspects of the nasal cavities can only be visualized by endoscopy. The nasal mucosa is normally pink in color and moist. With the aid of a light source it is possible to view 5–8 cm of the rostral nasal mucosa in the mature horse, and proportionately less in foals. Inflammation of the nasal mucosa will lead to mucosal edema, erythema, and variable volumes of purulent to mucopurulent exudate. Some lesions of the nasal mucosa are readily visible and characteristic, for example rhinitis sicca in horses with chronic grass sickness (Fig. 8.1). Marked ulceration of the nasal mucosa is characteristic of diseases such as epizootic lymphadenitis in horses.

The volume of air exhaled from the nostrils can be assessed by holding the back of the hand or some teased cotton wool in front of the nostrils. Manually occluding both nostrils for about 30 seconds will temporarily increase the rate and depth of breathing, making it easier to assess airflow. In the normal horse the flow of air from each nostril is approximately equal. A unilateral obstruction in a nasal cavity, such as that caused by a large sinus cyst, will reduce or stop the airflow from the ipsilateral nostril. Variations in the volume of expired air can be further assessed by occluding each nostril individually.

Fig. 8.1. Rhinitis sicca in a horse with grass sickness (dysautonomia). The serum exudation and crusting evident on the nasal mucosa are likely the result of desiccation of the mucosa because of dysfunction of the autonomic control of nasal gland secretion. This often painful complication is considered to be pathognomonic for grass sickness.

Malodorous breath can occur in horses with sinusitis (especially, but not exclusively, dental sinusitis), nasal mycosis, guttural pouch mycosis, and gangrenous pneumonia. Attempts should be made to determine the source of the odor. Malodorous breath is exhaled only from one nostril in horses with lesions rostral to the nasopharynx, while horses with dental, periodontal, or other oral diseases may have malodor originating from the mouth, and not from the nostrils.

Nasal discharges can be characterized as being unilateral or bilateral, continuous or intermittent, scant or profuse or malodorous. The nature of any discharge can further be categorized as serous; mucoid; mucopurulent; serosanguineous; hemorrhagic; and containing food or gastrointestinal contents. Inflammation of upper or lower respiratory tract mucosa characteristically increases mucus production, leading to a mucoid nasal discharge. However, with most equine inflammatory respiratory disorders the increased mucus production is accompanied by transmucosal migration of large numbers of leukocytes, primarily neutrophils. Consequently, the respiratory secretions, whether of upper or lower respiratory tract origin, are commonly mucopurulent. Purulent secretions occur in disorders such as sinusitis and strangles, when respiratory secretions contain so many degenerating leukocytes (predominantly neutrophils) that they are highly viscous. Whilst purulent discharge is traditionally associated with bacterial infection of the respiratory tract, it may also be a feature of other disorders including viral respiratory infections and recurrent airway obstruction (RAO). Purulent secretions can be white, yellow, or green, with the color *sometimes* being dependent on bacterial pigments and metalloenzymes.

Unilateral nasal discharge occurs in disorders of the nasal cavity and paranasal sinuses. While small volumes of discharge from the guttural pouch will cause a predominantly unilateral nasal discharge, large volume hemorrhage from the guttural pouch of a horse with unilateral guttural pouch mycosis will cause bilateral epistaxis. A small proportion of horses with pulmonary disease inexplicably have unilateral or predominantly unilateral nasal discharge (Dixon et al 1995). Most horses with a unilateral purulent nasal discharge have an underlying disorder, such as sinusitis caused by dental apical infection, compromised sinus drainage or the presence of inspissated pus within a sinus, with secondary bacterial infection. Consequently, antibiotic therapy based on culture and sensitivity testing of nasal swabs is rarely curative, and often simply delays the establishment of a specific diagnosis and institution of appropriate treatment, which usually involves surgery.

Lesions of the respiratory tract caudal to the nasal septum typically result in bilateral nasal discharge. Bilateral nasal discharge is usually indicative of pulmonary disease, with the respiratory secretions being transported up to the nasopharynx by the mucociliary escalator, or by gravity when the head is lowered below the level of the thoracic trachea, and also by coughing. Once transported to the nasopharynx these secretions are usually swallowed, but if large volumes flow into the nasopharynx (e.g. after lowering of the head) the discharge may exit both nostrils. In horses with concurrent upper and lower respiratory tract inflammation, such as those with viral respiratory infections, nasal discharge may originate from both the upper and lower respiratory tract.

Pharyngeal or esophageal dysphagia may lead to nasal discharge that contains food. This food and saliva will flow down both nasal cavities, especially when the head is lowered, usually within a minute or so after the ingestion of food or liquids.

Epistaxis

Epistaxis denotes hemorrhage from the nose, but does not indicate the origin of the hemorrhage. Hemoptysis (coughing up blood of pulmonary origin through the oral or nasal cavity) is usually associated with disease of the lower respiratory tract in other species but is a rare finding in horses. With exercise-induced pulmonary hemorrhage, epistaxis of pulmonary origin usually occurs without coughing. Unilateral epistaxis suggests a lesion rostral to the nasopharynx, while bilateral epistaxis indicates a lesion caudal to the nasal cavities.

In some cases of epistaxis, the origin of the blood may be obvious, but often other diagnostic procedures are required to detect the cause. Endoscopy of the nasal cavities, nasopharynx, larynx, trachea, and large bronchi is often necessary and usually diagnostic. Radiography of the head may be necessary in some cases to detect lesions of the sinuses (fractures and progressive ethmoidal hematoma), which may be the source of hemorrhage.

Submandibular lymph node

The submandibular lymph node groups (which in the horse comprises 70–150 small nodes) are usually not palpable or are barely palpable in healthy horses. Even when these lymph nodes are enlarged, they are often not readily detected unless the dorsomedial aspect of the more caudal aspects of the mandible is specifically examined for their presence. Occasionally these lymph nodes are swollen with neoplasia of the head, mainly as a result of local inflammation rather than by metastases. The presence of ipsilateral submandibular lymphadenopathy along with unilateral nasal discharge is confirmatory evidence of a unilateral upper respiratory tract disorder. Bilateral submandibular lymphadenopathy is occasionally the result of bilateral upper respiratory disorders, such as bilateral sinusitis or guttural pouch disorders, but is most commonly a response to generalized respiratory infections, such as equine influenza, herpesviruses 1 and 4, or strangles.

Respiratory sounds

In a normal resting horse, breathing is virtually inaudible. However, during fast exercise, the increased (>60-fold) airflow causes the respiratory sounds to become audible at a considerable distance from the horse. Normally, exercise-related expiratory sounds are almost twice as loud as inspiratory sounds. A common, loud expiratory sound in horses is caused by a snorting-like vibration of the nasal structures, including the *true* nostrils, and is termed "false nostril flutter" or "high blowing". These normal sounds often occur at the beginning of exercise, and can also occur in aggressive horses at rest. Very unfit horses may have increased levels of normal breath sounds, or coarse low-pitched noises, during fast work, and horses making such noises are referred to as being "thick winded". These noises normally disappear when the animal becomes fitter.

Horses with dynamic upper airflow obstructions, such as laryngeal paralysis or dorsal displacement of the soft palate, have normal breath sounds at rest when airflow is low, but make loud abnormal respiratory sounds during strenuous exercise, because of airflow turbulence at sites of dynamic airway obstruction. These exercise-induced sounds may be audible at more than 50 m from the exercising horse.

It is not always possible to exercise a horse to listen for exercise-induced abnormal noises, because of the lack of an exercise area or treadmill, inclement weather, or inter-current lameness. In such circumstances the importance of obtaining an accurate history cannot be overemphasized. Alternatively, the owner can make a video or audio recording of the horse making the noise at exercise. After prolonged and fast exercise, such as racing, even normal, fit horses will exhibit post-exercise dyspnea for 5–15 min, when they will continue to breathe rapidly and deeply and make loud but normal breath sounds. Prolonged post-exercise dyspnea is indicative of respiratory dysfunction.

When assessing horses with suspected upper airway obstruction, it is important to determine whether abnormal noises made during fast exercise occur during inspiration or expiration. This is readily achieved by assessing when the noises occur in relation to the horse's gait, because at the canter and gallop, locomotion and breathing are synchronized. Expiration occurs when the forefeet hit the ground, while inspiration occurs when the forefeet are being elevated.

Examination of the paranasal sinuses

Diseases of the paranasal sinuses often cause unilateral nasal discharge, submandibular lymph node enlargement, and epiphora. The thick lateral maxillary wall is not usually distorted in primary and dental sinusitis, unless gross sinus distension and bone softening occur. In contrast, the thin medial walls are readily distorted towards the nasal septum, leading to unilateral nasal obstruction.

Percussion of a sinus may be of value in assessing its contents. An exudate-filled sinus may lose the resonance of a normal air-filled sinus. However, a diseased sinus is not necessarily filled with exudate and, additionally, affected horses usually resent percussion of an inflamed and presumably painful sinus. In general, percussion is unreliable unless advanced sinus disease is present. It is more useful to place a hand over the maxillary sinuses on both sides and assess the maxillary bones for pain, swelling, increased heat and softening of the bones. With low-grade sinusitis all clinical examinations are unreliable. Therefore, if sinusitis is suspected the diagnosis should be confirmed by radiography, endoscopy, or sinoscopy (Chapters 10, 18 and 26).

Nasopharynx

The oropharynx is the part of the pharynx between the soft palate, the tongue, and the epiglottis. The nasopharynx lies medial to the vertical ramus of the mandible and the parotid salivary gland, while the laryngopharynx is rostral to the larynx. The omohyoid muscles, the cricothyroid ligament, and the hyoid apparatus fully cover the ventral aspect of the oropharynx. Because of the presence of these overlying structures, little information can be gained from external palpation of the pharynx. Swelling of the parotid region may indicate distension of the underlying guttural pouch, inflammation of the overlying parotid salivary gland, lymphadenitis of the retropharyngeal lymph nodes (e.g. strangles infection), or evidence of neoplasia (usually melanoma) of this region.

With use of sedation and a full mouth speculum, pharyngeal palpation may be performed *per os* in larger horses, depending on the relative sizes of the horse's oral

cavity and pharynx and the examiner's hand and arm. The base of the tongue, the large midline glossoepiglottic fold, the oropharyngeal walls and floor, the caudoventral aspect of the soft palate and the base of the epiglottis are palpable when the soft palate and epiglottis are in the normal breathing position. This examination will often cause the soft palate to become dorsally displaced, allowing palpation of the epiglottis and the rostral aspect of the larynx.

Pharyngeal dysphagia in the horse is manifested by food and saliva flowing down the nasal cavity, during or immediately after eating; coughing caused by food inhalation; and evidence of froth and masticated food in the horse's water bucket. Pharyngeal dysphagia can be the result of neuromuscular dysfunction, caused by lesions affecting its sensory or motor nerves (cranial nerves IX, X, and XI) or the pharyngeal muscles. Neuromuscular dysfunction may be part of a generalized neurological disorder, such as botulism, rabies, or lead poisoning (when generalized weakness will be present), or may be a local disorder such as guttural pouch mycosis, with damage to cranial nerves IX, X, or XI. The pharyngeal (gag) reflex is absent or weak when there is paralysis of the pharynx. This reflex can be assessed by touching the nasopharyngeal wall or soft palate with a nasogastric tube or endoscope and observing if a swallow reflex is induced.

Acute pharyngitis, as occurs with strangles, occasionally causes transient pharyngeal dysphagia because of marked retropharyngeal lymphadenitis. The other clinical signs of strangles, such as bilateral purulent nasal discharge, bilateral submandibular lymphadenitis and fever, will aid diagnosis. Swelling of the parotid and retropharyngeal lymph nodes frequently occurs with strangles but may not be readily detectable because of the presence of the large overlying parotid salivary gland. However, deep palpation of the parotid area may reveal focal areas of increased heat and pain resulting from inflammation of the parotid lymph nodes.

Soft palate

Only the rostral few centimeters of the oral aspect of the soft palate are visible on oral examination. Digital examination of the entire ventral aspect of the soft palate is possible in well-sedated horses. Consequently, the soft palate is usually examined by nasopharyngeal endoscopy.

Guttural pouches (eustachian tube diverticulae)

The guttural pouches are positioned dorsal to the nasopharynx and medial to the mandible and parotid salivary glands. Consequently, they cannot be visualized or palpated in the normal horse. Because of the limitations of clinical examination of this area, endoscopy is the technique of choice for examining the guttural pouches. Guttural pouch distension may be evident in foals with guttural pouch tympany, and occasionally in horses with empyema or chondroids. A very distended guttural pouch may protrude caudolaterally between the angle of the mandible and the larynx, with the floor of the guttural pouch lying subcutaneously, lateral to the larynx.

Larynx

Recurrent laryngeal neuropathy (RLN) causes abnormal breathing noises and reduced exercise performance. Abnormalities of phonation, as in the presence of an abnormal whinny, may also occur in some severely affected cases.

To assess for cricoarytenoideus dorsalis muscle atrophy, palpation of the dorsorostral aspects of the larynx will demonstrate increased prominence of the muscular process of the arytenoids. The degree of laryngeal muscling on each side should be compared. The fingers of both hands are advanced beneath the rostral aspect of each sternocephalicus tendon and are moved rostromedially along the dorsum of the larynx to the muscular processes of the arytenoid on each side.

Some degree of laryngeal muscle atrophy is present in most large horses as a result of subclinical RLN, but clinical signs may not appear until it is very marked. The thoracolaryngeal ("slap") test can also be used to assess the function of the recurrent laryngeal nerve and laryngeal adductor muscles. In this test, the dorsal laryngeal area on one side is palpated while the saddle area on the contralateral thorax is slapped. Alternatively, the larynx is examined endoscopically during this maneuver. In normal horses this maneuver induces transient adduction of the opposite side of the larynx. The absence of this reflex can indicate laryngeal paresis or paralysis. A further technique to assess laryngeal function is the "arytenoid depression maneuver", which consists of stabilizing the right side of the larynx with three to four fingers while pressing the left arytenoid muscular process in a rostromedial direction with the right index finger. This maneuver readily induces inspiratory stridor in horses with severe RLN.

In horses with cricopharyngeal–laryngeal dysplasia (fourth branchial arch defect), laryngeal palpation may reveal a short, possibly vertically oriented, larynx and an abnormal space may be palpable between the caudal aspect of the short thyroid cartilage and the cricoid cartilage (the thyroid cartilage normally overlaps the cricoid). Examination of affected horses when they eat may reveal belching, caused by ingress and egress of large volumes of air into the esophagus, because of the absence of the cricopharyngeal muscles.

The most common and effective clinical technique to assess laryngeal function is to listen for abnormal inspiratory noises at fast exercise. Initially during exercise,

horses with RLN make an abnormal "whistling-type" inspiratory noise. With further or faster exercise these whistling sounds change to coarse inspiratory noises resembling "wood sawing" (termed "roaring" by owners) and in cases with severe RLN these sounds become very loud and will become biphasic (occur during inspiration and expiration). Palpation of the larynx immediately after exercising a horse with severe laryngeal paralysis may transiently reveal fremitus as a result of turbulent airflow.

Horses should be examined for the presence of a laryngotomy scar, beneath the thyroid notch on the ventral aspect of the larynx, and the rostral sternothyrohyoideus area should be assessed for the presence of myectomy scars and the absence of muscle.

Trachea

The space between the caudal aspect of the cricoid cartilage and the first tracheal ring (the cricotracheal ligament) can be readily identified by palpation, both by the greater width of the cricoid cartilage as compared to a tracheal ring, and because the cricotracheal ligament is wider than the spaces between the tracheal rings (interannular ligaments). In some horses, even with their heads in the normal flexed position the cricotracheal space can be very large and the ligament may appear to be slack, and with digital pressure it can be readily pushed into the airway lumen. This anatomical feature has been considered by some authors to cause inspiratory airflow obstruction during fast work.

The cervical portion of the trachea is examined by inspection of the overlying skin to identify changes in shape or position, deformities, and scars. Palpation may detect pain, local swellings, deformities from a previous tracheostomy, and developmental defects such as dorso-ventral or lateral collapse, and misalignment of the free borders of some tracheal rings. Mild digital pressure on the trachea may elicit coughing in horses with tracheitis.

In many horses, particularly thoroughbreds, the first few tracheal rings have a slight, clinically insignificant degree of flattening, particularly of the ventral aspect. A prominent protrusion on the ventral aspect of these tracheal rings may be palpable in the cranial neck, where they are covered by the relatively thin sterno-thyrohyoideus muscles. In small ponies, especially Shetlands and miniatures, a common congenital defect is dorsoventral flattening of the tracheal cartilages of the caudal cervical and intrathoracic trachea. Palpation of the caudal cervical trachea may be very difficult because of the thick skin, greater muscle covering over the distal cervical trachea and frequently excessive body condition of these ponies. However, deep lateral palpation in the jugular groove may reveal an abnormal sharp lateral edge to the trachea. Deep midline pressure over the caudal cervical trachea may induce stridor in affected ponies;

however this procedure should be performed with care to avoid inducing asphyxia.

External trauma may cause tracheal rupture with ingress of air into tissues from external wounds, via tears in the tracheal mucosa; or from infections by gas-producing bacteria. This may lead to localized painful swellings on the ventral aspect of the neck and sub-cutaneous emphysema (manifested by crepitus on palpation). As a traumatic esophageal rupture can cause identical signs, clinical examination should be supported by endoscopic examinations of both the trachea and esophagus.

Clinical Examination of the Lower Respiratory Tract

Audiovisual inspection of breathing

The clinician should determine the rate, depth, and pattern of breathing, and listen for abnormal sounds associated with breathing. This is best performed by distant observation, with the horse in a quiet location and undisturbed, because anxiety can profoundly alter the breathing pattern and rate. Horses that are examined outwith their normal environment, such as in an out-patient clinic, are often anxious and have alterations in the rate, depth, and pattern of breathing. Under such circumstances, the clinician must endeavor to differentiate these physiological changes from those resulting from respiratory disease.

Breathing rate

The breathing rate is most easily determined by observing the movement of the costal arch while standing caudo-laterally to the horse. Most of the changes in thoracic volume that accompany resting breathing are the result of cranial and caudal movement of the diaphragm. Consequently there is very little change in chest circum-ference during quiet breathing, and determination of the breathing rate for normal resting horses is often difficult. To overcome this problem, the breathing rate may be determined by feeling the expiratory airflow with a hand placed near the external nares. Alternatively, in cold environments, the breathing rate is readily determined by observing the exhalation of condensed water vapor. The normal resting breathing rate is 8–12 breaths/min for horses and 15–20 breaths/min for ponies. Inspiration and expiration are normally of similar duration.

Depth of breathing

During quiet breathing, resting horses have relatively subtle movements of the nostrils, costal arch, and inter-costal and abdominal muscles. These movements are

exaggerated in horses with dyspnea. As horses with pulmonary disease often have a nocturnal deterioration in pulmonary function (Deegen & Klein 1985), clinical examinations performed during the day may underestimate the severity of the nocturnal pulmonary dysfunction. Clinicians should take into account this potential deterioration when considering case management.

Pattern of breathing

The horse has a unique breathing strategy that is evident upon close inspection of breathing. Most other mammals inhale actively by contraction of the external intercostal muscles and diaphragm but exhale passively because of passive lung recoil. In contrast, the normal horse has biphasic expiratory and inspiratory phases, with the first parts of inspiration and expiration being passive and the second parts being active (Koterba et al 1988). Thus the horse inhales initially by passive relaxation and recoil of the abdominal muscles to the resting equilibrium position, and then by active contraction of the diaphragm and external intercostal muscles. Exhalation comprises initial passive recoil towards the resting equilibrium position, followed by active contraction of the abdominal muscles and internal intercostal muscles. The end-expiratory abdominal lift or heave is often visible in normal resting horses. The biphasic breathing pattern, and in particular the end-expiratory abdominal heave, is markedly exaggerated in horses with expiratory dyspnea (such as occurs in RAO).

Normally each hemithorax moves equally and symmetrically during breathing. Asymmetric reduction in movement of one hemithorax may occur with ipsilateral space-occupying lesions, such as pleural effusion or pneumothorax, or with ipsilateral pleurodynia (pleural pain). Paradoxical breathing may occur in dyspneic neonatal foals; during inspiration the highly compliant thoracic wall collapses inwards while the abdomen expands.

Abnormalities in the rate, depth, and pattern of breathing

Abnormalities in the rate, depth, and pattern of breathing may be subdivided into five broad categories, as indicated in Table 8.1. Recognition of these abnormalities may enable the clinician to determine the cause of the underlying problem.

Differentiation of expiratory versus inspiratory dyspnea

Dyspnea is defined as a difficulty in breathing. Horses with dyspnea have an obvious increase in breathing effort and may appear distressed. Dyspnea may occur throughout

Table 8.1. Abnormalities in the rate, depth, and pattern of breathing
Rapid deep breathing
● Physiological causes including exercise and anxiety
● Pathological causes including lung disease, anemia, metabolic acidosis, pain
Rapid shallow breathing
● Anxiety
● Pleurodynia associated with chest wall injury or pleuropneumonia. As deep breathing increases the intensity of pleurodynia, horses with pleurodynia adopt a rapid, shallow breathing strategy
● Disorders that reduce the compliance of the lungs or chest wall, including;
(a) space-occupying lesions of the thorax, such as pleural effusion and pneumothorax, that limit lung compliance by reducing the intrathoracic volume available for lung expansion
(b) restrictive lung diseases, such as interstitial pneumonia and pulmonary fibrosis that reduce lung compliance directly. These disorders make it difficult for the horse to inspire deeply. Consequently, affected horses adopt a more energy efficient, fast and shallow breathing pattern
Slow deep breathing
● This is occasionally evident in horses with severe airway obstruction, when inspiratory or expiratory airflow is restricted to such an extent that the duration of inspiration and/or expiration is markedly prolonged and as a result the breathing rate cannot be increased
Slow shallow breathing
● This is indicative of central nervous system depression, or a compensatory response to metabolic alkalosis
Cheyne–Stokes breathing
● This is characterized by a cyclical waxing and waning of the rate and depth of breathing.

the breath cycle, or it may occur predominantly during inspiration or expiration. Horses with fixed airway obstructions, such as those with a solid mass within the nasal cavity, may have a relatively constant degree of dyspnea throughout the breath cycle, because the degree of obstruction remains constant throughout the breath cycle. The majority of horses with airway obstructions, however, have dyspnea that is significantly worse during inspiration (for most upper airway obstructions) or expiration (for most lower airway obstructions). This is because of dynamic airway collapse, whereby the degree of obstruction varies throughout the breath cycle as a consequence of changes in the transmural pressure gradient. Knowledge of the pathogenesis of expiratory and inspiratory dyspnea may enable the clinician to determine the likely anatomical site and nature of the underlying disease process (Table 8.2).

Table 8.2. Differentiating inspiratory *versus* expiratory dyspnea

Expiratory dyspnea

Signs
- Prolonged and labored expiratory phase
- Exaggerated expiratory contraction or "heave" of the abdominal muscles
- Heave line in chronically affected horses
- Pumping of anus
- Dilatation of external nares throughout the breath cycle
- Extension of the head and neck

Causes
- Obstruction of the intrathoracic airways

Inspiratory dyspnea

Signs
- Prolonged and labored inspiratory phase
- Increased inspiratory effort
- Exaggerated diaphragm and external intercostal muscle activity
- Dilatation of external nares throughout the breath cycle
- Extension of the head and neck

Causes
- Obstruction of the extrathoracic airways
- Restrictive lung disorders
- Space-occupying lesions of the thorax

Mixed inspiratory and expiratory dyspnea

Cause
- Fixed obstructions of the airways

Expiratory dyspnea

Horses with expiratory dyspnea have a prolonged and labored expiratory phase, and an exaggerated expiratory abdominal effort that is termed a "heave". The marked intra-abdominal pressure swings which result from severe expiratory abdominal efforts can cause rhythmic pumping of the anus. Horses with longstanding severe expiratory dyspnea may develop a heave line; a linear depression which develops ventral to the external abdominal oblique muscles, when these muscles hypertrophy (Fig. 8.2). Heave lines were previously common in horses with chronic severe RAO but are rare today as a consequence of improvements in the diagnosis and management of this disease. A heave line should be differentiated from the external abdominal oblique muscle hypertrophy that is present in performance horses as a result of athletic training.

Expiratory dyspnea is caused by obstruction of the intrathoracic airways (such as occurs in RAO), because the transmural pressure gradients cause the inflamed and narrowed intrathoracic airways to undergo progressive dynamic airway collapse during expiration (Fig. 8.3).

Inspiratory dyspnea

Inspiratory dyspnea results in a prolonged and labored inspiratory phase, and exaggerated activity of the diaphragm and external intercostal muscles during inspiration. Inspiratory dyspnea is most commonly caused by obstruction of the extrathoracic airways, such as bilateral laryngeal paralysis or cervical tracheal collapse. This is because the subatmospheric pressures that develop within the extrathoracic airways during inspiration cause dynamic collapse of airways that have lost their structural integrity. Disorders that reduce lung or chest wall compliance, such as restrictive lung disorders and space-occupying lesions of the thorax, may also lead to inspiratory dyspnea because these conditions compromise lung inflation.

Abnormal Respiratory Sounds

The clinician should listen for respiratory sounds which are audible without the aid of a stethoscope. Their presence usually indicates respiratory tract disease.

Coughing

Coughing is probably the most reliable clinical sign of pulmonary disease, although it is inexplicably absent in some horses that have pulmonary disease and excessive respiratory secretions. Coughing rarely results in expectoration of respiratory secretions from the oral cavity, most of the respiratory secretions being swallowed. Consequently differentiation of productive and nonproductive coughing has limited diagnostic value in equine medicine, and in any case endoscopy will invariably show excessive respiratory secretions in the trachea of horses with any type of pulmonary disease (Dixon et al 1995). Horses with pleurodynia may have a characteristic, soft cough.

Wheezes and crackles

Wheezes and crackles may be heard at the nostrils of horses with severe respiratory tract disease. Stridor is a term used to describe a particularly loud monophonic inspiratory wheeze that may be heard considerable distances from the horse. It is indicative of an extrathoracic airway obstruction.

Grunts or groans

These loud expiratory sounds, produced by sudden laryngeal opening after a period of breath-holding against a closed glottis, are usually an indication of pain.
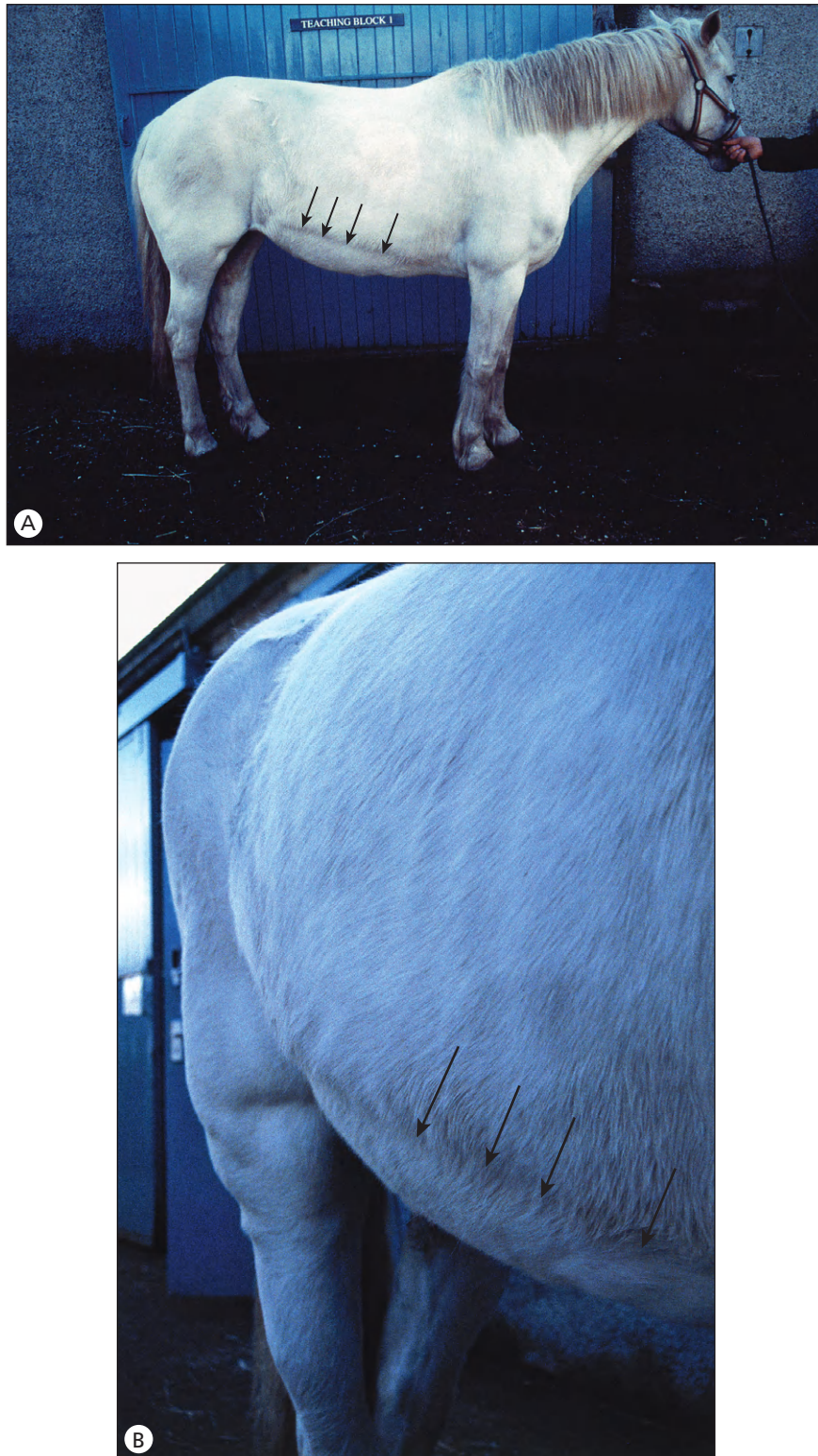
Fig. 8.2. A horse with a "heave line" (arrows). This is a linear depression which develops ventral to the external abdominal oblique muscles, when these muscles hypertrophy in horses with longstanding severe expiratory dyspnea.

A   **Inspiration**

Transmural pressure gradient on inspiration tends to
• collapse extrathoracic airways
• dilate intrathoracic airways

B   **Expiration**

Transmural pressure gradient on expiration tends to
• collapse intrathoracic airways
• dilate extrathoracic airways
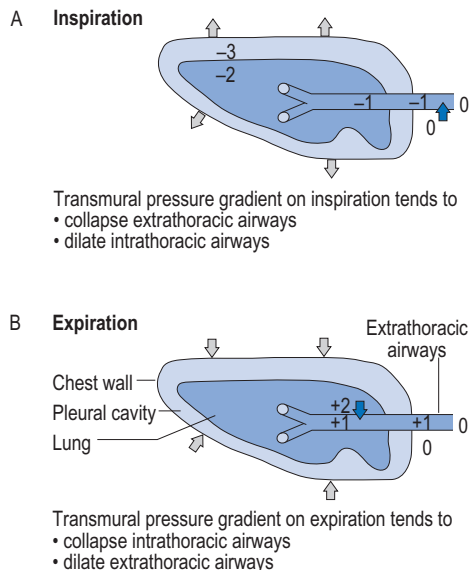
Fig. 8.3. Schematic diagram showing the pathogenesis of dynamic airway collapse. During inspiration (A), the pressure outside the extrathoracic airways exceeds that within the airways. If this transmural pressure gradient overcomes the rigidity of the airways, dynamic airway collapse will result. Conditions that compromise the rigidity of the extrathoracic airways, including nasal paralysis, laryngeal paralysis, and cervical tracheal collapse, may induce airway collapse and cause inspiratory dyspnea. Conversely, during expiration (B) the transmural pressure gradient favors dynamic collapse of the intrathoracic airways. Conditions that lead to narrowing of these airways, such as RAO, exacerbate airway closure and cause expiratory dyspnea. Redrawn from McGorum et al 2000, with permission.

Auscultation of the Lower Respiratory Tract

Auscultation is the most frequently used technique for examining the respiratory tract. When performed by an astute clinician it can provide useful diagnostic information. However, as with all clinical techniques, auscultation has limitations. While it is important to understand these limitations, the clinician must avoid dismissing thoracic auscultation as a technique to be performed only as a diagnostic ritual, in deference to tradition, rather than as a truly valuable technique. The major limitation of auscultation is the inaudibility of breath sounds detectable over the equine thorax, especially when examining obese horses under noisy field conditions. A further limitation is the considerable confusion that exists in the nomenclature and interpretation of lung sounds. This has resulted from over-interpretation or over-simplification of Laënnec's original description of breath sounds (Robertson 1957), and as a result of poor understanding of the mechanisms of sound production and transmission within the respiratory tract. Thankfully,

recent simplification of the terminology of lung sounds has led to more accurate interpretation of auscultatory findings. The terms bronchial sounds, vesicular sounds, alveolar sounds, sonorous sounds, rhonchi, moist rales, dry rales, squeaks, whistles, and crepitation are now considered obsolete. The mechanisms of breath sound production and transmission in domestic animals have been well reviewed (Kotlikoff & Gillespie 1983, 1984).

Auscultation should be performed in quiet surroundings, and with the horse minimally restrained. The diagnostic sensitivity of the examination may be enhanced by the use of an electronic stethoscope, which increases the amplitude of breath sounds. A systematic pattern of auscultation is essential. The clinician should auscultate the distal cervical trachea and both lung fields, with each site being examined for a minimum of one complete breath cycle. During auscultation, the clinician should observe the costal arch to (1) determine the stage of the breath cycle at which breath sounds occur, and (2) assess the relative audibility of inspiratory and expiratory sounds.

Auscultation should also be performed while the horse is hyperventilating because hyperventilation considerably improves the audibility of breath sounds and hence increases the sensitivity of the examination. Clearly, forced hyperventilation is contraindicated in horses with severe dyspnea or pleurodynia. Hyperventilation is readily achieved using a rebreathing bag, which is tolerated by most horses (Fig. 8.4), but other clinicians induce hyperventilation by occluding the horse's external nares for 30–60 seconds. This method is less useful than the rebreathing bag because it induces only transient hyperventilation. Furthermore, it often induces swallowing and chewing, resulting in referred noise, which can mask breath sounds. The clinician should suspect respiratory tract disease if forced hyperventilation induces adventitious breath sounds, coughing, or pleurodynia, or if the time for the breathing rate to return to normal is prolonged.

During auscultation, the clinician should attempt to address the following points:

● Is the audibility of breath sounds heard on auscultation of the distal cervical trachea and thorax normal?
● Are there regional differences in the audibility of the breath sounds heard over the thorax?
● Are adventitious breath sounds audible?
● If so, which adventitious sounds are audible, what is their location, and at what stage of the breath cycle do they occur?

Normal breath sounds

Certain sounds, termed normal breath sounds, always accompany the movement of air within airways. These sounds are generated predominantly by turbulent airflow

Fig. 8.4. The audibility of breath sounds detected by auscultation may be increased by using a rebreathing bag. This induces prolonged hyperventilation and considerably improves the sensitivity of thoracic auscultation.

Fig. 8.5. Schematic diagram showing the effects of pulmonary consolidation, pneumothorax, and pleural effusion on the transmission of high and low frequency breath sounds from their source in the large airways to the stethoscope. Redrawn from McGorum et al 2000, with permission.

in the large (>2 mm) airways. Breath sounds are transmitted efficiently up and down the lumina of the larger airways as airborne pressure waves, with relatively little sound attenuation. Consequently, breath sounds audible over the trachea or over the thorax represent a mix of sounds originating from many different sites throughout the respiratory tract, including the nares, nasal cavity,

pharynx, larynx, trachea, and larger intrathoracic airways. In contrast, small airways (<2 mm) transmit sound waves poorly and probably do not contribute to the generation or transmission of breath sounds.

Normal breath sounds audible at the distal cervical trachea

The normal breath sounds produced in the large airways are clearly audible on auscultation of the distal cervical trachea because they are transmitted efficiently through the thin peritracheal tissues to the stethoscope. In the normal horse they are soft blowing sounds which should be neither harsh nor accompanied by adventitious sounds. They are heard predominantly during early inspiration and early expiration. The inspiratory and expiratory noises normally have similar amplitude.

Normal breath sounds audible over the lung fields

Only a small fraction of the breath sounds produced in the large airways reaches a stethoscope placed against the thorax. The remainder is lost by attenuation and reflection as sound is transmitted from the large airways, through the parenchyma and the relatively thick equine thoracic wall, to the stethoscope (Fig. 8.5). Consequently, breath sounds audible over the thorax are considerably quieter than those audible over the distal cervical trachea. Indeed, the breath sounds detected over the thorax during normal resting breathing are often barely audible and consequently are difficult to interpret. The acoustic transmission properties of the lung and thoracic wall are such that this loss of sound is normally greater for high-frequency sounds than

for low-frequency sounds. Normally, breath sounds are louder during inspiration than expiration, and are slightly louder over the right thorax than the left thorax.

Increased audibility of normal breath sounds

The audibility of breath sounds is determined largely by two factors, namely:

- the amplitude of breath sounds produced within the large airways
- the proportion of sound which is lost during sound transmission.

Increased audibility of normal breath sounds over the entire lung field most commonly reflects hyperventilation, which increases the amplitude of breath sounds by increasing the velocity of airflow in the large airways. There are numerous causes of hyperventilation including respiratory tract disease, anxiety, exercise, metabolic acidosis, fever, severe anemia, and cardiac failure.

A focal increase in the audibility of normal breath sounds may be detected over pulmonary masses and areas of pulmonary consolidation. The acoustic properties of these lesions favor efficient transmission of high-frequency breath sounds from the surrounding healthy airways to the overlying chest wall (Fig. 8.5). Breath sounds heard over consolidated lung are thus similar to those audible over the distal cervical trachea.

Decreased audibility of normal breath sounds

Decreased audibility of breath sounds usually results from increased attenuation of sound as it is transmitted from the large airways to the stethoscope. Reduction in the audibility of breath sounds over the entire thorax is common in obese horses, because the thicker chest wall considerably reduces breath sound transmission. In contrast, breath sounds of thin horses and foals are much more audible. The clinician must therefore interpret the audibility of the lung sounds with reference to the body condition of the animal. Rarely, a generalized reduction in breath sounds may reflect reduced airflow velocity, as occurs in horses with hypoventilation.

Regional loss of breath sounds occurs when the pleural cavity contains air, fluid, or displaced abdominal organs (Fig. 8.5). In such circumstances, breath sounds are lost largely by reflection at the tissue/air or tissue/fluid interfaces, because these interfaces act as near-complete acoustic barriers. Some intestinal sounds are auscultated in the normal thorax but increased intestinal sounds may be auscultated in horses with a diaphragmatic hernia.

From the above discussion it will be evident that the audibility of breath sounds is influenced by physiological factors such as body condition and by pathological

factors such as the presence of pleural effusion. While physiological factors influence the audibility of the lung sounds over the entire thorax, pathological processes usually result in regional differences in the audibility of breath sounds and are accompanied by adventitious breath sounds. The latter findings should alert the clinician to the presence of disease.

Adventitious breath sounds

Adventitious breath sounds are abnormal sounds that are superimposed on normal breath sounds (Table 8.3). As they are audible only in horses with respiratory tract disease, they are a useful indicator of respiratory tract disease, but provide limited information regarding its etiology. Consequently, the clinician should be cautious

Table 8.3. Adventitious respiratory sounds
Wheezes
Prolonged (> 250 ms) musical sounds produced by air flowing through narrowed airways, causing the walls to vibrate between the closed and barely open positions - **Expiratory wheezes** – indicate obstruction of the intrathoracic airways such as occurs in RAO - **Inspiratory wheezes** – audible in horses with atelectasis, pulmonary consolidation or restrictive pulmonary diseases - **Stridor** – a loud monophonic inspiratory wheeze indicating obstruction of the extrathoracic airways, such as occurs in extrathoracic tracheal collapse
Crackles
Short duration, interrupted, non-musical sounds - **Coarse crackles** – loud, short duration (typically 10–30 ms), non-musical, "bubbling or crackling" sounds audible over the distal cervical trachea. Probably caused by air bubbling through, and causing vibrations within, respiratory secretions within the large airways - **Fine crackles** – compared with coarse crackles, these are shorter duration (typically 1–10 ms), lower amplitude and higher pitch. Probably caused by sudden explosive opening of a series of airways which had become abnormally closed during expiration 1. Late inspiratory fine crackles: occur in restrictive pulmonary diseases 2. Early inspiratory fine crackles: occur in obstructive pulmonary diseases
Pleural friction rubs
Variable sounds produced as inflamed visceral and parietal pleurae rub together
Expiratory grunts
Loud expiratory sounds produced by sudden laryngeal opening after a period of expiration against a closed larynx. They indicate pain.

when attributing a particular adventitious sound to a specific disease process or etiology. Conversely, as significant respiratory disease may occur in the absence of adventitious sounds, the absence of adventitious sounds does not preclude respiratory tract disease. The nature, anatomical location, and audibility of adventitious breath sounds may all vary considerably with time as a result of alterations in the underlying airway dysfunction, breathing pattern, or clearance of airway secretions.

Adventitious noises tend to occur consistently at the same stage of the breath cycle, over many consecutive breaths. The clinician should determine the stage of the breath cycle at which they occur, because this may yield information regarding the nature and location of the underlying lesions. The anatomical location of the point of maximal intensity of adventitious sounds should also be determined because this usually indicates the site of the lesion.

Wheezes

These are prolonged (>250 ms) musical sounds that occur when air flows through narrowed airways, causing the airway walls to vibrate between the closed and barely open positions (Fig. 8.6). Airway narrowing may be caused by extraluminal compressive lesions (e.g. neoplasms), thickening of the airway walls (e.g. mucosal inflammation or edema), or intraluminal obstructions (e.g. broncho-

Fig. 8.6. Schematic representation of mechanisms underlying the production of crackles and wheezes. (A) Wheezes are produced by air flowing through narrowed airways causing their walls to vibrate between the closed and barely open positions. (B) Crackles are caused by a series of collapsed airways suddenly opening as a result of increasing traction from surrounding parenchyma. Redrawn from McGorum et al 2000, with permission.

spasm, mucus accumulation, foreign bodies). Airway narrowing is further exacerbated by the Bernouilli effect, whereby acceleration of the air column as it flows through the narrowed airway causes a reduction in the intraluminal pressure. The airway narrowing, in turn, causes deceleration of the airflow, reducing the Bernouilli effect, and opening up the airway. This rapid cyclical opening and closing of the airway generates the wheeze. Many healthy humans and most human asthmatics can generate expiratory wheezes simply by performing a violent forced expiration, which induces dynamic collapse of the central airways at low lung volumes.

Two types of wheeze are recognized.

- **Monophonic wheeze** – this is a single note of constant pitch, location of origin and timing within the breath cycle. As it usually indicates partial obstruction of a single airway by a solitary space-occupying lesion such as a neoplasm, it is an uncommon finding in horses.
- **Polyphonic wheezes** – these comprise several different notes of different pitch and timing. They indicate obstruction of multiple airways and are commonly detected in horses with RAO.

Wheezes are frequently audible at the thoracic wall, cervical trachea, and external nares, because they are transmitted efficiently up and down the large airways, and through the thoracic wall, with relatively little sound attenuation.

Wheezes arising from fixed airway obstructions may be audible throughout the breath cycle. However much more commonly, wheezes are confined to either expiration or inhalation because of exacerbation of airway obstruction as a result of dynamic airway collapse (Fig. 8.3). Expiratory wheezes indicate partial obstruction of the intrathoracic airways, as occurs in RAO. In contrast, late inspiratory wheezes indicate atelectasis, pulmonary consolidation or restrictive lung diseases. In these latter disorders, wheezes are produced by air entering previously collapsed airways as the lung expands and the airways open during late inspiration. As noted, stridor is a term used to describe a particularly loud monophonic inspiratory wheeze that may be heard considerable distances from the horse without the use of a stethoscope. It is indicative of extrathoracic airway obstruction.

Crackles

These are short duration, interrupted, non-musical sounds. Two types can be recognized:

- **Coarse crackles** – these are loud, explosive, short duration (typically 10–30 ms), non-musical, "rattling or bubbling" sounds. They are probably the most common adventitious breath sound heard in the horse. They are most readily detected over the distal cervical trachea, especially during induced hyperventilation, and may

be heard during inspiration and expiration. They are possibly caused by air bubbling through, and causing vibrations within, respiratory secretions in the larger intrathoracic airways, including those that are pooling within the dependent part of the rostral thoracic trachea ("tracheal sump").

- **Fine crackles** – compared with coarse crackles, fine crackles are of shorter duration (typically 1–10 ms), lower amplitude and have a higher pitch. Fine crackles can be simulated by rolling a lock of hair between the fingers, close to the ear, or by "rustling" cellophane. Most fine crackles are probably caused by sudden explosive popping open of a series of airways that have become abnormally closed during expiration (Fig. 8.6). Thus fine crackles tend to occur consistently at a particular transpulmonary pressure. The audible sounds are the result of sudden equalization of the downstream and upstream airway pressures, or the sudden alteration in the tensions of the airway walls. In some horses, air bubbling through secretions in the large airways can cause fine crackles. Fine crackles are detectable mainly in peripheral and dependent lung areas. Fine crackles may be detected in early or late inspiration. Late inspiratory crackles occur in restrictive lung diseases, which reduce the lung compliance and cause airway closure at low lung volumes. Crackles are produced by the sudden opening of these collapsed airways during late inspiration. In contrast, early inspiratory crackles occur in horses with obstructive pulmonary diseases that lead to expiratory airway collapse. The crackles are produced by the airways as they pop open explosively during early inspiration.

Pleural friction rubs

The gliding movement of the visceral and parietal pleurae is normally silent because of the lubricant properties of the pleural fluid. However, when inflamed visceral and parietal pleurae rub together, frictional resistance may produce pleural friction rubs. These sounds vary considerably, from sounds resembling fine crackles to classical harsh pleural friction sounds that can be simulated by rubbing two sheets of sandpaper together. This variability makes it difficult to recognize pleural friction sounds definitively, and to differentiate them from crackles originating from pulmonary disease. Pleural friction rubs are usually heard during both inspiration and expiration, and tend to recur consistently at similar stages in the breath cycle, features which are diagnostically useful. Most pleural friction rubs are transient phenomena, disappearing when the pleurae are separated by pleural effusion. Thus the absence of pleural friction rubs does not preclude the presence of pleuritis. The clinician can differentiate between pleural friction rubs and cardiac murmurs by determining whether the sounds are synchronous with the breath or with cardiac cycles.

Extraneous noises not associated with the breath cycle

If the stethoscope is not held firmly against the horse's body during auscultation, it can rub on the hair and produce sounds resembling crackles.

Gastrointestinal and cardiac sounds are frequently audible over the lung fields of normal horses. Consequently their presence should not arouse suspicion of diaphragmatic herniation. Gastrointestinal sounds may be differentiated from breath sounds because they are sporadic, variable in intensity and duration, and bear no temporal association with the breath cycle. The clinician may differentiate cardiac and breath sounds by determining whether the sounds coincide consistently with the cardiac or breath cycle. To determine the source of the unidentified sounds, it is occasionally useful to transiently stop the horse breathing, by occlusion of the external nares. If the unidentified sound persists despite the cessation of breathing, it is clearly not associated with the breath cycle.

Muscular tremors, such as occur in horses that are anxious or cold (blanket taken off for thoracic auscultation in cold environments), may preclude satisfactory thoracic auscultation.

"Thumps" produced by contraction of the diaphragm may be heard over the thorax and flanks in horses with synchronous diaphragmatic flutter. It is probable that acid–base and electrolyte disturbances make the phrenic nerve sensitive to the depolarizing electrical activity of the adjacent myocardium. The diaphragmatic contraction and resultant "thump" are thus synchronous with the heartbeat and not with the breath cycle.

Acoustic Percussion of the Thorax

Acoustic percussion is a useful non-invasive technique for detecting pleural and superficial parenchymal lesions. Whilst it should be performed as part of the routine clinical examination, it is especially indicated when auscultation has revealed regional variations in the audibility of lung sounds or there is other evidence of pleural disease. While percussion is a useful clinical technique, it is considerably less sensitive than ultrasonography for detecting pleural and superficial parenchymal lesions and only relatively large lesions can be detected. Furthermore, as the percussive sound wave penetrates only to a depth of several centimeters, percussion will only detect pleural and superficial parenchymal lesions. As with most clinical techniques, considerable practice using normal and abnormal animals is required to attain diagnostic proficiency with percussion.

Percussion may be performed by the direct technique using a plexor and a pleximeter, but as these are not always available, the indirect technique is described. The right-handed clinician should hyperextend the middle finger
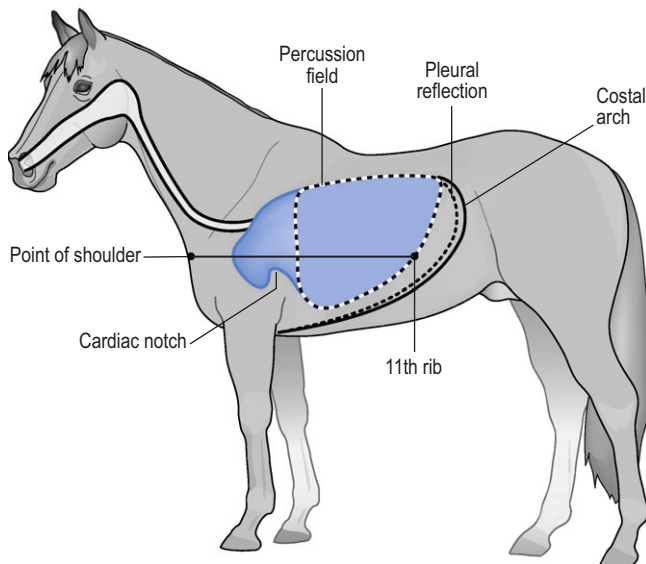
Fig. 8.7. Schematic diagram showing the line of costodiaphragmatic pleural reflection (black dotted line), the cardiac notch and the outline of the equine lung field as determined by percussion (black and white dotted line). On percussion, the ventral limit of the lung field at the 11th rib is level with the point of the shoulder. The anatomical outline of the lung, however, varies during the breath cycle. Redrawn from McGorum et al 2000, with permission.

Table 8.4. Abnormal findings on acoustic percussion of the thorax
● Dull area ventrally – pleural effusion (most common), diaphragmatic hernia, marked cardiomegaly, marked pericardial effusion
● Dull area at any focal site – large pleural or pulmonary abscess or neoplasm
● Increased resonance dorsally – pneumothorax
● Increased resonance at any site – emphysematous bullae, although these are unlikely to be of sufficient magnitude to be detectable by percussion
● Coughing – percussion induces coughing only if there is disease of the underlying lung parenchyma
● Pleurodynia – percussion is painful in horses with diseases of the underlying thoracic wall, pleurae or lung parenchyma

From McGorum et al 2000, with permission.

of the left hand and should press the distal phalanx firmly against the thoracic wall. To avoid dampening the percussive wave, other parts of the left hand should not contact the thorax. This phalanx is then struck sharply with the tips of the middle three fingers of the right hand, while keeping these three fingers together in a rigid, semi-flexed position. The right hand is immediately withdrawn to avoid damping the resultant sound wave.

Percussion is best performed at peak inspiration, when the contrast between normal and abnormal sounds is most easily detected. Percussion should be performed in a systematic pattern, usually commencing at the cranio-dorsal aspect of the thorax and working in a dorsal to ventral direction down each intercostal space, before repeating the examination over the caudally adjacent intercostal space. It is essential to percuss and compare both sides of the thorax, as abnormalities may be confined to one hemithorax.

Percussion over the lung fields in a normal horse (Fig. 8.7) results in a low-pitched hollow sound that is termed resonant, while percussion outwith this area produces a dampened, higher pitched tone that is described as dull. Percussion yields a more resonant tone in individuals with thin thoracic walls, such as foals and thin adults.

Abnormal percussion findings are summarized in Table 8.4. The presence of fluid or solid tissue masses within the superficial lung parenchyma, pleural cavity or thoracic wall attenuate the sound wave, resulting in a flat or soft,

higher pitched tone that is described as dull. Pneumo-thorax causes increased resonance over the dorsal thorax. While many texts describe the use of percussion to detect enlargement of the lung fields in horses with RAO, this feature is rarely detectable in the author's experience.

Palpation of the Thorax

Thoracic wall palpation may detect chest wall injuries, fractured ribs, subcutaneous emphysema, or pleurodynia. The clinician must be cautious when interpreting the response to thoracic palpation, as many normal horses resent this procedure. Horses with pleurodynia may also have an anxious facial expression, are reluctant to move, especially in a tight circle, or to lie down and may persistently point one forelimb or arch their back. They often have rapid shallow breathing, a soft and suppressed cough, and an expiratory groan.

Additional Clinical Features of Respiratory Tract Disease

As respiratory tract disease may result in additional clinical signs, a full clinical examination should be performed. Other signs include:

● Nasal discharge – usually bilateral but may be unilateral if scant and serous, mucoid, mucopurulent, purulent, hemorrhagic, or food-containing in nature.
● Pyrexia – this is common in viral, bacterial, and neoplastic conditions of the respiratory tract.
● Weight loss – this is common in chronic bacterial lung infections, respiratory tract neoplasia, and in horses with chronic severe dyspnea.
● Cyanosis – an increase in the quantity of circulating reduced hemoglobin imparts a bluish coloration to the

mucous membranes. Cyanosis usually indicates profound arterial hypoxemia ($P_{A}O_{2}$ < 40 mmHg, assuming that the hemoglobin concentration is within normal limits). It is best appreciated when viewed under daylight, and may be unrecognizable under fluorescent lighting.

- Peripheral subcutaneous edema – an accumulation of fluid within the subcutaneous tissues of the ventral thorax is common in horses with pleuropneumonia. Edema of the head may occur in horses with cranial thoracic masses as a result of impaired venous and lymphatic drainage from the head, and with purpura hemorrhagica following strangles.

- Subcutaneous emphysema – air may track into the subcutaneous tissues following traumatic or surgical penetration of the upper airway or chest. Alternatively, it may occur in horses with very severe pulmonary disorders that lead to air trapping or emphysema, when air tracks from the ruptured alveoli through the bronchovascular sheaths to the lung hilus and mediastinum and thereafter into the subcutaneous tissues. Light palpation of the affected skin reveals a characteristic crepitation.

- Jugular vein distension –when the horse's head is in the normal standing position, only that part of the jugular vein in the lower third of the neck should be distended. Distension above this point indicates increased central venous pressure or obstruction of venous return such as occurs in horses with cranial thoracic masses.

- Anemia – this may be detected in horses with internal carotid or pulmonary hemorrhage or with chronic bacterial or neoplastic diseases.

- Peripheral lymphomegaly – palpable bilateral enlargement of the intermandibular lymph nodes is common in horses with bacterial or viral respiratory diseases. Unilateral enlargement occurs with ipsilateral neoplasia, sinusitis and guttural pouch empyema. Generalized peripheral lymphomegaly may occur in horses with multicentric lymphosarcoma.

- Hypertrophic osteopathy (Marie's disease) – affected horses have symmetrical, firm swelling of all four limbs and shifting leg lameness.

REFERENCES

Deegen E, Klein HJ 1985 On the variability of lung function measurements in the horse – a preliminary report. In: Deegen E, Beadle RE (editors) Lung Function and Respiratory Diseases in the Horse. International Symposium in Hannover, Germany. Hippiatrika Verlagsgesellshaft, Calw, pp.72–73

Dixon PM, Railton DI, McGorum BC 1995 Equine pulmonary disease: a case control study of 300 referred cases. Part 3: Ancillary diagnostic findings. Equine Veterinary Journal 27: 428–435

Koterba AM, Kosch PC, Beech J et al 1988 Breathing strategy of the adult horse (*Equus caballus*) at rest. Journal of Applied Physiology 64: 337–346

Kotlikoff MI, Gillespie JR 1983 Lung sounds in veterinary medicine. Part I: Terminology and mechanisms of sound production. Compendium on Continuing Education for the Practicing Veterinarian 5: 634–639

Kotlikoff MI, Gillespie JR 1984 Lung sounds in veterinary medicine. Part II: Deriving clinical information from lung sounds. Compendium on Continuing Education for the Practicing Veterinarian 6: 462–467

McGorum BC, Dixon PM, Radostits OM, Abbott JA 2000 Clinical examination of the respiratory tract. In: Radostits OM, Mayhew IG, Houston DM (editors) Veterinary Clinical Examination and Diagnosis. WB Saunders, London, pp.213–244

Robertson AJ 1957 Rales, rhonchi and Laënnec. Lancet ii: 417

9 Collection and Analysis of Respiratory Tract Samples

Jennifer L Hodgson and David R Hodgson

Introduction

There have been significant advances in our understanding of equine pulmonary diseases over the past two decades, with much progress as a result of advances in ancillary diagnostic testing. However, these techniques should not replace the procurement of an accurate history and performing a thorough clinical examination. The latter will frequently dictate appropriate diagnostic tests to be performed. Alternatively, a lack of specific clinical signs associated with respiratory tract disease, yet evidence of poor performance, may be indicative of subclinical or low-grade pulmonary pathology. In this situation ancillary diagnostic testing may detect subtle changes in the respiratory tract that are not discernible by routine clinical evaluation.

Recent research has focused on the development of "in-field" diagnostic tests for equine practitioners. This chapter outlines the availability of, and requirements for, "field" techniques for collection of samples from the respiratory tract of horses. Naturally the quality and diagnostic value of any sample collected is directly related to the care and skill used by the clinician in its gathering. Thus, technical prowess and a solid understanding of the potential errors in the processing, evaluation, and interpretation of these tests will improve the outcomes achieved (Hoffman & Viel 1997).

A variety of samples may be collected in the field from horses with respiratory disease. These include swabs, washings, aspirates or biopsies from sites in the upper respiratory tract (URT), as well as tracheobronchial washings and aspirates (TA), bronchoalveolar lavage (BAL) and pleural fluid (PF) collection. These samples may be evaluated by various methods including, but not limited to, cytological and biochemical analysis, bacterial culture, viral immunology and virus isolation, and histopathology. The indications, collection techniques, handling, evaluation, and interpretation of these samples will be discussed.

Indications

The indications for collection of samples from different sites within the respiratory tract will depend on the history, clinical signs, and likely differential diagnoses. In addition, the ease of performing the technique and the type of horse (performance versus pleasure) may influence selection of the laboratory technique.

Indications for sampling the nasal cavity, nasopharynx, and guttural pouches

Diseases of the nasal cavity may result in nasal discharge, epistaxis, dyspnea, inspiratory stridor, and malodorous breath. Chronic unilateral nasal discharge usually results from a unilateral lesion of the URT and is seldom the result of a primary bacterial infection. If a profuse bilateral nasal discharge is present, particularly associated with coughing, it is commonly indicative of pulmonary disease. However, a small percentage of horses with pulmonary disease have unilateral nasal discharge (Dixon 1997). Diseases of the nasopharynx can be associated with abnormal respiratory noise, exercise intolerance, dyspnea, and dysphagia.

When these signs are present, important steps in the diagnostic pathway will be collection of a relevant history followed by a thorough physical examination. In many cases ancillary diagnostic aids, including endoscopy, ultrasonography and radiography of the affected areas, are indicated to further evaluate lesions of the URT (see Chapters 10, 11 and 18 respectively, for further details). Based on this examination, specific sampling from relevant sites may be undertaken as outlined in Table 9.1.

Indications for sampling the lower airways and pleural cavity

Indications for TA and BAL include coughing, poor performance, the presence of mucopus in the trachea, epistaxis post-exercise, fever of unknown origin and possibly dyspnea (see Table 9.1). When choosing one or both of these procedures the clinician needs to be cognizant of the differences between them. Tracheal aspirates obtain secretions from the larger airways, including the trachea and bronchi, and also from more distal airways, moved to the trachea by mucociliary clearance. Bronchoalveolar lavage harvests secretions from the smaller, more distal airways and specifically from the region lavaged. In certain instances, where a particular diagnosis has a high degree of certainty based on history

	Site					
Clinical sign	**Sinonasal cavity**	**Naso- pharynx**	**Guttural pouch**	**Lungs**	**Pleural cavity**	**Examination techniques**
Nasal discharge (unilateral)	+++	++	+++	+	–	Physical exam, endoscopy, radiography likely to be most diagnostically rewarding. Swab, wash or fine-needle aspirate/biopsy of lesion(s) in nasal cavity or nasopharynx. Wash from GP
Nasal discharge (bilateral)	++	++	+	++	+	Physical exam, endoscopy, radiography likely to be most diagnostically rewarding. If evidence of LRT involvement include TA, BAL. Culture of samples from nasal cavity, nasopharynx and GP washings for diagnosis of strangles
Epistaxis (unilateral)	+++	++	+++	+	–	Physical exam, endoscopy (esp. of GPs), radiography likely to be most diagnostically rewarding. If evidence of LRT involvement include TA, BAL
Epistaxis (bilateral)	+	++	+++	+++	+	As for unilateral epistaxis
Dysphagia	–	+++	+++	–	–	Endoscopy and radiography of nasopharynx, GPs and neck
Swelling of sinonasal or parotid area	+	++	++	–	–	Physical exam, ultrasound, endoscopy and radiography likely to be most diagnostically rewarding. Possible aspirate or biopsy of lesion in nose. Culture of swabs from nose ± washings from GP for strangles
Horner syndrome	–	–	+++	–	+	Physical exam including neurological exam, endoscopy and radiology. Specific examination of GPs
Inspiratory stridor	+++	+++	++	–	–	Physical exam, endoscopy and radiography. Fine-needle aspirate or biopsy of mass if observed in nares, naso- pharynx or parotid area. Nasal and GP washing and culture for strangles
Dyspnea	+	+	+	+++	+++	Physical exam, endoscopy and radiography. Possible ultrasound of thorax. If evidence of LRT involvement may include TA, BAL ± thoracocentesis
Cough	–	–	–	+++	+	As for dyspnea
Poor exercise performance	+	+++	+	+++	+++	As for dyspnea
Fever of unknown origin	+	+	++	+++	+++	As for dyspnea
Pleurodynia	–	–	–	+	+++	As for dyspnea with particular attention to ultrasound of thorax ± thoracocentesis

Table 9.1. Possible sites of origin and approach to diagnosis for differing clinical manifestations of disease relating to the respiratory tract

GP = guttural pouch; TA = transtracheal aspirate; BAL = bronchoalveolar lavage; LRT = lower respiratory tract.

and clinical signs, one or other of these techniques may be indicated, e.g. BAL in cases of suspected "heaves" or exercise-induced pulmonary hemorrhage (EIPH); TA in suspected bacterial pneumonia, pleuropneumonia, or fever of unknown origin. In other situations, where the diagnosis is unresolved, such as cases of poor performance or coughing during exercise, collection of samples using both techniques is recommended to broaden assessment of the health of the lower airways. It is important to note that no significant correlation between TA and BAL cytology has been found (Derksen et al 1989, Malikides et al 2003); therefore the cell population sampled by one technique is not representative of that obtained by the other. Additionally, recent studies have shown that inflammation of the airways may be regionally localized; therefore a combination of TA and BAL is more likely to detect airway inflammation.

When there is an indication to perform both procedures, the TA should be performed first, particularly if bacterial culture is to be attempted. This is an important consideration because BAL will result in transient bacterial contamination of the distal trachea with oropharyngeal flora.

Contraindications for TA and BAL include horses or foals with severe respiratory distress, cyanosis, marked hypovolemia or presence of significant dysrhythmias. In these situations the combination of the restraint required for, and stress-induced by, the procedure may have untoward effects. Similarly, horses with known defects in hemostasis should be assessed carefully before being subjected to these procedures.

Pleural effusion is the most common indication for thoracocentesis (see Table 9.1). Pleural effusion may be suspected based on history, clinical signs, thoracic auscultation, and percussion. Confirmation of pleural effusion is made with ultrasound.

Collection Techniques

Nasal, nasopharyngeal, and guttural pouch samples

Lesions within the nasal passages and nasopharynx usually require endoscopic examination for adequate visualization before sampling (Traub-Dargatz 1997). It should be remembered that sedation may distort the nasopharynx as a result of relaxation of the surrounding soft tissues. Therefore, the initial endoscopic examination of this area should be conducted without sedation if possible. Radiography may also help define the extent of a lesion prior to sample collection. Samples from lesions in the nasal cavity, nasopharynx, and pharynx may be evaluated by cytological and histopathological examinations and culture (bacterial or fungal) (Clinkenbeard et al 2002).

Lesions of the mucosal surfaces of the nasal cavity and nasopharynx are more frequently sampled than deeper structures. For lesions close to the external nares, collection of direct imprints, fine-needle aspirates and/or biopsies is possible. Alternatively, superficial lesions such as atheromas may be accessible via percutaneous aspiration. Local secretions may be collected using swabs inserted through the external nares, or using a flexible endoscope. Exudates within these sites may be collected via a transendoscopic catheter. Masses and fungal plaques can be sampled with the endoscopic biopsy instrument.

The varied structures (cartilage, bone, adipose tissue, salivary glands, lymphoid tissue) that underlie the mucosal surfaces may, on occasions, be sampled in the field. Core biopsies, surgical biopsies, or fine-needle aspirates can be used to obtain cells from these structures if pathological processes are suspected.

Microbiological samples

Complete bacteriological examination of nasal or nasopharyngeal samples is rarely warranted because of the presence of a normal bacterial flora. In certain well-defined circumstances isolation of specific pathogens may be indicated; e.g. isolation of *Streptococcus equi* ss. *equi* from suspected cases of strangles. Microbiological samples can usually be collected from the nasopharynx or pharynx without sedation. Nasal washes are more effective than swabs for the detection of small numbers of organisms because a greater surface area of the internal nares is sampled. The technique involves instilling about 50 ml of warm normal saline via a 15-cm piece of sterile soft rubber tubing (5–6-mm diameter) about 12 cm into the internal nares and collecting the washings. These are decanted into sterile containers and centrifuged; the pellet obtained is then cultured. An alternative technique for collection of microbiological samples is the use of swabs. It is preferable to use a guarded swab to sample these sites to collect from the specific site intended and to bypass, as much as possible, the normal flora of the nasal cavity. The latter will contaminate samples and rapidly overgrow any pathogens during culture.

Samples from the URT may also be collected to confirm viral respiratory tract infections. Successful detection of respiratory viruses is influenced by the time of sampling, and techniques used for collection, transport, and processing of samples. In general, success is greatest when nasopharyngeal mucus samples are collected within 48 h of the onset of illness, when the horse is febrile, and the nasal discharge is serous and not mucoid. Rapid transport to, and immediate processing by, the laboratory are critical to optimize chances of virus isolation.

Nasopharyngeal mucus samples can be obtained using a sterile 5 cm by 5 cm Dacron or cotton gauze swab inserted through a loop in a twisted stainless steel wire (60 cm long). Ideally the wire/swab combination is housed in a 40 cm length of soft rubber tubing for protection during insertion. Alternatively a guarded mare uterine swab may be used. The swabs are passed via the ventral nasal meatus, inserting the swab as far back as possible in the horse's nasopharynx (~30 cm in 500-kg horse). Once in the nasopharynx, the swab is advanced out of the soft rubber tubing, rubbed against the nasopharyngeal mucosa, and then retracted into the tubing. After collection, the Dacron or cotton gauze swab should be placed immediately in virus transport media and transported to the laboratory on ice. Viruses are unlikely to survive if swabs are allowed to dry out. Also, there is increased chance of sample contamination if bacterial transport medium is used.

Some respiratory viruses (e.g. equine herpesviruses) may also be isolated from citrated or heparinized whole blood. If blood is collected for attempted virus isolation, at least 20 ml of venous blood should be obtained and transported chilled, but not frozen. Greatest success in virus isolation from blood is usually within 4–10 days after onset of respiratory signs.

Serological tests may be used to detect antibodies to respiratory viruses and therefore confirm infection. In contrast, these tests are rarely useful for diagnosis of bacterial diseases. Acute (taken as soon as possible after the onset of clinical signs) and convalescent (taken at least 10 days later) serum samples should be collected and

submitted to appropriate diagnostic laboratories. Antibody induced by vaccination can confound interpretation of serological results; thus vaccination history must be taken into account to ensure that detected rises in antibody titer reflect the presence of infection rather than vaccination.

Recent advances in the rapid diagnosis of viral respiratory infections have resulted from development of tests that detect the presence of viral antigens, viral nucleic acid, or virus-infected cells in respiratory secretions. Some of these tests are commercially available for field use (e.g. Directigen FLU-A assay) and others can be performed in specialized diagnostic laboratories (e.g. polymerase chain reaction, PCR). Most of these tests require nasopharyngeal swabs or whole blood collected into ethylenediamine-tetraacetic acid (EDTA), but specialized diagnostic laboratories should be contacted for specific requirements if doubt exists. In addition, even when rapid "horse-side" tests are used to diagnose equine influenza, a nasopharyngeal swab should be taken and sent to an appropriate laboratory for virus isolation. This will allow characterization of virus isolates and assist in determination of strains for vaccines.

Sampling from the guttural pouch

Samples may be collected from the guttural pouches for cytological evaluation and/or microbial culture and sensitivity testing. Several important structures are contained within the guttural pouches (Freeman 1991). These structures and their anatomical associations must be kept in mind when sampling this site.

Samples from the guttural pouch may be collected by blind catheterization, but with the ready availability of flexible endoscopes, endoscope-guided catheterization has become the preferred technique. Most clinicians find catheterization easier if the endoscope is placed in the nasal passage opposite to the side of the pouch to be sampled. This allows superior visualization of the pouch opening and therefore easier insertion of the catheter into the pharyngeal orifice under view. Two different methods have been described. One method involves inserting a biopsy instrument or cleaning brush in the biopsy channel of the endoscope as a guide. This guide is extended 2–3 cm beyond the end of the endoscope. The guide is inserted into the guttural pouch opening and the endoscope is rotated to open the guttural pouch flap, allowing the endoscope to be advanced into the guttural pouch. The guide is then removed. The second technique involves the use of a Chamber's mare catheter, which is placed into the guttural pouch and rotated to open the flap. The flexible endoscope is then passed dorsally or ventrally to the catheter and into the pouch with the Chamber's catheter then being withdrawn.

Once access to the guttural pouch has been achieved, samples for cytological, microbiological, or molecular (PCR) evaluation can be collected. These can be obtained by directly aspirating exudate present on the floor of the guttural pouch. If exudate is not present, 20–30 ml of sterile physiological saline should be infused through the tubing and onto the area of interest, with subsequent aspiration of fluid pooling on the floor of the pouch. In situations where concretions (chondroids) are present, these may be sampled using a trans-endoscopic biopsy forceps or basket forceps and subjected to microbial and histopathological examinations.

In horses where guttural pouch mycosis is suspected, lesions (mycotic plaques) are frequently visualized on the dorsal aspect of the pouch, often involving the internal carotid artery and adjacent neural structures. Biopsy of these lesions, although tempting, may place the patient at risk of severe hemorrhage. As a result, diagnosis of guttural pouch mycosis is based usually on history, clinical signs, and the endoscopic appearance of the lesion.

Sampling the lower respiratory tract

Samples from the lower respiratory tract (LRT) are primarily collected for cytological and microbiological evaluation. The three most commonly used techniques for "in-field" sampling of the LRT are TA, BAL, and thoracocentesis. The choice of technique and method of sampling can significantly affect bacterial numbers, cell numbers, and even types of cells obtained. For this reason, standardization of technique with regard to type of technique used, time of sampling (e.g. at rest or after exercise), volume and type of fluid instilled, sample handling and processing is recommended.

The timing of sample collection may influence results. Large increases in numbers of bacteria and inflammatory cells in the LRT can occur within 6 h of restricted head movement (cross tying, head elevation, transportation) (Raidal et al 1995). This accumulation is generally cleared within 12 h of horses being released from confinement, although clearance may be prolonged if horses are concomitantly dehydrated. This change in numbers of cells and bacteria must be considered when interpreting the results of samples collected from horses transported long distances before collection of samples. Similarly, collection of samples after exercise can influence results. Samples collected 30–60 min after moderately intense exercise may yield specimens of greater diagnostic value than resting samples (Martin et al 1999, Malikides 2004). Samples obtained at this time are more likely to contain extra secretions, more likely to adequately represent different areas of the respiratory tract, and are therefore more likely to reveal the presence of airway disease if present.

Tracheal aspirates

Several methods for obtaining cytological and microbiological samples from the tracheobronchial tree have

been developed; each having advantages and disadvantages. The most important consideration when choosing a technique is whether microbiological culture of the tracheobronchial secretions is indicated, such as in cases of suspected pneumonia. Aspirates obtained endoscopically invariably become contaminated by upper airway flora and therefore are unsuitable for microbial culture unless specific precautions are taken. Use of a double- or triple-guarded catheter passed through the biopsy port of the endoscope may circumvent this problem. Alternatively, the transtracheal (percutaneous) aspiration technique may be used.

A variety of needle/catheter combinations may be used to perform transtracheal aspiration, but maintaining asepsis is critical. The catheter/needle combinations may be purchased individually or pre-packaged. The latter usually include a catheter-over-needle (± stylet) and flushing/aspiration catheter. An alternative, but convenient, combination comprises a 12-gauge needle, 7.5-cm over-the-needle cannula and no. 5 French canine urinary catheter with the tip cut off obliquely. Sedation is generally indicated when performing a transtracheal aspiration, with α_2-agonists being commonly used. An area measuring approximately 6×6 cm over the middle third of the cervical trachea should be prepared for aseptic surgery. A bleb of local anesthetic (~0.5 ml 2% lidocaine) is injected subcutaneously over the midline and a stab incision is made through the skin and subcutaneous tissue with a No. 15 scalpel blade. The trachea is stabilized with one hand and the needle/cannula combination is introduced into the tracheal lumen between two cartilage rings. The needle (± stylet) is removed and the urinary catheter is passed down into the tracheal lumen to the level of the thoracic inlet where the washing and aspiration is performed. A cough response may be elicited upon stimulation of the tracheal mucosa. This may cause the catheter to retroflex into the proximal trachea and result in contamination or inadvertent sampling from the URT.

Usually 20 ml of sterile isotonic saline is instilled to obtain a satisfactory sample. However, larger volumes may be required in some cases to ensure sufficient sample recovery. In the adult horse, flushing and re-aspiration can be repeated three or four times if necessary to obtain a suitable sample. Other methods to facilitate retrieval of infused fluid include slow retraction of the catheter while applying gentle suction, or encouraging the horse to lower its head slightly to facilitate more proximal pooling of fluid. Once an adequate sample has been collected, the catheter should be withdrawn, maintaining the cannula *in situ* during this retraction to minimize contamination of peritracheal tissues.

An increasingly popular alternative for collection of TAs is via a fiberoptic endoscope or videoendoscope (see Fig. 9.1A,B). This technique has largely replaced the percutaneous technique because it can be performed less

invasively and has fewer side effects (Mair 1987). The method is well tolerated by horses and endoscopy allows visualization of the LRT at the time of sampling. Evaluation of the tracheal mucosa (degree of hyperemia) and its luminal contents (quantity and quality of mucus, mucopus, and blood) may assist in interpretation of cytological and microbiological results. Furthermore, if the length of the endoscope permits, the large bronchi may be visualized and purulent debris draining from a specific bronchus suggestive of pulmonary abscess may on occasions be recognized and sampled. However, it should be re-emphasized that samples collected using unguarded catheters will inevitably be contaminated with bacteria from the nasopharynx and biopsy channel, making them unsuitable for microbial culture.

To perform TA via an endoscope, the lowest point of the trachea, anterior to the carina and level with the thoracic inlet ("tracheal puddle or sump"), should be visualized.
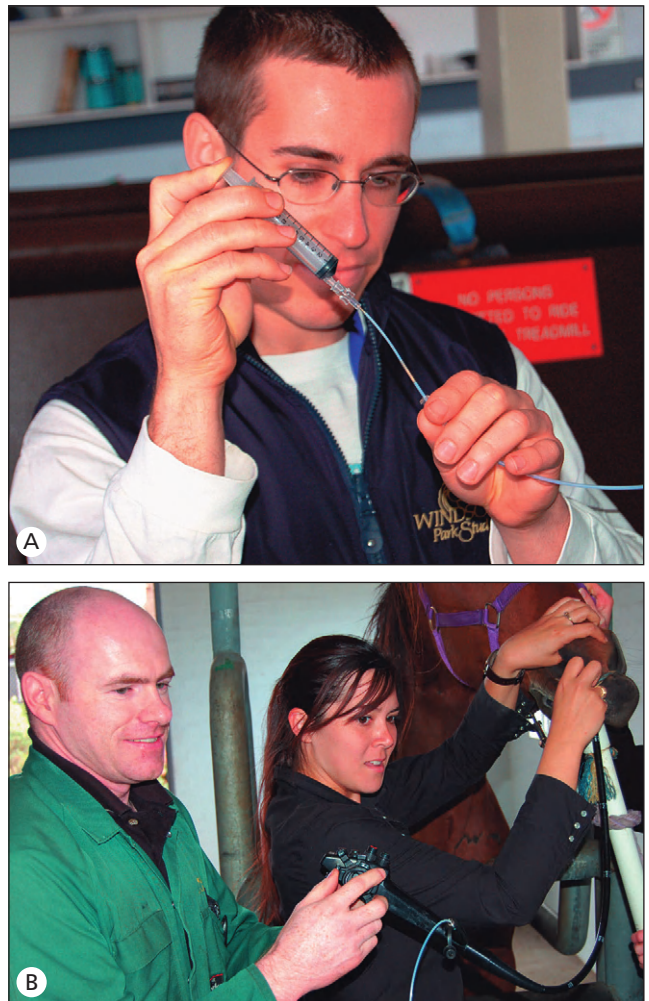
Fig. 9.1. (A) Tracheal aspirate being performed using a guarded catheter inserted via the biopsy port of a fiberoptic endoscope. (B) Tracheal aspirate being performed using a videoendoscope.

Continued
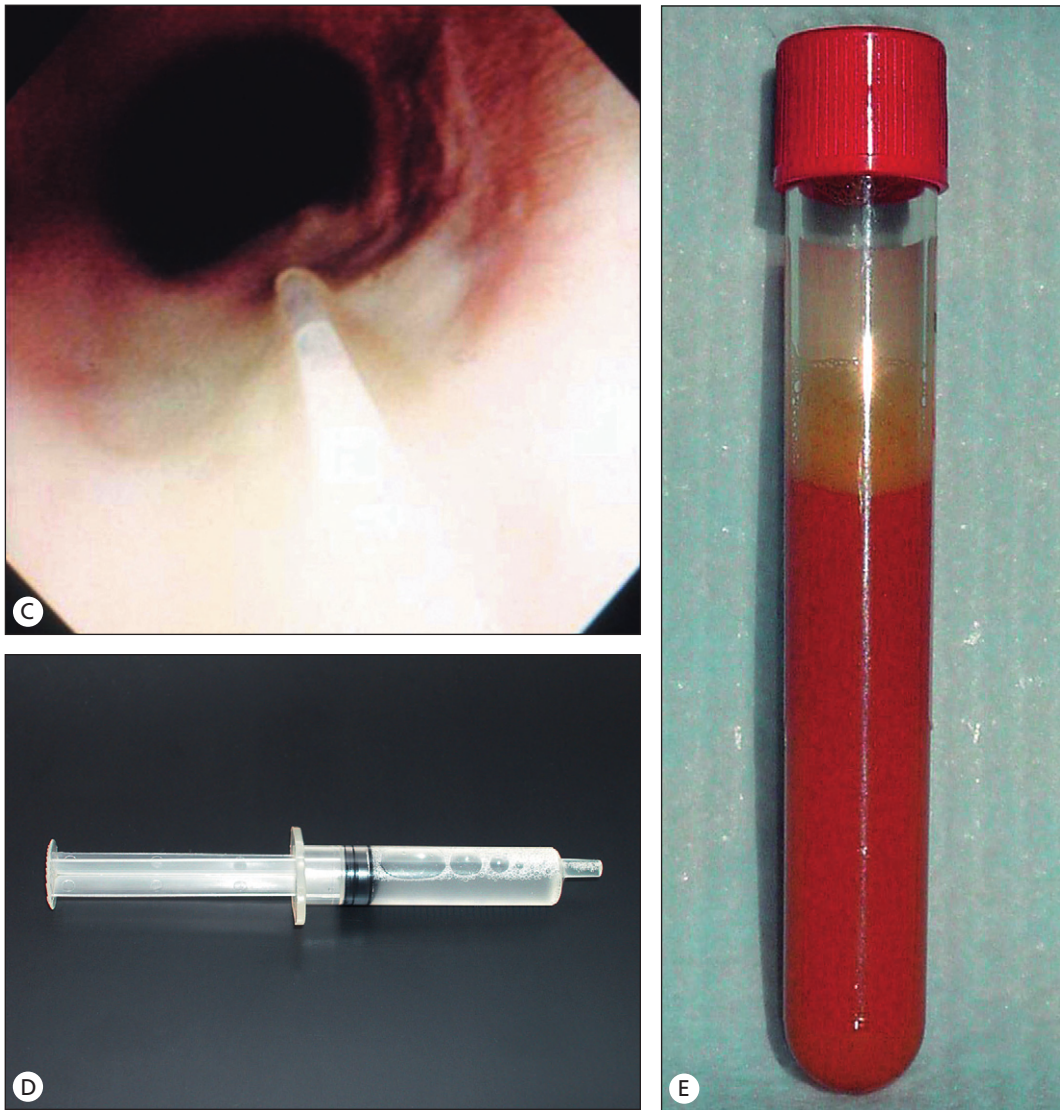
Fig. 9.1, cont'd. (C) Tracheal aspirate showing the tip of the aspiration catheter immersed in the "tracheal puddle." (D) Normal tracheal aspirate demonstrating a clear fluid with few mucous strands. (E) Tracheal aspirate from a horse with severe pneumonia showing turbid, reddish-brown fluid.

A small polyethylene catheter is passed through the biopsy channel and 10–15 ml of sterile isotonic saline is instilled. Fluid will accumulate in the "tracheal puddle" from where it can be aspirated (see Fig. 9.1C). The principal use of samples collected using this technique is for cytological evaluation.

More recently, a number of guarded catheter systems have been developed for collection of uncontaminated samples from the LRT via endoscopy. One example is a multi-lumen, telescoping catheter that contains a glycol plug in the outer catheter to maintain sterility as the catheter is advanced through the endoscope and into the trachea. Once in place, the plug is ejected and the inner catheter is advanced for aseptic retrieval of the specimen.

Another guarded catheter system involves a 5-gauge French inner catheter within an 8-gauge French guiding catheter. Again, these guarded catheters are passed through the biopsy channel and samples are aspirated whilst directly visualizing the collection site. Thorough disinfection of the endoscope, including the biopsy channel, is essential before each collection. The risk of sample contamination with organisms such as *Pseudomonas* spp. increases substantially if disinfection is not performed. For this purpose, 2% chlorhexidine in 70% alcohol is an effective disinfectant provided that the endoscope, including the biopsy channel, has 10–15 min contact time prior to use. The endoscope and biopsy channel should then be flushed thoroughly with sterile saline before sampling.

Although the advantages of guarded catheters are many, there remains some controversy regarding the adequacy of these samples for microbiological culture. Technical prowess definitely influences the quality of the sample obtained and factors that help prevent contamination include:

- rapid sample collection
- small volume of instilled sterile isotonic saline (10–15 ml)
- advancement of only the inner (sterile) catheter into the "tracheal puddle."

In addition, if the horse coughs frequently during collection, the TA has an increased risk of contamination with oropharyngeal organisms and is rarely appropriate for bacteriological culture. Finally, isolation of upper airway contaminants or transient bacteria, which are not causing inflammation, is possible with any method of aspiration and correlation of bacteriological, cytological and clinical findings should always be performed. Identification of the presence of squamous epithelial cells and inflammatory cells, quantification of the numbers of bacteria and differentiation of bacterial species is essential when attempting to determine the significance of the cultured isolates.

Bronchoalveolar lavage

Bronchoalveolar lavage collects samples from the distal airways and alveoli and is most frequently used for diagnosis of diffuse and/or chronic disease processes. In these cases, samples collected from either side of the lung are considered representative of the entire lung (McGorum et al 1993a). This constitutes the basis for the use of a "blind" field technique using a flexible, cuffed nasobronchial tube (see Fig. 9.2A). Alternatively, BAL may be collected using an endoscope, which allows more specific selection of the site for lavage, especially when there is suspicion of localized lung pathology. Samples obtained by BAL are most commonly evaluated cytologically, although culture of samples, on rare occasions, may also be indicated.

BAL is usually performed in the standing animal following mild sedation with an α_2-agonist. Concurrent administration of butorphanol tartrate (0.01–0.02 mg/kg body weight) may be used in horses with severe clinical signs of heaves and marked airway hypersensitivity to ameliorate the cough response. Application of a twitch is recommended and cleaning the external nares with moistened gauze before the procedure helps to reduce contamination.

The "blind" field and endoscopic techniques for BAL are technically similar except that endoscopy permits visual inspection of the airways prior to BAL, guidance of the endoscope during collection, and the use of biopsy tools (Hoffman & Viel 1997). Any accumulation of secretions or

blood within the trachea should be recorded and described as to the location, quantity, and character/color. If a pulmonary abscess is suspected, the bronchial segment of the lung from which the mucopus is emanating should be identified for later sampling.

The bronchoscope should optimally have a working length of at least 160–180 cm to allow lavage of the distal lung segments of adult horses. The depth of lung ultimately lavaged is dependent on the outer diameter of the endoscope. Bronchoscopes with a larger outer diameter (10–13 mm) will generally wedge within a fourth- to sixth-generation bronchus and allow recruitment of cells from a larger number of airways and alveoli (Hewson & Viel 2002). In contrast, a bronchoscope with a small outer diameter, or use of a catheter passed through the biopsy channel, results in lavage of only a limited number of small airways and alveoli because wedging occurs in a more peripheral bronchus or bronchiole.

Coughing may occur during passage of the endoscope near the carina because of the stimulation of cough receptors. In most normal horses this response abates within 5–10 seconds. In contrast, horses with airway inflammation and hypersensitivity (particularly heaves) may have a more prolonged period of coughing which may be alleviated by instillation of local anesthetic. Pre-warmed, 0.4% lidocaine (12 ml 2% solution diluted with sterile water to 60 ml) can be instilled onto the carina when the endoscope is advanced to this point. This decreases the cough reflex and after approximately 30 seconds the endoscope can be further advanced into the lower airways until a wedge is obtained in a segmental bronchus. This is detected as resistance to gentle attempts at further endoscope advancement. Once the bronchoscope is wedged, BAL can be performed using pre-warmed sterile normal saline. The fluid is instilled in two or three aliquots via the biopsy channel of the bronchoscope and retrieved using either gentle suction with 60-ml syringes or using a suction apparatus set at a pressure of -5 to -15 cmH$_2$O. Excessive negative pressure should be avoided because it can cause collapse of the airway and trauma to the respiratory epithelium resulting in decreased fluid recovery and hemorrhage into the sample. The volume of infused fluid impacts on the total and differential cell counts of the resulting BAL sample (Sweeney et al 1992). Smaller volumes typically yield a bronchial wash without retrieving cells from the alveolar space and therefore have a higher percentage of neutrophils. Larger volumes yield samples more representative of the respiratory epithelial lining fluid within the alveoli. A standard volume of 250–500 ml is currently recommended (Robinson 2001). In general, 50–80% of the instilled fluid can be retrieved, although smaller proportions may be obtained from obstructed airways because airway edema and bronchospasm result in lumen obstruction during suctioning. It is important to observe the presence of white
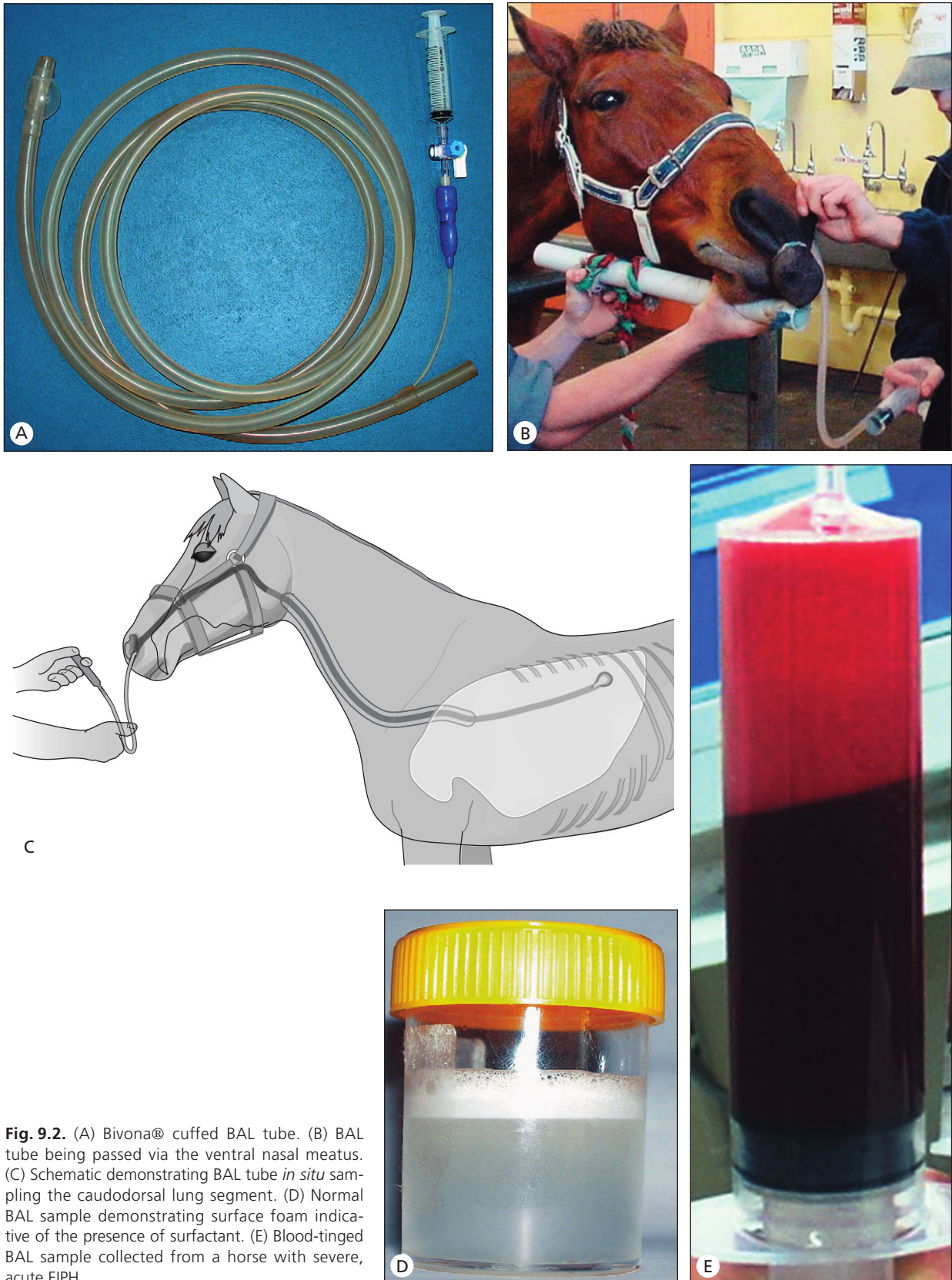
Fig. 9.2. (A) Bivona® cuffed BAL tube. (B) BAL tube being passed via the ventral nasal meatus. (C) Schematic demonstrating BAL tube *in situ* sampling the caudodorsal lung segment. (D) Normal BAL sample demonstrating surface foam indicative of the presence of surfactant. (E) Blood-tinged BAL sample collected from a horse with severe, acute EIPH.

foam (surfactant) on the surface of the sample as this indicates that alveoli have been sampled (see Fig. 9.2D).

Use of the "blind" BAL catheter technique is an alternative to bronchoscopy in the field if a suitable endoscope is not available. The non-endoscopic collection of BAL uses a flexible, cuffed nasobronchial tube with an outer diameter of 11 mm. With the horse's head extended the nasobronchial tube is passed through the nasopharynx into the trachea until a cough response is elicited (see Fig. 9.2B). As with the endoscopic technique, use of a topical anesthetic agent at this time may alleviate prolonged coughing. The tube is then pushed into the lungs "blindly" until an adequate wedge is detected as resistance to gentle attempts for further advancement. In most cases the tube will naturally travel to the right caudodorsal lung field because of the anatomy of the main-stem bronchus (see Fig. 9.2C). The cuff is then gently inflated using 5–10 ml of air, thus forming a complete wedge to prevent the backflow of infused fluid. Again, 250 ml (and up to 500 ml) of pre-warmed sterile saline (5 × 50 ml) is infused using 60-ml syringes, with 100 ml first infused and withdrawn, followed by each additional 50–100 ml being infused and withdrawn. The samples are pooled at the end of collection where aspiration should yield 60–80% of the instilled fluid.

Complications of BAL are minimal. A neutrophilic inflammatory response occurs within the lavaged lung, and can be detected if subsequent lavages are performed within 48 h (Sweeney et al 1994). This response is usually limited to the bronchus and lung segment lavaged, but occasionally may be noted also in the contralateral lung. On occasion a mild increase of rectal temperature has been reported in horses for less than 24 h after BAL, with no apparent adverse clinical effects. However, any persistent or exaggerated pyrexia accompanied by signs of depression should prompt further evaluation.

Thoracocentesis

Thoracocentesis can be performed in the standing horse with minimal restraint. Horses often have a fenestrated mediastinum, so PF collected from either side of the thoracic cavity should be similar. However, in pathological conditions mediastinal fenestrations may become obstructed with fibrin resulting in the two sides of the thorax being affected to different degrees. This will be reflected in differing physiochemical properties of PF from each hemithorax. Thus, in cases where pleural pathology is suspected, samples should be collected from both sides of the chest. The location of PF for sampling is best determined by ultrasound examination of the pleural spaces. If ultrasound equipment is not available, thoracocentesis can be performed using a "blind" technique based on anatomical landmarks.

Thoracocentesis should always be undertaken using aseptic technique regardless of the requirements of the sample. A 4 × 4-cm area of the thorax should be surgically
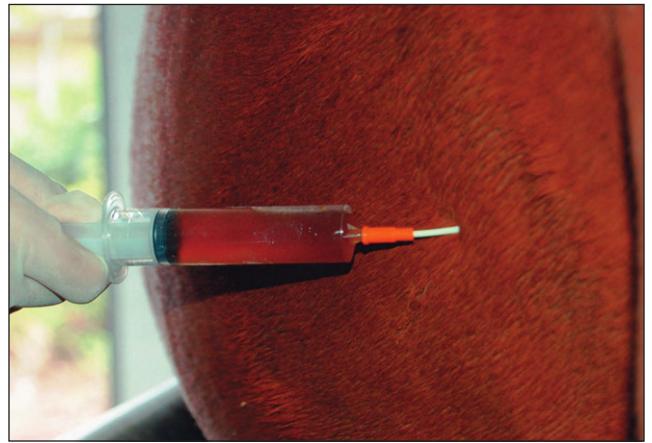
Fig. 9.3. Right-sided thoracocentesis using a 14-gauge Teflon catheter and 20-ml syringe in a horse with bacterial pleuropneumonia. Note reddish-brown, turbid fluid.

prepared. If the "blind" technique is used, the major objective is to insert the needle at a site sufficiently caudad to the heart, yet obtain access to the more cranioventral regions of the thorax. On the right hand side this site is located over the sixth or seventh intercostal spaces up to 10 cm dorsal to the level of the olecranon. On the left side, the sixth to ninth intercostal spaces may be selected, 4–6 cm dorsal to the olecranon. The needle or catheter should be inserted just anterior to the cranial margin of the rib as this reduces the risk of laceration of the intercostal vessels and nerves. Infiltration of local anesthetic (10–20 ml 2% lidocaine or prilocaine) into the subdermis, intercostal muscles, and, most importantly, the parietal pleura is regarded as essential to decrease the pain associated with this procedure. The parietal pleura have a high density of pain receptors that are irritated with pleural inflammation. Local anesthesia reduces pain and patient movement thereby increasing the likelihood of obtaining a diagnostically useful sample.

Although a variety of needles and cannulas are suitable for thoracocentesis, a 14-gauge, 13.3-cm Teflon catheter is ideal (see Fig. 9.3). Alternatively, a sterile teat cannula can be used, although a stab incision of the skin and muscle should be made to facilitate its insertion. Prior to insertion, a 10 or 20-ml syringe should be connected to the catheter/cannula. Entry of the catheter/cannula through the parietal pleura may be recognized by a palpable 'popping' and subsequent decrease in resistance to insertion. If using a Teflon catheter the needle stylet should be removed at this stage, then gentle aspiration performed (see Fig. 9.3). Alterations in the depth to which the catheter is inserted may be required to obtain fluid. In general multiple punctures of the parietal pleura should be avoided as this may result in leakage of PF within muscle planes and subcutaneous tissues causing localized cellulitis.

Using standard techniques, 2–8 ml of PF may be obtained from many normal horses, although no fluid is also common. If pleural effusion is present a sample can often be readily obtained on the first attempt. Only 1–2 ml of fluid is usually required for a total nucleated cell count, cytological examination, total protein measurements, and bacteriological testing (if required). However, if no fluid is collected another site may be selected or a sterile 30-cm bitch catheter or longer thoracic catheter can be used. Difficulty in collecting a sample usually indicates that the volume of PF is not increased, an incorrect site has been sampled, or that the effusion is loculated rather than diffuse. In these cases use of ultrasonography is encouraged to guide thoracocentesis. Changes to PF, especially red discoloration, occurring during collection indicate peripheral blood contamination and should be taken into account during interpretation.

Pleural fluid should be collected into an EDTA tube for cell counts, cytological analysis, and total protein measurements (refractometric) (DeHeer et al 2002). A subsample should also be deposited into plain (serum) tubes for biochemical analysis and bacterial culture. Alternatively, an appropriate liquid culture medium (e.g. blood culture media) may be inoculated directly after collection because this will facilitate isolation of causative bacteria, especially strict anaerobes. If this culture system is not available to the practitioner, at least one plain tube should be filled completely with PF to remove all air from the tube for subsequent anaerobic culture of the fluid. Ideally, the reference laboratory should be contacted for recommendations on handling and transportation of these samples.

Complications of thoracocentesis are not common. Improper technique may result in pneumothorax, lung laceration, hemothorax, cardiac dysrhythmias, or puncture of bowel, liver and heart. Mild pneumothorax resulting from aspiration of air through the catheter/cannula rarely causes clinical signs with small volumes of free air (up to 200 ml) in the thoracic cavity being resorbed rapidly. Occasionally, localized cellulitis surrounding the thoracocentesis site may occur.

Sample Handling, Transportation, and Processing

Sample handling

As samples from the respiratory tract are often collected in the field, there is a necessary delay whilst samples are transported to the laboratory. Every effort should be made to transport these samples in a timely manner to prevent deterioration of cellular morphology and bacterial proliferation. Minimal cellular deterioration will occur if processing occurs within 8 h of collection when samples are stored at room temperature (Pickles et al 2001). This can be extended to 24 h if samples are stored at 4°C. In addition, bacterial overgrowth of samples rarely occurs over 24 h in samples stored at 4°C. In contrast, samples that are not refrigerated are at risk of rapid bacterial overgrowth with pathogenic or contaminant organisms, potentially confusing interpretation. Finally, strictly anaerobic bacteria, often isolated from horses with pneumonia, rarely survive any storage regimen, particularly if at cooler temperatures.

If longer delays are anticipated or refrigeration of the sample is not possible, samples for cytological evaluation may be of diagnostic use if a subsample is diluted in an equal volume of fixative solution. In these cases the laboratory where the sample is to be processed should be contacted to determine which fixative is preferred, as the subsequent staining technique will influence the choice of fixative. In addition, a portion of the sample should be left undiluted in an EDTA tube for total red blood cell (RBC) and nucleated cell counts.

Samples for microbial culture should always be processed as soon as possible. Realistically, the time between sample collection and processing may range from minutes to hours, but delays of longer than 24 h are likely to preclude valid microbiological results. Therefore culture after this period is not recommended. Sample drying (all microorganisms), exposure to a noxious atmosphere (oxygen for obligate anaerobes), and bacterial overgrowth are the major concerns if samples are not analyzed promptly. Moistness may be maintained in swabs by placing them in transport medium composed of a balanced salt solution, usually in a gelled matrix. Because this medium does not contain any nutrients, microorganisms in the sample multiply poorly and therefore relative numbers and ratios are preserved. Some organisms will remain viable for up to a day, but the exact duration of survival will depend on the microorganism involved. For example, β-hemolytic streptococci do not survive as long as *Escherichia coli*. This must be taken into consideration when interpreting culture results. Samples placed in fluid culture media may support bacteria for longer periods but the original relative proportions of bacteria will be lost because of the differential growth rates of bacterial species. Those samples suspected of containing strictly anaerobic bacteria (e.g. pleural effusions, lung abscesses) should be cultured immediately if these bacteria are to be recovered. Swabs are an inferior method of transport for anaerobes compared to fluid media. However, if swabs are used for collection of samples, one swab should be placed in an anaerobic transport medium and maintained at room temperature. Do not refrigerate swabs suspected of containing strict anaerobes because some species do not tolerate reduced temperatures.

Specimens submitted for viral isolation should be placed in virus transport media in sealed containers for safety during transport. Submitted tissues should be stored on ice (4°C), or alternatively may be frozen (−20°C) if

histopathology is not required because freezing destroys tissue morphology. Tight plastic wrapping of the specimens should be avoided because it enhances autolysis and necrosis, which decrease the concentration of infectious viral particles. All samples submitted to a diagnostic laboratory should be clearly identified.

Slide preparation and staining

In all situations it is optimal if smears are made as soon as possible after sample collection. This is recommended because the smears will serve as a reference point for cellular morphology and microbial populations at the time of sampling. Smears should be submitted to the laboratory with all other samples.

Smears are made from swabs by gently rolling the swab across a clean glass microscope slide and allowing it to air dry. Rolling the swab reduces the risk of rupturing cells, which often occurs if the swab is rubbed or dragged across the slide. Samples collected by brushing of mucosal surfaces can be gently impressed on the slide. Similarly, rubbing or dragging the brush across the slide surface should be avoided. A variety of techniques can be used to make smears from fine-needle aspirates and biopsies. These techniques are beyond the scope of this chapter and readers are referred to the extensive coverage provided in texts on diagnostic cytology (Cowell & Tyler 2002).

Samples collected using washes (e.g. nasal or guttural pouch washes, TA or BAL) should first be assessed grossly to determine the amount of mucus, mucopus or blood present before making smears. If the sample is clear, or contains only few strands of mucus, centrifugation is required before smear preparation because of its low cellularity. Cells can be harvested by centrifugation of approximately 1–5 ml of sample for 5 min at 350 *g*. The supernatant fluid is poured off, the pelleted material is gently resuspended, and a drop of suspension is applied to a clean glass slide with an applicator or pipette. The sediment can then be spread using a "blood (line) smear technique". Alternatively, cells may be concentrated using a cytocentrifuge or using the perspex block and cell sedimentation technique (Nicholls & Pirie 2001). The latter technique yields slightly greater lymphocyte numbers than cytocentrifugation, produces excellent quality slides for interpretation of cell morphology and is reported to be more suitable for use in the field.

If the sample is turbid, contains many mucus strands, or is dark red, a direct smear may be prepared from either the freshly collected sample or the fixed diluted sample after thorough mixing. Dilutions of a turbid TA may also be performed using sterile isotonic saline, and the diluted sample can be processed by cytocentrifugation. Addition of saline to thick tenacious samples helps to dilute the cellular and mucous elements, making interpretation easier. All smears should be air-dried as rapidly as possible to prevent alterations in cell morphology; a small desk-top fan will assist rapid drying.

A variety of routine hematological stains may be used if cytological preparations are to be examined within the practice setting. In general, the use of a simple stain such as Diff Quik is sufficient for routine analysis of samples from the respiratory tract, although Wright–Giemsa, May–Grünwald or Leishmann's stains also may be used. In more complex cases, the use of special stains may be indicated. Special stains can assist with further identification of cytological elements. Examples include Gram stain for bacteria, non-specific esterase (α-naphthyl acetate esterase) to differentiate immature macrophages from large lymphocytes, and Perl's Prussian blue for hemosiderin. In addition, the metachromatic granules of equine mast cell are refractory to modified Wright–Giemsa stains (Diff Quik) and require specific staining (e.g. Leishmann's or Toluidine Blue).

Finally, when utilizing outside laboratories, it is best to determine in advance of specimen collection any special requirements for sample storage, shipment, and submission favored by the particular laboratory. These requirements will be influenced by the analyses requested. In most instances, air-dried, direct or concentrated line smears of fluid, together with aliquots in EDTA and plain tubes, and samples in culture transport media are appropriate.

Evaluation

Gross examination

The gross appearance of fluid samples (TA, BAL or PF) should be assessed initially for turbidity, presence of flocculent debris, odor, clot formation, and color. In addition, BAL fluid (BALF) should be examined for a surface layer of foamy surfactant indicating that the sampling process was adequate (see Fig. 9.2D).

Normal TAs, BALF and PF should appear clear or mildly turbid (see Figs 9.1D, 9.2D). Turbidity of TAs and BALF from horses with clinical respiratory disease ranges from clear to opaque (see Figs 9.1D and E, 9.2D and E). Increased turbidity and the presence of flocculent material reflects increased mucus, cells and cellular debris. Occasionally, pieces of plant material or debris may be observed in TAs and BALF if the horse had undertaken strenuous exercise before sampling. Increased turbidity of PF reflects increased cellularity and protein content. Flocculent material visible within PF usually comprises strands of fibrin. Rarely ingesta may be observed in PF secondary to either accidental enterocentesis or gastrointestinal rupture.

All fluids should be odorless normally. A putrid smell may be associated with anaerobic infections or necrosis of lung tissue and is consistent with a guarded prognosis. However, the absence of a foul odor does not rule out disease processes.

Pleural fluid from healthy horses contains a negligible quantity of fibrinogen and will not clot. Blood contamination of, or protein exudation into, PF will increase fluid fibrinogen content resulting in clot formation when the sample is exposed to air.

Tracheal aspirates and BALF are normally clear or colorless, whereas PF is pale yellow. Color of the fluid samples in clinical cases will vary with numbers and relative proportions of erythrocytes and nucleated cells and presence of biochemical constituents such as hemoglobin or lipid. Thus, color is a useful gauge because it may indicate the type of underlying pathology. For example, shades of pink to red will occur with the presence of red cells or free hemoglobin in the specimen. Tracheal aspirates or BALF from horses with recent EIPH may appear pink or red (see Fig. 9.2E), whereas more long-standing hemorrhage may result in brown-tinged fluid because of the presence of hemosiderin. Reddish brown, port wine, or muddy-colored PF may be associated with ischemic tissue injury, necrosis, or neoplasia. Milky or opalescent discoloration of PF is the result of increased nucleated cells or elevated lipid content, as occurs in chylous and pseudochylous pleural effusions. Exudative pleural effusions are more likely to be discolored and turbid, attributable to their increased cellularity and protein content. In these circumstances, it is diagnostically useful to examine the sample's sediment and supernatant fluid. In the field this can be done by allowing the fluid to sediment by gravity, whilst in the laboratory microhematocrit or routine centrifugation can be used. The height of the sediment in the tube is usually proportional to the cellularity of the fluid, while the color varies according to the relative numbers of RBCs and nucleated cells present. The sediment may be red when RBCs predominate or brown to off-white or gray when nucleated cells dominate. Red, reddish-brown, amber or brown discoloration of the supernatant fluid usually reflects damage to RBCs that occurred before collection.

When PF grossly resembles whole blood, comparison of packed cell volume (PCV) of the fluid with venous blood should be performed. In addition, determination of clotting times and cytological appearance may be useful to determine the source of the blood (venous versus thoracic cavity).

Biochemical evaluation

Biochemical evaluation is routinely performed only on PF, and not on TAs or BALF. Generally, protein content is measured, though a variety of additional biochemical variables may be determined when specific pathological conditions are suspected.

- **Total protein content** of PF is routinely measured using a refractometer on samples collected into EDTA. Chemical methods (e.g. biuret technique) may be used,

though mostly only in reference laboratories. The total protein concentration of normal PF is < 25 g/l (2.5 g/dl) (DeHeer et al 2002). With small sample volumes (i.e. EDTA tubes are less than a quarter full), erroneous results for fluid protein concentrations and cell counts may be obtained. Protein concentrations as determined by refractive index may be artificially increased (solute effect of the EDTA), whereas protein concentrations as determined chemically (e.g. biuret technique) may be artificially decreased (dilutional effect).

- **Glucose and lactate concentrations and pH** may be measured to assist in differentiating septic from non-septic pleural exudates. If these analytes are to be assayed, samples should be collected into tubes containing fluoride–oxalate to prevent cellular metabolism of glucose. Water-soluble molecules of low molecular weight, such as glucose and lactate, readily diffuse from the circulation into PF, resulting in similar concentrations in blood and PF in normal horses. Increased anaerobic glycolysis by metabolically active cells (leukocytes or neoplastic cells) or bacterial organisms may decrease glucose concentrations, increase lactate concentrations, and decrease pH. Consequently, decreased PF glucose (< 0.4 g/l or 40 mg/dl), increased lactate (greater than a paired blood sample) and decreased pH (< 7.0) are considered by some to be useful in predicting sepsis, even in horses where PF microbial cultures demonstrate no growth. Pleural fluid glucose concentrations > 0.6 g/l (> 60 mg/dl) may be interpreted to suggest an uncomplicated (non-septic) pleural effusion (DeHeer et al 2002).

- Pleural fluid **triglyceride and cholesterol concentrations** are useful in distinguishing chylous from pseudochylous effusions. Chylous effusions are characterized by triglyceride concentrations greater than, and cholesterol concentrations less than, paired serum values. Conversely, elevated PF cholesterol and low triglyceride values are expected in pseudochylous effusions.

Mucus

Evaluation of the quantity of mucus within a TA or BAL is best performed in conjunction with endoscopy of the lower airways. This will facilitate accurate estimation of the amount of mucus present in the airways, as opposed to just the quantity of mucus collected. Endoscopic evaluation of the quantity and quality of mucus in the lower airways is described elsewhere in this book (see Chapter 5).

The mucociliary clearance mechanism in normal horses is efficient, such that mucus elimination keeps pace with production (Whitwell & Greet 1984). Consequently, the healthy LRT contains little or no mucocellular material and low numbers of free cells. Tracheal aspirates and BALF from normal horses are translucent and light gray, with a few fine strands of clear mucus, which may appear as

flocculent material. Cytologically, scant amounts of mucus should be observed, which appears as fine strands of pink to light blue amorphous or fibrillar material on Diff-Quik-stained preparations.

In cases where increased amounts of mucus are observed endoscopically, cytological evaluation is essential for accurate interpretation. For example, horses with a history of chronic coughing may have TAs that appear grossly mucoid and gray-white, giving the impression of septic bronchitis. However, cytology frequently demonstrates large numbers of active macrophages in a copious amount of mucus, with insignificant numbers of bacteria cultured (Beech 1991).

The quantity of mucus in the lower airways increases with pulmonary irritation. In these cases the TAs or BALF will contain variable amounts of thicker, more tenacious, gray to cream mucus. Cytologically, the mucus may be thick and inspissated (deeply basophilic staining) or form airway casts (Curschmann's spirals). These may be found in increased numbers in chronic conditions and in severe suppurative inflammatory disease, although low numbers may be observed in normal horses. Additionally, trapped, degenerating leukocytes may be observed within thick mucus strands.

Cell counts

Quantification of the total number of cells/ml of sample retrieved may help indicate overall cellularity as well as assisting interpretation of relative numbers of individual types of inflammatory cells. However, a number of factors will influence the accuracy of these counts. Total nucleated cell counts (TNCC) are accurate when samples are obtained without prior infusion of fluid, such as for PF. In contrast, only an estimate of the cell count within the lung secretions can be deduced from TA and BALF cell counts as the infused saline dilutes cell concentrations. Furthermore, the saline dilution factor varies depending on the amounts of saline infused and retrieved. A number of techniques have been developed to estimate the effect of saline dilution including the urea dilution technique (McGorum et al 1993b). However, urea concentration can increase markedly in alveolar fluid with pulmonary inflammation affecting the accuracy of results. Alternatively, a crude method to control for saline dilution is through routine use of standard techniques and repeatable sites for performing lavages/aspirates, combined with attempts to ensure 40–80% of the instilled fluid is retrieved (Hewson & Viel 2002). Finally, large amounts of mucus will also influence the TNCC because of the trapping of cells.

Despite these shortcomings, TNCC and RBC counts should be performed as precisely as possible using either a Neubauer hemocytometer counting chamber or an automated cell counter. If an automated cell counter is used, flocculent samples may be filtered to remove excess mucus strands and other debris before analysis. However, filtering of TAs or BALF can cause a significant reduction in TNCC as well as selective loss of specific cell types including epithelial cells, macrophages and mast cells (Hewson & Viel 2002). In addition, results from automated cell counters should be interpreted with caution as the broad range of cell sizes and clumps of epithelial cells within the sample may be outside the calibration range of the instrument. Total nucleated cell and erythrocyte counts of PF are performed as for a blood sample.

The TNCC varies appreciably in TAs and BALF based on the collection technique and the method used to estimate cell counts. In addition, laboratories may have variable ranges for TNCC depending on the horse population they test. In general, a TA from a clinically normal horse usually contains $<10^9$ cells/liter, with few to no RBCs present. The reference range for BAL fluid is usually $<10^9$ cells/liter, though an upper limit of 4×10^8 cells/liter is used in some laboratories (Hewson & Viel 2002). The TNCC for normal PF should be less than 8×10^9 cells/liter and is frequently much lower (DeHeer et al 2002).

Cytology

Cytological evaluation of samples obtained from the respiratory tract is reliant on an appropriate knowledge of the cells routinely present in various sites and their normal morphological appearance. In addition, determining the relative proportions of these cells aids the interpretation of pathological changes. Relative proportions are determined by performing a differential cell count on at least 200–300 consecutive cells. Additionally for PF, the percentage values should be related to the TNCC, total protein concentration, and volume of fluid present to optimize an accurate interpretation.

Before undertaking a differential cell count, the entire slide should be examined under low (10× objective) and high (40× objective) power to identify regional discrepancies. These commonly occur in association with cells trapped in mucus strands and between the base and the tip of a slide. If discrepancies are detected, all regions of the slide should be included in the differential cell count. When assessed by an experienced cytologist, evaluation of a slide under the 40× objective is usually sufficient to be able to evaluate individual cells for their cytological features including nuclear chromatin pattern and presence of nucleoli. Practitioners may find performing the final differential cell count under oil immersion (100× objective) easier to more accurately identify the specific morphological characteristics of each cell and to detect any intra- or extra-cellular microorganisms.

Tracheal respiratory secretions are an accumulation of discharges from all areas of the lung, with variable delay in their transit to the trachea. These delays may be marked when pulmonary disease is present because of decreased
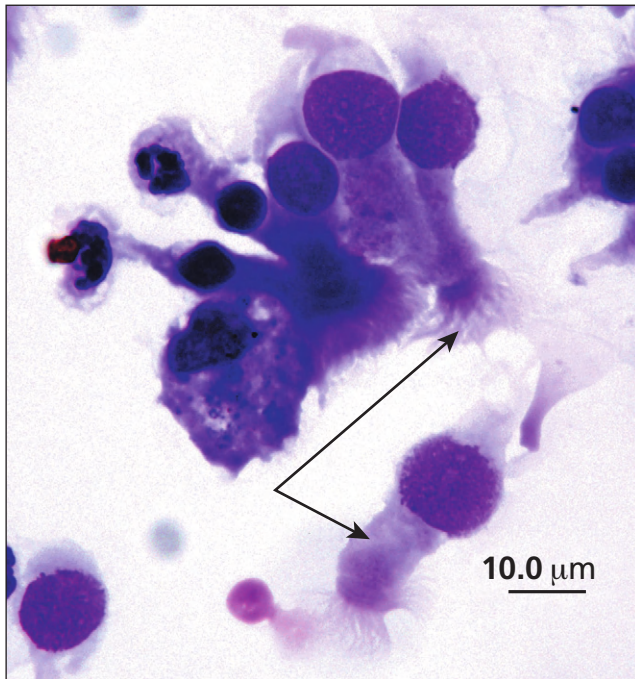
Fig. 9.4. A small cluster of ciliated columnar epithelial cells. Note hair-like cilia extending from apical ends of cells (arrows). Diff Quik (×400).
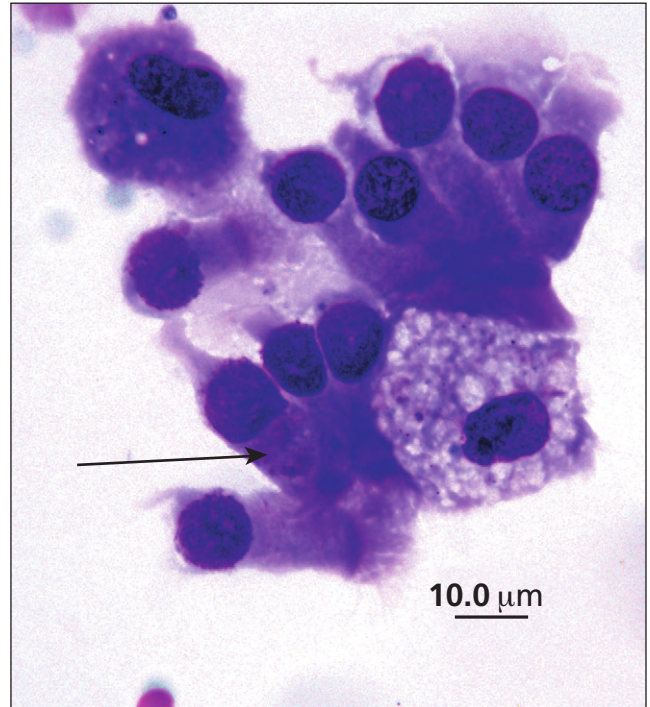
Fig. 9.5. Goblet cell. Note the numerous, purplish-staining, cytoplasmic mucin granules in the apical end of the cell (arrow). Diff Quik (×1000).

mucociliary clearance rates. Consequently, cells collected by TAs are frequently degenerate and more difficult to differentiate. Cytological examination is also hampered by the presence of dark-staining mucus strands in the background (Dixon 1997, Hodgson & Hodgson 2002b). In contrast, cells observed in cytological preparations of BAL and PFs are better preserved and usually easier to identify (McGorum & Dixon 1994).

Red blood cells

Although low numbers of RBC are frequently observed in TAs, BALF and PF collected from clinically normal horses, they are usually considered to reflect minor iatrogenic hemorrhage during collection. Accordingly, erythrophagocytosis by macrophages is not a feature of fluid samples from healthy horses. The supernatant fluid should also be clear, with no evidence of hemolysis in normal specimens.

Epithelial cells

The epithelial cells detected will differ depending on the site of collection (Clinkenbeard et al 2002). Thus, the types of epithelial cells observed can help confirm the actual site of collection, or potential contamination from other areas during collection.

Samples from the normal URT consist of exfoliated epithelial cells characteristic of the area sampled together with low numbers of inflammatory cells. The nasal epithelium caudal to the vestibule progresses from stratified squamous epithelium to pseudostratified ciliated and non-ciliated columnar epithelium containing numerous goblet cells. Columnar epithelial cells appear as medium-sized, elongated cells with basophilic cytoplasm and central to basal nuclei. Ciliated cells have pink-staining, hair-like cilia extending as a fringe from the apical end of the cell (see Fig. 9.4). Goblet cells are non-ciliated, mucus-producing cells found interspersed between ciliated columnar epithelial cells (see Figs 9.1B and 9.15). Goblet cells vary in size and shape, depending on the number of secretory granules in their cytoplasm. The nucleus is generally located at one end of the cell (basal), with red to purplish-staining secretory granules at the other (apical) end. Free secretory granules may be observed in some preparations.

The pharynx is lined primarily by pseudostratified ciliated columnar epithelium, but also has areas of stratified squamous epithelium. The mucosa of the guttural pouches and paranasal sinuses consists of transitional epithelium and simple ciliated columnar or cuboidal epithelium containing goblet cells (Chiesa et al 1999). Cytologically, cuboidal epithelial cells of transitional epithelium appear as medium-sized cuboidal cells with rounded borders, basophilic cytoplasm, and large central nuclei composed of finely stippled chromatin with areas of condensed chromatin.

Samples collected from the LRT similarly reflect the normal respiratory epithelium of this region, together with inflammatory cells present as the first line of respiratory
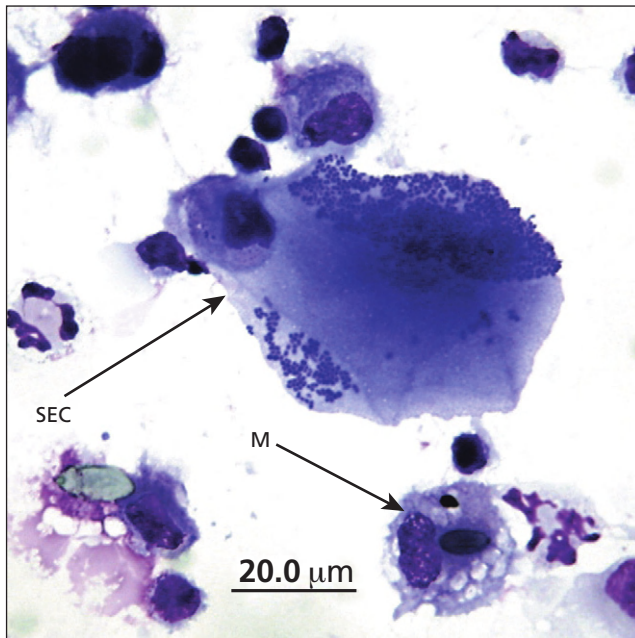
Fig. 9.6. Squamous epithelial cell (SEC) with surface bacteria (cocci). A macrophage ingesting a pollen granule is also present (M). Diff Quik (×1000).

defense mechanisms. Tracheal aspirates collect cells from the entire LRT and should thus contain cells from all levels of the pulmonary tree. This should include columnar and cuboidal epithelial cells and pulmonary alveolar macrophages. Limited interpretation is possible if cells from all levels are not represented and TAs should be regarded as diagnostically inadequate if there is scant cellularity, or if epithelial cells predominate in the absence of macrophages.

Tracheal aspirates from normal horses contain low numbers of epithelial cells, although increased numbers may be observed in samples obtained endoscopically. The epithelial cells are predominantly ciliated, with their size reflecting the site of origin. Columnar cells originate from the larger airways (trachea and bronchi) and cuboidal cells from the smaller airways (distal bronchi and bronchioles). Ciliated columnar epithelial cells are present commonly in small clumps or clusters and a thin, cone-shaped pedicle may be observed on the basilar border, particularly if cells are harvested by mechanical trauma. Goblet cells may be observed interspersed among the tracheobronchial epithelial cells. They are rarely observed in TAs from normal horses, though their numbers may be increased in samples collected by endoscopic methods.

Squamous epithelial cells should not be present in TAs from normal horses. When present they represent oropharyngeal contamination at the time of sampling or result from aspiration of oropharyngeal secretions. These cells are frequently covered by bacteria, and therefore provide a significant source of microbial contamination.

Identification of the presence of these cells is important to be able to interpret the results of microbial culture accurately. They are large flat cells that usually stain lightly basophilic. Their cytoplasmic borders are often straight with distinct corners (see Fig. 9.6).

Epithelial cells may also be identified in BALF but are less common than in TA. In this fluid, ciliated columnar epithelial cells, cuboidal non-ciliated cells, and squamous epithelial cells are most commonly observed. The latter cells occur as a result of oropharyngeal contamination at the time of sample collection.

Inflammatory cells

Occasional inflammatory cells (macrophages, lymphocytes, neutrophils, eosinophils) may be observed in samples from the URT of normal horses. In the LRT, these cells are present in higher numbers, and in BALF and PF samples they are the predominant cell types. In TAs, moderate numbers of inflammatory cells, in particular pulmonary alveolar macrophages, should be observed.

Large variations in the proportion of inflammatory cells in TA and BALF from normal horses are reported. This may reflect differences in sampling technique and sample processing, but also is influenced by the geographical location of the horse at the time of collection. In general, stabled horses will have a higher proportion of inflammatory cells, most likely a response to low-level irritation by inhaled agents. These mild elevations in the proportions of inflammatory cells, often accompanied by a mild increase in the amount of mucus, probably represent a normal response to these noxious stimuli and in all probability do not contribute to decreased respiratory function. However, if prolonged, this irritation may act as a risk factor for the development of more permanent respiratory tract pathology (Malikides 2004). Clear-cut reference values for normal horses under different environmental conditions need to be established for BALF and particularly TAs to better define abnormalities in these samples.

Defining cut-off values for normal percentages of inflammatory cells is difficult because of the considerable variation between studies. In general, however, it is considered that BALF should have <5% neutrophils, <2% mast cells and <0.5% eosinophils (Hoffman 1999, Robinson 2003). Wider ranges in the proportions of lymphocytes (30–60%) and macrophages (40–70%) are reported; therefore ascribing cut-off values for these cell types is more complicated. Tracheal aspirates should have <20% neutrophils, <1% eosinophils, <10% lymphocytes and very few mast cells (Hodgson & Hodgson 2002a). Finally, wide variation in the proportions of inflammatory cells in PF may occur in normal horses, making interpretation of any changes in the proportion of these cells difficult. Reported proportions include neutrophils 32–91%, lymphocytes 0–22%, large mononuclear cells 5–66%, and eosinophils 0–9% (DeHeer et al 2002).
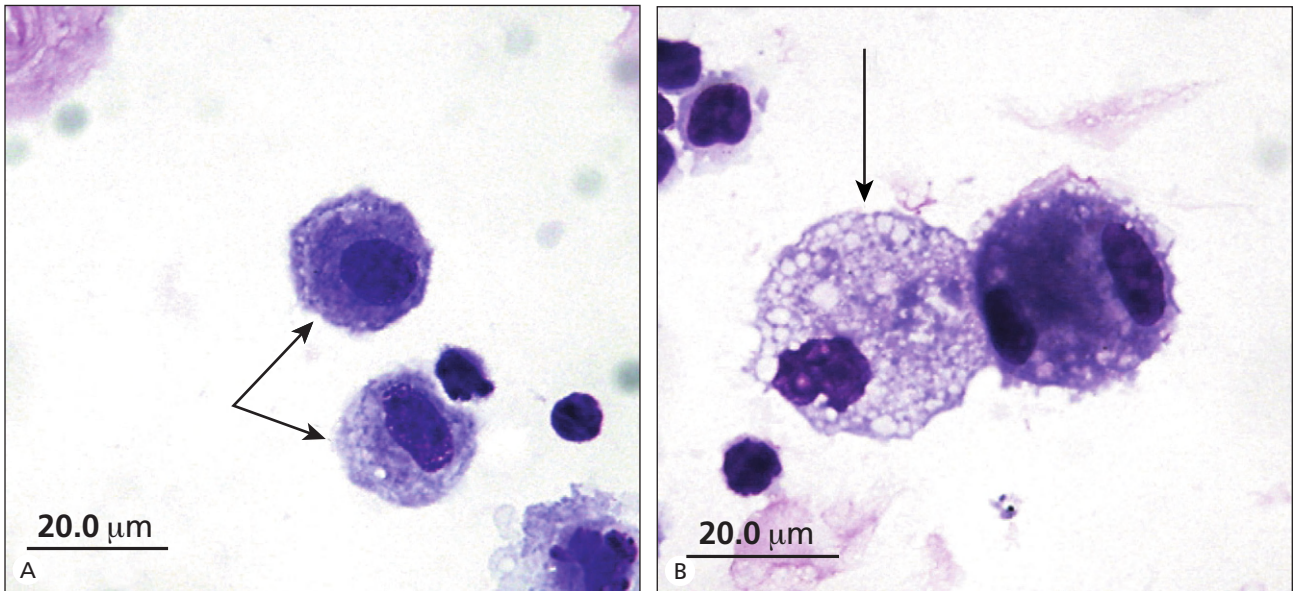
Fig. 9.7. (A) Two non-active macrophages in BALF (arrows). Diff Quik (×1000). (B) Activated macrophage with increased vacuolation (arrow). The adjacent cell is a binucleate macrophage. Diff Quik (×1000).

Continued

Macrophages and hemosiderophages

Pulmonary alveolar macrophages are the most abundant inflammatory cell in TAs and BALF from normal horses. Typically non-reactive macrophages have an indented oval nucleus, with a relatively homogeneous chromatin pattern and moderate amounts of gray cytoplasm (nucleus to cytoplasm ratio is typically <1 : 3) (see Fig. 9.7A). Their nucleus can be quite pleomorphic, varying from elongated, round, convoluted, to lobulated. These cells may contain phagocytosed debris such as erythrocytes, apoptotic cells, fungal spores, hemosiderin, and pollens (see Fig. 9.7C). In low amounts, such debris is considered normal because pulmonary alveolar macrophages constitute one of the first pulmonary defense mechanisms.

The activity of macrophages present in fluid samples may vary considerably. Reactive macrophages tend to have abundant, more basophilic cytoplasm with ruffled cytoplasmic margins. In addition, they often have prominent cytoplasmic vacuoles and inclusions (see Fig. 9.7B). Macrophages with finely vacuolated cytoplasm are not considered abnormal, but marked increases in cytoplasmic vacuolation, or large vacuoles that distort the cell and displace the nucleus, are usually present only when there is pulmonary disease. This increase in cytoplasmic reactivity occurs in response to infective, irritant, and small airway allergic disease or airway obstruction. The cytoplasmic inclusions reflect the amount and type of endogenous and exogenous materials present in the lower airways. In these cases, the presence of many macrophages with ingested cellular debris or whole cells is common and reflects increased cellular turnover. However, care must be taken in the interpretation of ingested elements. For example,

intracellular fungal spores or hyphae may be observed (see Fig. 9.13), but should not be interpreted as evidence of fungal pneumonia. More commonly, fungal elements are simply phagocytosed inhaled airborne fungal elements. In the case of fungal pneumonia, other cytological or clinical evidence of disease must be evident to confirm this rare diagnosis.

Multinucleated macrophages (giant cells) are commonly observed in low numbers in TAs and BALF from horses with no evidence of inflammation (see Fig. 9.7D). Increased numbers of these cells may occur in chronic inflammation, but this is an inconsistent finding.

Following respiratory tract hemorrhage, RBCs within the airways are rapidly phagocytosed by pulmonary alveolar macrophages (erythrophages; see Fig. 9.7E). The RBCs are subsequently degraded and their heme pigment is reduced to hemosiderin, giving rise to hemosiderophages (see Fig. 9.7F). This pigment is easily recognized in preparations using routine hematological stains, but may be confirmed with special stains such as Perl's Prussian Blue, which positively stains ferric iron. In Diff-Quik-stained smears the color of the heme pigment is dependent on its age, olive green pigment indicating more recent hemorrhage, with the pigment becoming more golden brown as it ages. The amount of pigment within hemosiderophages varies and some cells may contain a few granules, whilst others contain substantial pigment deposits.

Large mononuclear cells

Macrophages in PF are frequently difficult to differentiate from other large mononuclear cells and therefore are categorized in one group. This category incorporates non-reactive

20.0 μm

C

E

10.0 μm

F

20.0 μm

D
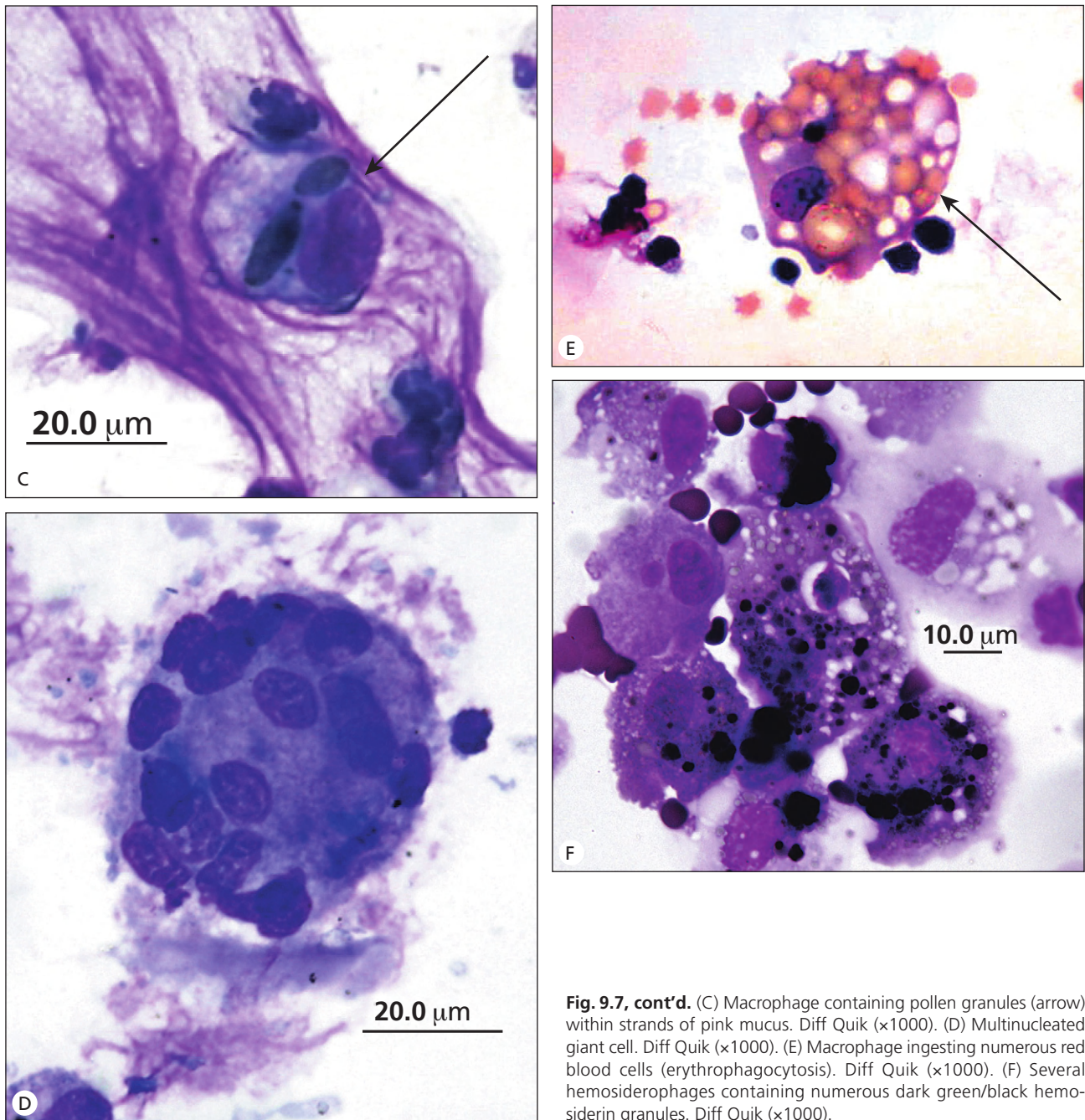
Fig. 9.7, cont'd. (C) Macrophage containing pollen granules (arrow) within strands of pink mucus. Diff Quik (×1000). (D) Multinucleated giant cell. Diff Quik (×1000). (E) Macrophage ingesting numerous red blood cells (erythrophagocytosis). Diff Quik (×1000). (F) Several hemosiderophages containing numerous dark green/black hemosiderin granules. Diff Quik (×1000).

(tissue) macrophages of blood monocyte origin, reactive (tissue) macrophages, and mesothelial cells. They may also be referred to collectively as mononuclear phagocytes, because all have phagocytic potential. All of these cells are large, with a moderate nuclear to cytoplasmic ratio and abundant, somewhat basophilic cytoplasm. Their nucleus is usually oval with a finely reticular chromatin pattern.

Mesothelial cells may occasionally exhibit distinctive features. For example, a fine eosinophilic "corona" or halo of glycocalyx may be evident along the cell margin. In transudative effusions, mesothelial cells may occur in sheets or rafts, with a uniform cellular appearance that is polygonal to rhomboid shape (see Fig. 9.8A). In exudative effusions, mesothelial cells may become reactive or transformed and may exhibit hyperplastic and eventually dysplastic features indicative of increased proliferation, including increased cytoplasmic basophilia, multinucleation, prominent nucleoli, and mitotic activity (see Fig. 9.8B) (DeHeer et al 2002). Care must be taken when interpreting these cellular changes because hyperplastic/dysplastic features can begin to mimic neoplasia in severe inflammatory conditions.
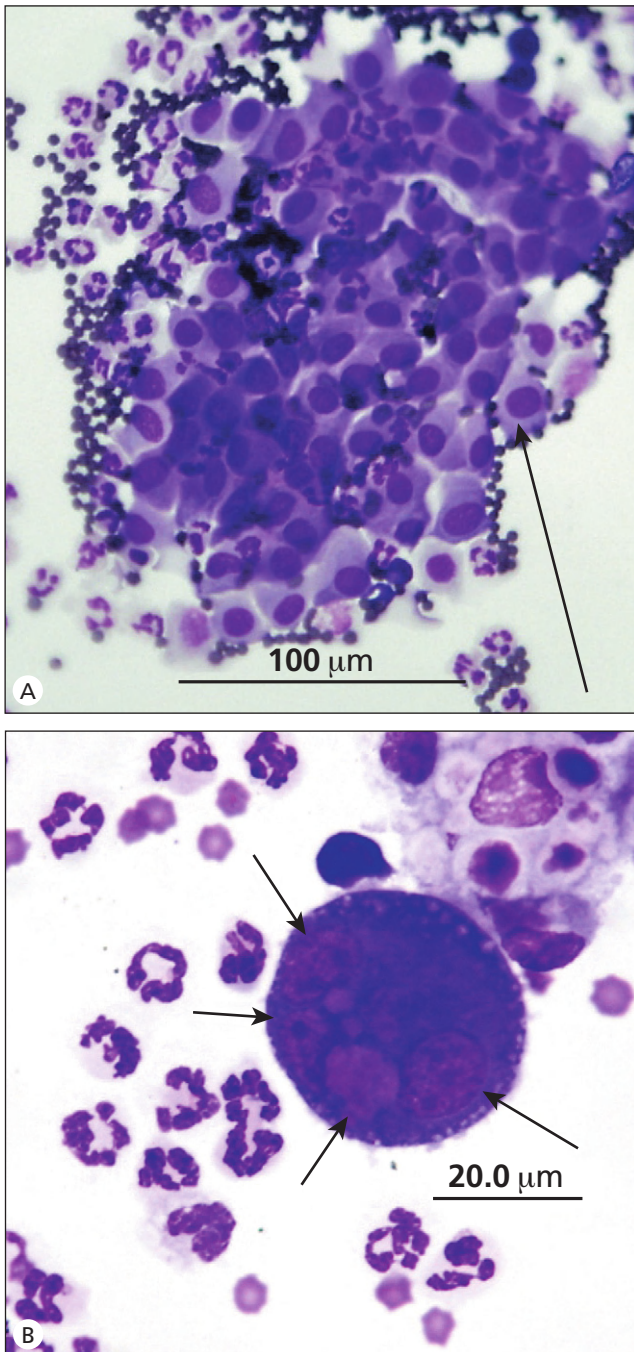
Fig. 9.8. (A) Large raft of mesothelial cells in thoracic fluid (arrow). Diff Quik (×400). (B) Large activated mesothelial cell with intensely basophilic cytoplasm, multiple nuclei (arrows) and prominent nucleoli. Diff Quik (×1000).

Lymphocytes

Lymphocytes are present in low numbers in normal TAs and PF, but occur in higher proportions in BALF. They appear as small to medium-sized spherical cells with a scant rim of basophilic cytoplasm. Their nucleus is relatively large and may be slightly indented with a coarse

chromatin pattern. In TAs, they may be difficult to differentiate from some small macrophages, "stripped" epithelial cell nuclei, and "end-on" epithelial cells. As a result this group may be referred to as small mononuclear cells. Plasma cells (or reactive lymphocytes) are antigenically stimulated lymphocytes. They are larger than neutrophils and are recognized readily by their royal-blue cytoplasm, which may contain a pale-staining Golgi apparatus. The nuclear chromatin pattern is moderately granular and nucleolar rings (a slight clearing of the chromatin where the nucleolus is located) are seen occasionally.

Neutrophils

These cells usually have a lobulated nucleus with a coarse chromatin pattern and relatively colorless cytoplasm. Although a population of well-preserved neutrophils resides in horses' airways, the relative proportion of these cells is considered to be normally low. However, neutrophils respond to a variety of stimuli, and their numbers may fluctuate rapidly. In addition, neutrophils generally are found in higher proportions in TA than in BALF from normal horses. This possibly reflects the greater exposure to noxious influences occurring in the larger airways. The proportion of neutrophils normally present in PF is highly variable (30–90%) and the total number of neutrophils per unit volume is a more reliable indicator of pathological influences on this cell line in PF.

Neutrophils entering the airways and thoracic cavity do not return to the bloodstream. Consequently, *in situ* cell aging and death are normal events for this cell. Low numbers of aged neutrophils are often observed with moderately hypersegmented to pyknotic nuclei. In addition, leukophagocytosis of senescent neutrophils by macrophages may be observed. Neutrophils in normal fluid samples from the LRT exhibit little to no phagocytic activity *per se.*

Neutrophils may appear normal, with few degenerate or toxic changes, in some disease conditions such as heaves. In contrast, other pulmonary diseases may cause large increases in the proportions of degenerative or toxic neutrophils. The most common degenerative change of neutrophils is karyolysis which involves:

- cell swelling
- nuclear swelling with loss of nuclear segmentation
- indistinct nuclear margins
- decrease in nuclear staining intensity from purple to pink
- eventual lysis of the cell.

The degree of nuclear disruption is roughly proportional to the degree of degeneration (see Fig. 9.9A,B). Degenerate neutrophils are observed most commonly in samples from horses with infectious diseases, but care must be taken in their interpretation because samples collected by TA will

A

B

Fig. 9.10. Tracheal aspirate containing many eosinophils (arrow). Diff Quik (×1000).
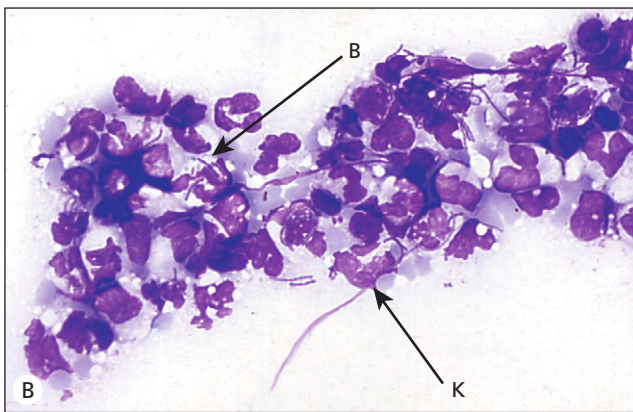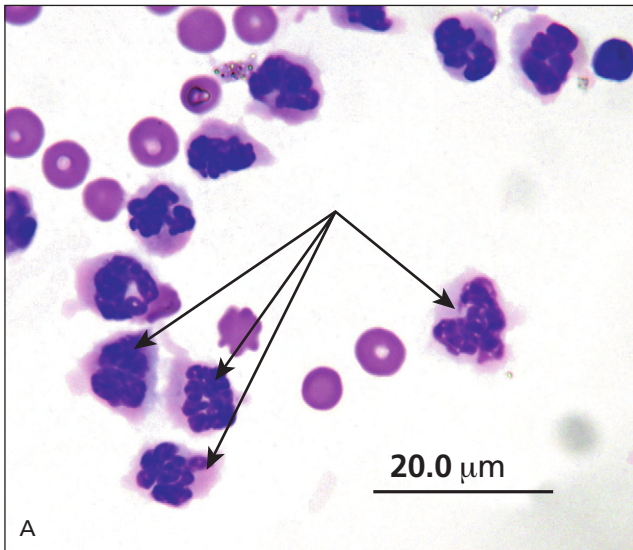
Fig. 9.9. (A) Mild karyolysis of neutrophils (arrows). Diff Quik (×1000). (B) Neutrophils demonstrating moderate to marked karyolysis (K). Some neutrophils contain intracellular bacteria (B). Diff Quik (×1000). K = karyolysis, B = bacteria.

contain more degenerate neutrophils than BALF even in normal horses.

Another morphological change that may be observed in neutrophils is apoptosis, which is characterized by a condensed nucleus and minimal cytoplasm. Apoptotic neutrophils may be observed in increased numbers in horses with heaves or non-infectious inflammatory airway disease (IAD).

The more "classical" toxic changes observed in circulating neutrophils (such as increased cytoplasmic basophilia, vacuolation, or Döhle bodies) may be present in PF samples in horses with septicemia or endotoxemia. Such toxic changes are considered to be "pre-existing", occurring during production (myelopoiesis), rather than subsequent to migration into the pleural cavity. If these changes are accompanied by visualization of phagocytosed bacteria they are compatible with septic pleuritis. The presence of band neutrophils or more immature granulo-

cytic cells may be interpreted to suggest acute inflammation and mobilization of the neutrophil storage and maturation pools. These are observed more commonly in PF, but may also occur in TAs and BALF.

Eosinophils and basophils

The morphology of eosinophils and basophils is the same in TA, BALF, and PF as in peripheral blood smears. Eosinophils are slightly larger than neutrophils with a lobulated nucleus and uniformly large, round, bright-red cytoplasmic granules (see Fig. 9.10). Basophils also are slightly larger than neutrophils with a lobulated nucleus and numerous, fine, dark-purple granules that may obscure nuclear detail.

Mast cells

Mast cells may be identified by their characteristic staining granules, which are more prominent when metachromatic stains (e.g. Leishmann's) are used (see Fig. 9.11). In normal horses, mast cells in TAs are rare (Hughes et al 2003). This is in contrast to BALF where higher numbers of mast cells may be observed. This difference may be explained by the predominant distribution of equine mast cells within the smaller airways and alveoli.

Cytological changes associated with delayed processing

A number of changes to cellular morphology may be observed in smears prepared after a delay of several hours, even in samples from normal horses. Macrophages can become vacuolated *in vitro* and perform erythrophagia, thus complicating the distinction between true hemorrhagic effusions and specimens contaminated with peripheral blood. Nucleated cells may exhibit aging
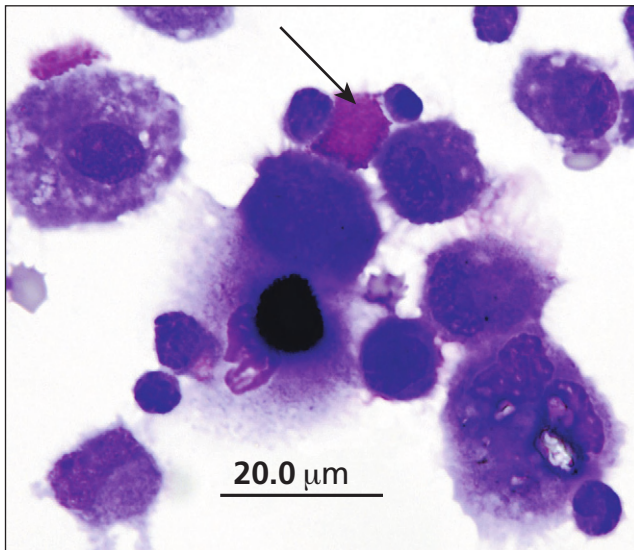
Fig. 9.11. Mast cell (arrow) in BALF. Leishmann's stain (×1000).
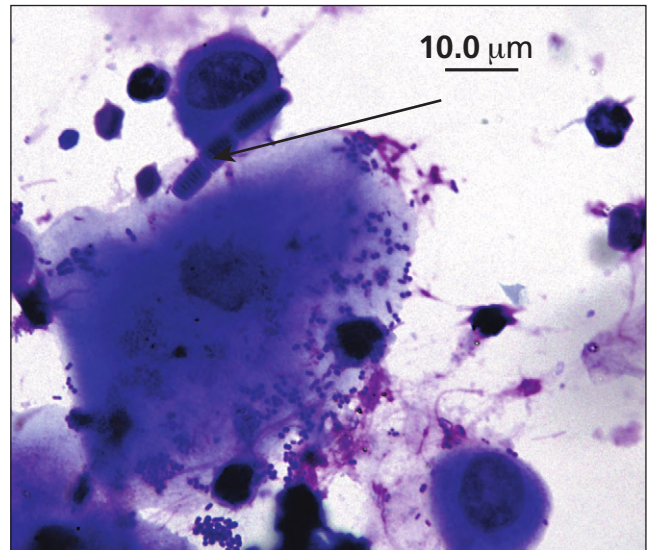
Fig. 9.12. Several rows of *Simonsiella* spp. (arrow) on the surface of a squamous epithelial cell. Diff Quik (×1000).

changes such as hypersegmentation and pyknosis, which can resemble the changes associated with chronic inflammatory processes. Neutrophil nuclear hyposegmentation artifact may also be observed following delayed processing of EDTA-preserved specimens. A similar phenomenon occurs in normal TA fluid samples. The potential for these changes must be kept in mind when evaluating and interpreting these samples.

Incidental findings

A variety of non-significant objects and artifacts may be observed in samples from the LRT. Bacteria normally inhabit the URT and oral cavity and may occur transitorily in the lower airways, especially after exercise or transportation or if contamination occurs during sampling. Consequently these bacteria, together with squamous epithelial cells, may be observed in TAs and BALF collected subsequent to these events. Perhaps the most conspicuous members of the normal flora of the oral cavity are *Simonsiella* spp., which appear as giant rod-like structures (see Fig. 9.12). These are composites of multiple *Simonsiella* rods apposed side-to-side. This bacterial species, and other normal flora of the URT, do not elicit a significant inflammatory response in these sampling sites unless clearance is impaired or if aspiration-induced pneumonia has occurred.

Various other microorganisms and airborne debris may be observed in TAs, BALF, and URT samples from normal horses. These reflect the horse's environment and mucociliary function at the time of sampling. Fungal spores, macroconidia, fruiting bodies, and occasional hyphae of saprophytic ("barn mold") fungi are the most common elements observed and may be seen extra- and intra-cellularly in macrophages (see Fig. 9.13). A common
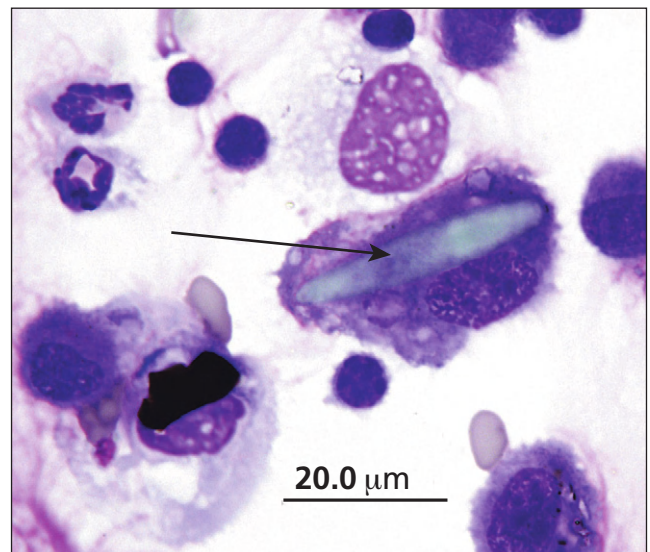
Fig. 9.13. Fungal spore within a macrophage which is distorting the cell (arrow). Diff Quik (×1000).

example of a fungal contaminant is the saprophytic fungus *Alternaria*. This fungus has large macroconidia (> 1–2 RBC diameters in size) seen as green to turquoise, round to elliptical structures. It is important to note that the presence of these fungal elements does not indicate fungal infection. Other extraneous materials are frequently observed including pollen and plant material, hairs and pigmented debris. Rarely, mites, small larvae (not lungworm) or egg-like structures may be seen. Grass, dirt, and artificial racetrack surface material may occur in samples collected post-exercise.

Glove powder (corn starch) may occasionally contaminate PF samples. This appears as variably sized, round to hexagonal particles with a central fissure or cross. Particles are usually clear (non-staining) but on occasion may have a bluish hue.

Evaluation of samples for bacterial and fungal infections

Upper respiratory tract

As a result of the presence of normal flora in the URT, culture of samples from the nasopharynx is rarely helpful unless a specific pathogen is being investigated (e.g. *S. equi* ss. *equi* or viral pathogens). In addition, these samples do not reflect the bacterial population of the LRT, only those of the URT. Thus, they offer minimal diagnostic usefulness in the evaluation of LRT infections.

Culture of samples from the nasal cavity, nasopharynx, and guttural pouches should be directed at specific pathogens that are not part of the normal flora. As identification of individual bacterial pathogens based on their cytological appearance is not reliable, culture should be used to identify the organism involved. When attempting to isolate *S. equi* ss. *equi*, samples should be cultured on Columbia CNA (colistin, nalidixic acid) agar with 5% sheep or horse blood to enhance isolation. Alternatively, where available, PCR based on primer sequences from the *SeM* should be used because this technique is three times more sensitive than culture for detection of *S. equi* ss. *equi* (Timoney 2004).

Isolation of fungal pathogens from the URT should also be directed at specific pathogens. Specialized culture media (e.g. Sabouraud dextrose agar) is required for their isolation. In addition, care should be taken as some cultured fungal pathogens may be infective for humans (e.g. *Coccidioides immitis)* and if suspected, culture of these organisms should be performed at reference laboratories.

Lower respiratory tract

Bacterial cultures of TAs and PF samples are helpful in diagnosing LRT infections and ideally should be performed in all suspected cases of pneumonia or pleuropneumonia. Samples collected by BAL may occasionally be cultured, particularly when collected from a suspected lung abscess and when the endoscope is guided into an airway where mucopus is draining. In foals, this technique may also be used to diagnose *Pneumocystis carinii* infection, which is interstitial in nature (Leguillette et al 2002). BAL samples collected "blindly" are rarely of use for microbial culture because they are contaminated with oropharyngeal flora. This BAL technique also harvests cells from the caudodorsal lung fields, which are rarely involved in bacterial infections. Ideally, any sample submitted for microbial culture should be obtained before antimicrobial administration or after a sufficient period is allowed for

tissue clearance of previously administered antimicrobial agents (at least 24 and preferably up to 72 h).

Aerobic and anaerobic culture should be performed on TAs and PF that have cytological evidence of airway inflammation. Quantitative cultures, which determine the number of colony-forming units (cfu) for each bacterial species, provide additional information when culturing TAs. Tracheal aspirates collected in an appropriate fashion from normal horses, or from horses with airway inflammation without a bacterial etiology, usually yield $<10^3$ cfu/ml and frequently no bacteria at all. If $>10^3$ cfu/ml are cultured, it is likely that these bacteria are contributing to the disease process, and identification of each species present in high numbers will assist in interpretation of their significance. Furthermore, the greater the cfu/ml isolated, the more likely the significance of the bacteria isolated. Quantitative cultures are not performed on PF samples because the thoracic cavity is normally a sterile site and sample collection using appropriate techniques rarely involves contamination. In addition, culture of PF samples usually involves liquid culture media, allowing bacterial proliferation, which precludes quantification of the numbers of bacteria that were present at the time of sampling.

Identification of isolated bacteria allows differentiation of possible pathogens from probable contaminants. The bacteria isolated commonly from cases of pneumonia and pleuropneumonia are listed in Table 9.2. The significance of isolation of various bacteria is discussed in the section *Interpretation.*

Isolation of fungal pathogens from the LRT, like the URT, should be directed at specific pathogens. In addition, characteristic cytological findings will assist in interpretation of these isolates.

Diagnosis of parasitic infections

Parasitic bronchitis and pneumonitis has decreased in prevalence as a result of improved anthelmintics. The most likely parasite involved in pulmonary disease of foals and young horses is the roundworm *Parascaris equorum*. In contrast, the lungworm *Dictyocaulus arnfieldi* occurs predominantly in adult horses pastured with donkeys. Parasitic pneumonitis may be difficult to diagnose definitively, but a history of a poor anthelmintic regimen or close association with donkeys may provide clues. In addition, large numbers of eosinophils and activated alveolar macrophages may be observed on cytological preparations of TAs and BALF (see Fig. 9.10), but are not specific for parasitic infections as they may also be observed in other forms of eosinophilic pneumonia. Lungworm larvae may occasionally be observed in TAs or found in feces using the Baermann technique (Zinkl 2002). Fecal flotation may also be used to diagnose roundworm infection, but a negative result does not rule out their involvement as the pre-patent period for

Table 9.2. Bacterial and fungal isolates from lower airway samples of horses	
Common pathogens	*Streptococcus equi* ss. *zooepidemicus* *Actinobacillus* spp. *Pasteurella* spp. *Rhodococcus equi* (foals)
Less common pathogens	*Escherichia coli* *Klebsiella pneumoniae* *Enterobacter* spp. *Bordetella bronchiseptica* *Streptococcus pneumoniae* *Streptococcus dysgalactiae* ss. *equisimilis* *Streptococcus equi* ss. *equi* *Bacteroides* spp. *Fusobacterium* spp. *Peptostreptococcus* spp. *Mycoplasma* spp. *Aspergillus* spp. *Coccidioides immitis* *Histoplasma capsulatum* *Cryptococcus neoformans* *Blastomyces dermatitidis*
Likely contaminants	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Proteus* spp.
Definite contaminants	*Staphylococcus epidermidis* (and other coagulase negative staphylococci) *Bacillus* spp. *Alternaria* spp.

this parasite is 71–110 days, while pulmonary migration occurs 7–14 days after ingestion of infective larvae (Darien 1994).

Diagnosis of viral infections

A presumptive diagnosis of viral respiratory tract infections may be based on history and clinical signs. However, confirmation requires viral isolation or detection by serological or molecular techniques. These procedures are routinely performed in diagnostic laboratories and will vary between regions and countries. Advice should therefore be sought regarding the most appropriate procedures available.

Interpretation

Collection of appropriate samples from the respiratory tract is a key step in the investigative pathway. However, accurate interpretation of findings from these samples is critical if an appropriate diagnosis, strategy for management and prognosis are to be ascribed to the affected horse.

Interpretation of samples from the URT

A number of characteristic cytological findings may be associated with pathological processes of the URT.

Airway irritation

Goblet cells are rarely observed in washes of the normal nasopharynx. Conditions that irritate the mucosal lining of the URT can result in goblet cell hyperplasia and increased production of mucus, which appears cytologically as mats of homogeneous, pink-to-red staining material or as a finely mottled pink background. Free mucin granules may also be seen in smears from irritated mucosa.

Airway inflammation

The cytological hallmark of inflammation is increased numbers of inflammatory cells (neutrophils and macrophages in particular). Normally, only small numbers of inflammatory cells (<5%) are associated with the mucosa, submucosa, and associated structures of the URT (Chiesa et al 1999). Noxious stimuli, such as foreign bodies, trauma causing tissue necrosis, or infectious organisms provoke an inflammatory response and will result in increased proportions of inflammatory cells. If >25% of cells present in cytological preparations are inflammatory cells, a source of irritation should be considered and investigated.

Bacterial infections

Bacterial infections are typically associated with intense infiltration of neutrophils into infected tissues and these cells commonly constitute >85% of cells in smears (see Fig. 9.14A). Thus, increased proportions of neutrophils in cytological preparations should prompt suspicion of possible bacterial infection, even if bacteria are not observed. As many bacteria produce toxins, neutrophils may have degenerative or toxic changes. However, not all bacteria produce toxins (e.g. Actinomycetes, *Actinobacillus* spp.), and absence of degenerative changes does not preclude bacterial infection. Bacteria appear as a collection of uniform rods or coccoid structures that stain blue (Gram positive) or red (Gram negative) on grain stain (see Fig. 9.14B). Free mucin granules, free cilia, stain precipitate or necrotic debris can be mistaken for bacteria in smears. Bacteria can be located extra- or intra-cellularly (see Figs 9.14B,C). If bacteria are located only extracellularly and only small numbers of neutrophils are seen, this is more likely to reflect the presence of normal bacterial flora or bacterial contamination of the sample rather than true bacterial infection. In contrast, the presence of phagocytosed bacteria, especially in combination with a neutrophilic infiltration and degenerative changes of the neutrophils, indicates either a primary or secondary bacterial infection. The significance of bacteria isolated from the URT is discussed later in this section.
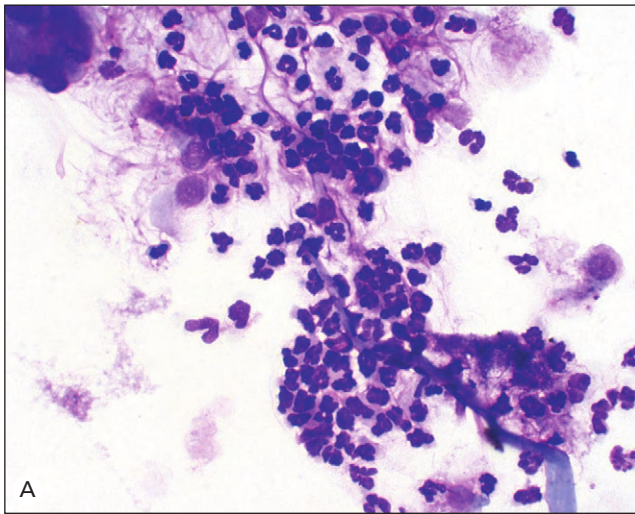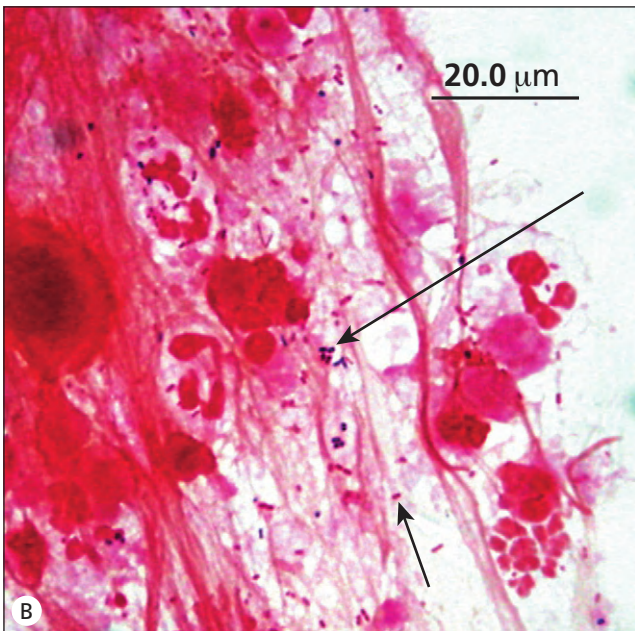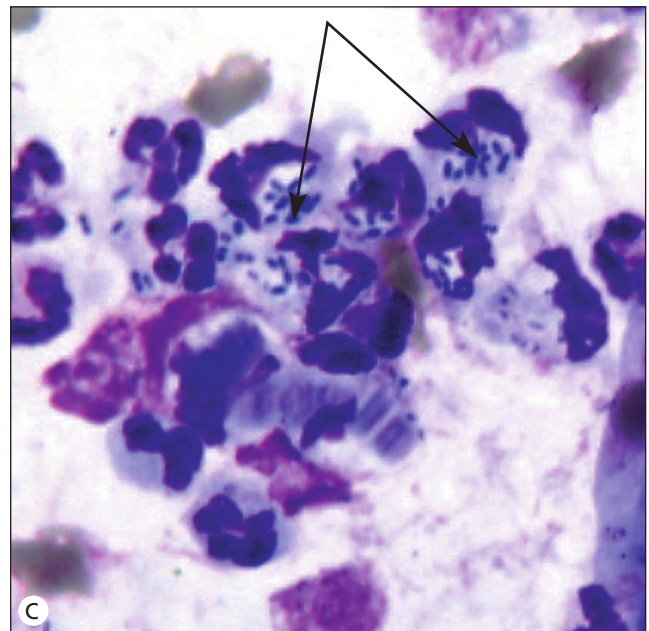
Fig. 9.14. (A) Smear demonstrating predominance of neutrophils (>85%). Many of these cells are trapped within mucus (pink, amorphous strands). Diff Quik (×400). (B) Gram stain demonstrating multiple intra- and extra-cellular bacteria in a smear of a tracheal aspirate obtained from a horse with mixed bacterial pneumonia. Gram-positive cocci (long arrow) and Gram-negative rods (short arrow) are present. Gram stain (×1000). (C) Multiple neutrophils with intracellular bacteria staining dark purple (arrows). Diff Quik (×1000).

Hypersensitivity reactions

Inflammatory processes involving hypersensitivity (allergic) reactions can result in increased numbers of eosinophils, basophils, or mast cells in cytological smears. These may occasionally be observed in samples obtained from the URT. Often the cause of the hypersensitivity reaction is not identified.

Lymphoid hyperplasia

Antigenic stimulation, in the absence of an inflammatory stimulus, is associated with increased numbers of small lymphocytes and plasma cells. Pharyngeal lymphoid hyperplasia, like other forms of benign lymphoid hyperplasia, has no cytological features distinguishing it from the normal appearance of lymphoid tissue. In these cases, fine-needle aspirate or biopsy samples reveal a cell population that has >80% small lymphocytes.

Cysts and hematomas

Cytological examination of samples from fluid-filled structures within the dermis, mucosa, salivary glands, or associated ducts present in the URT often helps to identify the process involved. Atheromas, which are epidermoid or sebaceous cysts of the nasal diverticulum, are distinguished by large numbers of squamous epithelial cells (see Fig. 9.6), cholesterol crystals, and a variable amount of sebum. Cysts involving the mucosa of the URT contain cells characteristic of their mucosal linings and may also

contain mucin. Mild to chronic inflammatory processes are often associated with cystic structures, therefore increased numbers of neutrophils are common. Hematomas will contain RBC, leukocytes and platelets. The RBC will be fresh or in various stages of catabolism with erythrophagocytosis and hemosiderin, bilirubin or biliverdin crystals present.

Polyps and neoplasia

Nasal polyps are usually secondary to chronic inflammation and not of neoplastic origin. They consist of fibrous tissue surrounded by a mucosal lining. Neoplasms involving the URT are usually carcinomas, arising from the mucosa and its associated glands, or tumors of structures underling the mucosa such as osteosarcomas or lymphosarcomas. Specific cytological diagnosis of neoplasms of the URT is best performed by a trained cytologist.

Interpretation of TAs and BALF

Evaluation of samples collected by TA and BAL may be used to assess pulmonary pathology in horses with clinical signs consistent with pulmonary disease or to assist in the evaluation of horses with poor performance. However, this evaluation should not be performed in isolation, but should be interpreted in association with history, clinical signs, and the results of other diagnostic tests. In addition, care must be taken when assessing results as controversy remains as to their significance. This is highlighted by differing definitions of normal and abnormal cytological findings between investigators/laboratories, unknown significance of mild increases in numbers of inflammatory cells and mucus (particularly in performance horses), and variable interpretation of significance of results of bacterial culture.

Mucus

The amount of mucus present in the lower airways increases with pulmonary irritation. Specific causes of increased mucus or mucopus include bacterial, fungal, or parasitic pneumonia, chronic bronchitis, heaves, and IAD. The significance of mild increases in the amounts of mucus in the airways remains unresolved. This is particularly the case in horses with increased amounts of mucus, but no change or mild increases in the number of neutrophils and many activated macrophages. Furthermore, these findings may be present in horses not exhibiting overt signs of respiratory disease. In contrast, recent studies suggest that mucus scores ≥ 2 affect racing performance due to impaired lung function (Holcombe et al 2006).

Total nucleated cell counts

Samples from horses with airway inflammation can have mild, moderate, or marked elevations in TNCC. These fluids may be white, gray, yellow, or brown because of this increased cellularity. Maximum increases in TNCC usually occur in cases of bacterial pneumonia or pleuropneumonia, heaves or lungworm infections.

Red blood cells

Free RBCs and hemosiderophages may be identified in TAs or BALF after pulmonary hemorrhage. Free cells and hemosiderophages usually result from EIPH whereas iatrogenic hemorrhage, occurring at the time of collection, yields just free cells in the sample.

Epithelial cells

An increase in the number of epithelial cells in samples is relatively rare. Increased numbers of epithelial cells, in the absence of increased numbers of inflammatory cells, have been reported in cases of unusual pulmonary lesions such as chronic fibrosing alveolitis. In addition, an increase in the epithelial cell population, together with an increase in the inflammatory cell population, is reported in association with respiratory viral infection.

Changes to the morphology of epithelial cells are more common and help indicate pulmonary pathology. Mild changes can occur in normal horses and include nuclear changes and the loss of some cilia. These probably represent normal "wear and tear" or turnover of cells, and care must be taken in their interpretation. Pathological changes to epithelial cells (epithelial atypia) result from inflammation. In addition, in cases of infectious respiratory tract disease, there may be direct damage to the epithelium by viruses or bacterial toxins. Alterations to epithelial cells are best detected with polychrome stains (e.g. Pollack's trichrome) as these provide superior staining of nuclear morphology. Finally, it must be remembered that there are many causes of airway inflammation, and therefore the presence of epithelial atypia is not diagnostic for a specific etiology.

A range of atypical features has been described in epithelial cells in TAs and BALF from horses with respiratory disease. These include cytoplasmic swelling, vacuolation (or ragged appearance of the cytoplasm), nuclear degeneration and hypochromasia, and pyknotic nuclei. Early squamous metaplasia, dysplasia or hyperplasia of bronchial or bronchoalveolar cells may be observed in chronic inflammatory processes. A variety of "irritation forms" of bronchial epithelial cells have been described and include enlarged prominent nuclei, and rounded clusters of ciliated epithelial cells (see Fig. 9.15), which may represent fragments of hyperplastic bronchial mucosa. Cytoplasmic and intranuclear inclusions have been reported in suspected viral respiratory tract infections, but are an inconsistent finding and therefore not of value in the specific diagnosis of these infections.

A specific epithelial change associated with respiratory viral infection in humans is ciliocytophthoria. This involves loss of the terminal plate of ciliated epithelial cells resulting
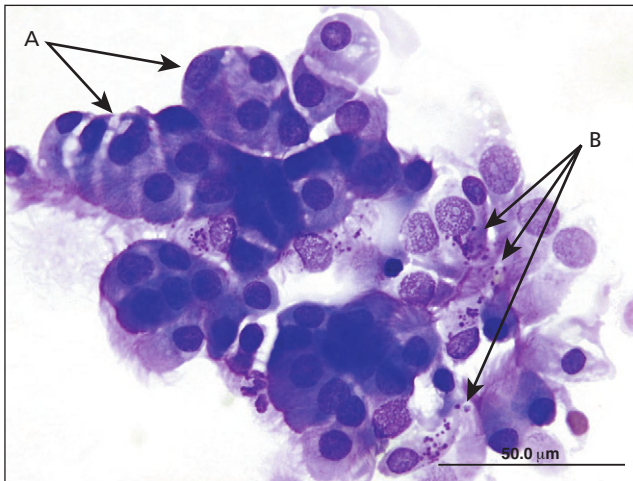
Fig. 9.15. Numerous rounded clusters of bronchial epithelial cells representing irritation forms (A). Note increased numbers of goblet cells (B). Diff Quik (×1000).

in the presence of a spherical cell with pyknotic fragmented nuclear material and pink inclusions together with a small, anucleate, rounded cytoplasmic fragment (or tuft) bearing the cilia. Both major cell changes are required for application of the term "ciliocytophthoria." This phenomenon is reported to occur in horses with suspected viral respiratory tract infections, though it is not a consistent feature. In addition, isolated ciliated tufts and epithelial cells with cytoplasmic damage may be seen in samples from horses with acute inflammation (e.g. acute bronchitis, heaves, and IAD) as a result of fragmentation of ciliated cells.

Macrophages/hemosiderophages

Macrophages constitute the predominant inflammatory cell population in TAs and BALF. Therefore, increased proportions of these cells are difficult to detect. Occasionally increased amounts of mucus, increased TNCC and increased numbers of activated macrophages may be observed, but the significance of these changes is currently not clear.

All causes of respiratory tract hemorrhage caudal to the larynx will result in hemosiderophages, but the most common cause is EIPH. Whilst good correlation has been reported between the presence of hemosiderophages in BALF and the presence of pulmonary hemorrhage in the caudodorsal lung lobes at necropsy, these cells are observed in a large proportion of horses in training, including those that are clinically normal (McKane et al 1993). The "acceptable" number of hemosiderophages in TAs and BALF is controversial because their number may not reflect the total amount of blood that has entered the airways. One reason is that much of the blood is likely to be cleared by mucociliary clearance and swallowed. In addition, hemosiderophages are cleared slowly, and may be present many months after pulmonary hemorrhage has occurred. Therefore, the numbers of hemosiderophages,

and amount of hemosiderin within individual pulmonary alveolar macrophages, should be interpreted with caution and in conjunction with other diagnostic tests (e.g. endoscopy, history).

Lymphocytes

The interpretation of increased lymphocyte ratios is complicated by the wide range of proportions observed in BALF from normal horses. Although increased proportions have been reported in racehorses with exercise intolerance and in other horses with chronic coughing, their significance remains unclear (McGorum & Dixon 1994). A narrower range of lymphocytes occurs in TAs, but no consistent correlation has been made between an increased proportion and a specific disease processes.

Neutrophils

The dilemma associated with interpretation of neutrophil percentages is to determine what value (if any) represents a significant change, particularly for TAs where numbers may fluctuate rapidly. Large variations in the proportion of neutrophils in TAs from normal horses are reported. However, recent studies have shown a strong association between the presence of increased proportions of neutrophils, signs of respiratory disease (coughing), and an increased likelihood of isolation of significant numbers of bacteria (Chapman et al 2000, Christley et al 2001). In addition, if evidence of lower airway inflammation (increased mucus, increased cell count) is taken into account in an inflammation score, horses with high scores have high relative proportions of neutrophils (>20–30%). Finally, the TNCC should be taken into account when interpreting the significance of neutrophils in TAs. High proportions of neutrophils in samples with a low TNCC are usually less significant than when the TNCC is elevated and the majority of these cells are neutrophils. In contrast, proportions of neutrophils in BALF from normal horses are less variable and good agreement exists between the presence of increased proportions of neutrophils in BALF and histological evidence of airway inflammation.

Neutrophils are usually the most common cell present in TA samples where there is an increase in TNCC. For example, they are the predominant cell observed in horses with bacterial pneumonia or pleuropneumonia and the percentage of neutrophils is usually >40%, and often exceeds 90% in acute cases. Elevated total and relative numbers of neutrophils also may be observed in cases of heaves, IAD, EIPH, acute viral infections, chronic bronchitis, and summer-pasture-associated obstructive pulmonary disease. However the increase in percentage is variable. Cases of interstitial pneumonia usually have low neutrophil numbers in TAs, but these may be elevated in BALF. Horses with clinical heaves will demonstrate elevated total numbers and proportions of neutrophils in BALF, but horses in remission will have normal BALF cytology.

Such horses may be differentiated from normal horses after being housed for at least 5 h in an inadequately ventilated stable containing poorly saved straw or hay, which induces a marked increase in neutrophils and other signs of heaves.

The presence or absence of degenerative changes to neutrophils may assist in the interpretation of increased proportions of this cell line, particularly when overall cell numbers are low. In some disease conditions (e.g. heaves, EIPH, and some cases of IAD) neutrophils are mostly mature with no degenerate or toxic changes. In contrast, degenerate neutrophils are commonly observed in samples from horses with bacterial or fungal pneumonia or pleuropneumonia because of the production of cytolysins by the infective agent. In these cases, careful examination of neutrophils for intracellular organisms is often diagnostically rewarding.

Eosinophils

Low numbers of eosinophils are present in TAs and BALF of normal horses. Increased proportions of eosinophils may be observed in lungworm infection or during ascarid migration. In these cases the relative percentage of eosinophils may be up to 85% of cells. Smaller elevations in the proportions of eosinophils occur in the absence of parasitic infections, and are often interpreted as evidence of a type I hypersensitivity response to inhaled allergens. In these horses concurrent increases in the proportions of mast cells may be observed. Frequently these elevations, particularly of eosinophils, are transitory and may not be observed in samples collected 24 h later. A number of both acute and chronic pulmonary disorders associated with elevated eosinophil ratios in TA and BALF have been described in horses and termed eosinophilic pulmonary disease (see Chapter 43). Elevated eosinophil counts in BALF of horses with heaves are an inconsistent finding, although heaves is proposed to have an underlying allergic etiopathogenesis. In young racehorses with poor performance, increased airway hyperresponsiveness at rest has been correlated with elevations in eosinophils in BALF (Hare & Viel 1998).

Mast cells

Mast cells are seldom seen in TAs, so alterations are better detected in BALF. An increased percentage of mast cells in BALF has been correlated with increased hyper-reactivity to histamine bronchoprovocation in horses with decreased exercise tolerance (Hoffman et al 1998). The increase in airway hyperreactivity is the result of the release of pro-inflammatory mediators such as histamine, leukotrienes and platelet-activating factor from mast cells. However, care must be taken in interpretation of elevated proportions of this cell population because high proportions of mast cells (>20%) also have been reported in clinically normal horses.

Adjuncts to interpretation of cytological changes in TA and BAL

A number of alternative approaches to cytological evaluation of LRT samples have been described that may assist interpretation.

Patterns of respiratory cytological changes

Evaluation of cellular patterns may help analysis of samples from the LRT. This approach takes into account subjective and quantitative, as well as morphological, features of all material in a specimen, and as such may be more useful than differential counts alone (Freeman & Roszel 1997a,b). Pathological processes such as bronchitis and bronchiolitis (which may be neutrophilic or eosinophilic, acute or chronic), alveolar edema, and pulmonary hemorrhage may be identified in this way. Alternatively, some patterns are suggestive of a specific disease process such as bacterial or fungal broncho-pneumonia, lungworm infection, EIPH, heaves, or interstitial pneumonia. However, care must be taken in the interpretation of cytological patterns because significant overlap exists between different diagnoses. Therefore, the conclusion derived from the cytological patterns observed must be consistent with other diagnostic tests as well as the clinical presentation of the horse.

Compound inflammation and hemorrhage scores

Compound inflammation scores have been applied to TAs in the assessment of airway inflammation. This system provides a semi-quantitative evaluation of the amount of mucus present within the airways (assessed endoscopically), the TNCC and the relative proportion of neutrophils. Each variable is assigned a score between 0 and 3 and a total score >6 is determined to be indicative of significant airway inflammation. Good correlation between a high airway inflammation score (>6) and the probability of growing significant numbers of bacteria has been shown (Chapman et al 2000).

Similarly, a compound EIPH score has been used to determine the likelihood of significance of pulmonary hemorrhage (Chapman et al 2000). This score takes into account the amount of blood in the airways, bloodstaining of the sample, and the numbers of hemosiderophages present. However, similar limitations to interpretation of this score exist as those discussed for the presence of hemosiderophages.

Interpretation of microbial culture of samples

Determination of the significance of bacteria isolated from the respiratory tract can be challenging. There are several factors that will assist evaluation and interpretation of these samples.

First, as a result of the presence of normal flora in the URT, culture of bacteria from this site is not significant *per se*. This is the case regardless of the numbers cultured. Only isolation of bacteria not routinely present in this site, and which are capable of causing the clinical signs observed, can be considered significant (e.g. *S. equi* ss. *equi*). In contrast, isolation of bacteria that are part of the normal flora, even if they are potential pathogens of this site (e.g., *S. equi* ss. *zooepidemicus*, *Pasteurella* spp.) cannot be interpreted as significant.

Second, the normal bacterial flora of the URT ceases at the larynx and the LRT is considered a sterile site. However, bacteria may occur transitorily in the LRT, especially post-exercise or after transportation, or may be introduced at the time of sampling. Thus, isolation of bacteria from TAs or BALF may represent infection, a transient lower airway population or contamination at the time of sampling. It is essential for appropriate management of these cases to differentiate between these scenarios. Differentiation can be assisted by:

- presence of clinical signs consistent with pneumonia or pleuropneumonia
- identification of isolates as known pathogens of the LRT in sufficient numbers (i.e. $>10^3$ cfu/ml)
- cytological evidence of inflammation.

Clinical signs consistent with pneumonia include fever, signs of depression, tachypnea, abnormal respiratory sounds, coughing, and nasal (often purulent) discharge. However, absence of these signs does not preclude bacterial infection, especially in milder cases of IAD caused by bacteria. The major bacteria and fungi capable of causing LRT infections in horses are included in Table 9.2. Quantification of these isolates was discussed earlier. Neutrophilic exudates are consistently observed in samples from horses with bacterial infections of the URT and LRT. These exudates are not present if non-pathogenic species or commensals are present, either transitorily (LRT) or as part of the normal flora (URT). Tracheal aspirates from horses with bacterial LRT infections will have increased mucus, increased TNCC, and increased relative and absolute neutrophil counts with possibly degenerative neutrophils and intracellular bacteria. No significance can be ascribed to bacteria isolated without this cytological evidence of inflammation. In addition, it is preferable not to culture samples with large numbers of squamous epithelial cells, even when there are many neutrophils present because this is evidence of oropharyngeal contamination. If such samples are cultivated, and large numbers of bacteria are isolated, it is not possible to assign any significance to these isolates. Re-collection of the sample is recommended.

Upper respiratory tract

Isolation of *S. equi* ss. *equi* from nasopharyngeal swabs or washes can confirm a diagnosis of strangles or identify carrier horses. However, cultures may be negative if collected during the incubation period or early clinical phase of disease as *S. equi* ss. *equi* is normally not present on the mucosa until 24–48 h after the onset of fever. In addition, the culture of nasal swabs or washes may fail to detect organisms sequestered in the guttural pouches of carrier horses as nasal shedding is sporadic (Newton et al 1997). In these cases, carriers may be identified by endoscopic examination of the guttural pouches to confirm the presence of inflammation, mucopus, chondroids, or retropharyngeal lymph node abscessation. If present, mucopus or chondroids should be cultured. Alternatively, where available, PCR based on primer sequences from the *SeM* can be used and this technique, combined with culture of guttural pouch samples, greatly increases the detection rate of carriers. However, care must be taken with interpretation of PCR "positive" samples from the guttural pouch because long-term carriers may remain PCR "positive" for months after the last culture of viable organisms is achieved. This indicates that bacterial DNA persists for a considerable period after death of the organism (Timoney 2004). In contrast, in convalescing horses, nasal swabs and washes become PCR negative shortly after viable organisms cease to be detectable. This can be explained by rapid mucociliary clearance of dead bacteria from the nasopharynx. PCR may also be used to detect *S. equi* ss. *equi* from nasal swabs in suspected cases of strangles, or it can be used as a method for rapid detection of *S. equi* ss. *equi* in animals about to be exported or introduced to strangles-free premises (Timoney 2004).

Pathogenic fungi (e.g., *Aspergillus* spp., *Pseudoallescheria boydii*) may be cultured from the URT, but care must be taken in interpretation of their isolation to ensure that they are not contaminants. Samples from horses with mycotic rhinitis, sinusitis, or guttural pouch mycosis typically contain large numbers of neutrophils and activated (epithelioid) macrophages. Multinucleated inflammatory giant cells, lymphocytes, plasma cells, and reactive stromal cells may also be seen. Occasionally these samples will contain increased numbers of eosinophils. If fungal elements are identified in smears in the absence of inflammation, no significance can be ascribed to their subsequent culture. Mycelium-producing fungi are readily recognized as filamentous structures having a width greater than ½ RBC diameter (wider than filamentous bacteria) and some may have septal divisions (see Fig. 9.16). However, determining the species of mycelium-producing fungi using cytological features is not reliable and the organisms must be cultured if specific identification is desired. Mycotic rhinitis may occasionally be associated with infection by *Rhinosporidium seeberi* or *Cryptococcus neoformans* and a presumptive diagnosis of these infections can be made by identification of characteristic fungal elements in nasopharyngeal swabs in association with an intense inflammatory reaction.
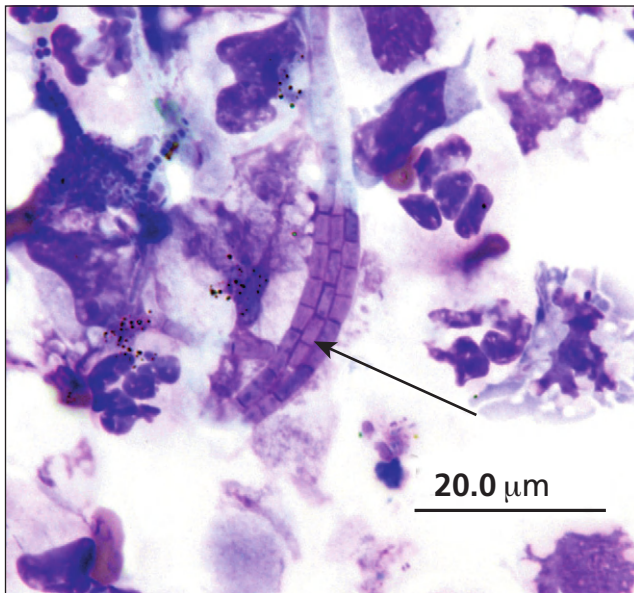
Fig. 9.16. Multiple branching septate fungal hyphae (arrow) trapped within strands of mucus. Diff Quik (×1000).

Lower respiratory tract

A variety of bacteria can cause LRT infections and frequently these infections are mixed (Table 9.2). Bacteria that are most commonly isolated from uncomplicated lower airway infections in adult horses include streptococci (both α- and β hemolytic), *Pasteurella* spp., *Actinobacillus* spp. and occasionally *Bordetella bronchiseptica*. Bacteria in the Enterobacteriaceae family (e.g. *E. coli*, *Klebsiella pneumoniae*) are more commonly secondary invaders and may be isolated following induction of antimicrobial therapy or when pneumonia is severe. Anaerobic bacteria (e.g. *Bacteroides* spp., *Fusobacterium* spp., *Peptostreptococcus* spp.) may be isolated from cases where sufficient necrosis of lung tissue has occurred such as within lung abscesses or when pleuropneumonia is present. *Mycoplasma equirhinis* and *M. felis* have been implicated in some outbreaks of equine respiratory infection. While it is difficult to culture these agents, some laboratories now perform serological evaluation to assist diagnosis of these infections. Pneumonia in foals may be caused by all the isolates causing disease in adults, as well as *Rhodococcus equi*. The diagnosis of *R. equi* infection is discussed in Chapter 24.

Pathogenic bacteria that rarely cause LRT disease but are common contaminants during sampling include coagulase-positive *Staphylococcus* spp., *Pseudomonas* spp., and *Proteus* spp. In particular, *Ps. aeruginosa* is a major contaminant commonly residing in inadequately sterilized endoscopes. This organism is rarely involved in LRT infections. Isolation of non-pathogenic bacteria (e.g. *Bacillus* spp., coagulase-negative *Staphylococcus* spp.), especially if

part of a mixed culture, indicates contamination at the time of sampling.

Occasionally, pathogenic fungi may be cultured from TAs or BALF (Table 9.2). The most frequently identified pathogenic fungus from the LRT of horses is *Aspergillus* spp. (Zinkl 2002), although the primary pathogens *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum* and *Cryptococcus neoformans* are also occasionally isolated in certain countries. Contaminating fungi, especially *Alternaria* spp., must be distinguished from true pathogens. The presence of an appropriate inflammatory reaction (see discussion of URT), together with free and ingested fungal elements, will help with this distinction. The fungal parasite *Pneumocystis carinii* can also cause acute interstitial pneumonia in immunosuppressed foals or secondary to *R. equi* infections (Leguillette et al 2002). Mixed inflammatory reactions (neutrophils and macrophages) together with the distinctive intact cysts can be identified in TAs or BALF. Finally, the majority of respiratory fungal infections in horses are secondary to immunosuppression, other severe diseases of the lung, or severe systemic diseases such as enterocolitis, peritonitis, endotoxemia or septicemia. Therefore, investigation of potential underlying disorders should be conducted to appropriately assess and treat these secondary infections.

Interpretation of virus isolation and serology

Virus isolation or detection of viral antigens or nucleic acid will confirm the presence of specific viruses in clinical samples. However, positive results should be discussed with the laboratory to apportion appropriate significance to this finding. A number of serological tests for respiratory viruses have been developed and include assay for equine herpesviruses 1, 2 and 4, equine influenza, equine rhinoviruses A and B, equine arteritis virus and adenovirus. The specific type of test, availability of tests, and the interpretation of results will vary between laboratories. However, in most cases, a four-fold or greater increase in titer between acute and convalescent serum samples is considered significant and may be used to indicate retrospectively that a viral infection has occurred. Alternatively, a lack of an increasing titer does not rule out a viral infection as titers rise rapidly during infection and may be increased at the time of onset of clinical signs. Therefore the expected increase in a convalescent sample may not be demonstrable. Although serological diagnosis of viral infections may not be of immediate value in the clinical management of cases, it may yield valuable information on the etiology of infections, particularly if virus isolation has proven unsuccessful. Such epidemiological information has apparent advantages in monitoring and designing effective vaccination programs, particularly for large susceptible groups of horses, such as those kept in training yards.

Interpretation of pathological changes in pleural fluid

The purpose of thoracocentesis is to evaluate the physicochemical, bacteriological, and cytological components of fluid accumulating within the pleural space. Subjective assessment of the color, turbidity, odor and volume of PF often provides sufficient evidence for a provisional diagnosis. This may allow initiation of therapy, before complete laboratory results are obtained. For example, if PF appears grossly turbid, discolored and has a foul odor, pleuropneumonia with involvement of anaerobic bacteria should be strongly suspected. Conversely, if PF appears grossly normal and of low volume, it is probably normal, because most pleural effusions in horses are exudative and will be visibly abnormal. However, when fluid volume is substantially increased, or if samples are moderately contaminated by peripheral blood, such diagnostic inference is more difficult. To further evaluate PF in such cases, total protein concentration, TNCC, and cytological examination are required.

Evaluation of PF is first directed at determining the type of fluid as this will help establish the underlying pathogenesis. Pleural fluids may be characterized as transudates or exudates (Table 9.3).

- Transudates are characterized by low (normal) cellularity and protein concentration.
- Modified transudates are rare forms of pleural effusions and are modified predominantly by an increase in protein concentration.
- Exudates are the most common type of pleural effusion and arise when there is an increased pleural capillary permeability and compromised lymphatic drainage. This results in fluid with increased TNCC and protein concentration.

Pleuropnemonia/pleuritis

Exudative thoracic effusions in horses are most commonly caused by bacterial infections, with fungal pathogens (e.g. *Coccidioides* spp., *Blastomyces dermatitidis*) involved only rarely. The bacterial organisms isolated most commonly are similar to those causing pneumonia (Table 9.2). Delayed or inappropriate treatment is likely to result in a chronic disease process characterized by involvement of strict anaerobes and poor response to therapy (Raidal 1995).

In cases of pleuropneumonia, PF volume, TNCC, and protein concentration will all be increased, but the degree of rise is not a good guide to prognosis. Neutrophils are the predominant leukocyte in most septic pleural effusions, but some anaerobic infections may contain numerous bacteria

Table 9.3. Physical, biochemical, and cytological features of pleural effusions in horses			
	Transudate	**Modified transudate**	**Exudate**
Color	Pale straw	Usually pale straw, but may be whitish pink if chylous	Varies from reddish brown to off-white according to number and relative proportion of RBCs and nucleated cells
Turbidity	Clear to slightly turbid	Clear to slightly turbid	Turbid
Protein (g/liter)	< 25 (usually < 15)	20–50	> 30
TNCC (× 10^9/liter)	< 5	5–15	> 15
Pathogenesis	↓ colloid osmotic pressure ↑ capillary hydrostatic pressure	↑ capillary hydrostatic pressure ↑ lymphatic hydrostatic pressure	↑ capillary permeability ↓ lymphatic drainage
Frequency	< 10%	Rare	> 90%
Causes	Hypoalbuminemia Congestive heart failure Hepatic disease	Congestive heart failure Neoplasia Lung lobe torsion Acute esophageal perforation Hepatic disease Chylothorax	Bacterial or fungal pleuropneumonia or pleuritis Penetrating chest wound Neoplasia Thoracic esophageal perforation Inhaled foreign body Vasculitis
Cytological findings	Unremarkable. Normal proportions and morphology of cells except reactive mesothelial cells may be present	Unremarkable except in chylous effusions where small lympho-cytes predominate	May be classified as suppurative (neutrophilic), chronic suppurative (pyogranulomatous) or chronic (granulomatous)

with few identifiable cells. This is most likely the result of the presence of potent bacterial cytotoxins. Degenerative changes of neutrophils are observed commonly and may be subjectively classified as mild, moderate, or marked depending on the degree of karyolysis (see earlier under *Evaluation: Neutrophils*). The more marked the degree of karyolysis, the more likely an effusion is the result of bacterial infection. Conversely, normal neutrophil morphology does not exclude a bacterial etiology. Neutrophil morphology may remain normal despite a marked inflammatory response if the infected tissue is walled off from the section of the pleural cavity sampled, if the bacteria produce few cytotoxins, or if antimicrobial therapy is initiated. Careful examination of Gram-stained preparations for bacteria should always be undertaken if an exudate is detected. These fluids should always be cultured, even if no bacteria are observed.

Large mononuclear cells may be plentiful and reactive in septic thoracic effusions, particularly when the effusion is more chronic. Mesothelial cell hyperplasia and reactivity may mimic neoplastic changes and care must be taken in their interpretation. Low numbers of lymphocytes are usually present in exudative effusions, although numbers may increase with chronicity. Increased numbers of plasma cells and large atypical lymphocytes may be observed when there is chronic antigenic stimulation. However, these atypical lymphocytes are present in relatively low numbers as compared to effusions associated with lymphoma where they are the predominant cell type.

Hemorrhagic effusions

Although RBCs are frequently seen in PF collected from clinically normal horses, they are considered to be the result of contamination at the time of sampling. Accordingly, erythrophagocytosis is not a feature of normal PF. The supernatant fluid is also clear and non-hemolysed in normal samples. The presence of RBCs and their breakdown products in PF may be the result of iatrogenic contamination, hemorrhagic diapedesis or intrapleural hemorrhage. Distinction between these different causes is clinically important so that appropriate management of cases of hemorrhagic effusions can be implemented.

Changes in the degree of red discoloration of PF during sample collection indicate peripheral blood contamination at the time of sampling. When PF resembles whole blood, comparison of PCV and clotting times with venous blood, and the cytological appearance of the PF may be useful to determine the source of RBCs. Failure of the sample to clot or presence of significant erythrophagia is indicative of true hemothorax. Contaminated specimens often have a PCV significantly less than peripheral blood, and platelet clumps may be visualizd microscopically.

Hemorrhagic diapedesis may be associated with pleuritis or neoplasia. The gross appearance of the fluid will vary depending on the relative numbers of RBCs and inflammatory cells, but is often reddish brown, port wine or muddy colored. The supernatant fluid may appear discolored as a result of hemolysis. Cytologically, erthryophagocytosis may be observed.

Hemothorax is rare in horses, but the various potential causes are described in Chapter 46. The cytological features associated with hemothorax will differ depending on the time at which samples are collected post hemorrhage and the degree of hemorrhage. If samples are collected shortly after intrathoracic hemorrhage, the PCV and total protein concentration of PF is usually less than that in peripheral blood, but may be similar if the hemorrhage is severe. The supernatant fluid is usually clear and cytologically the relative numbers of inflammatory cells will be similar to those in peripheral blood smears except for a decrease in the numbers of platelets. In contrast, the supernatant of PF collected subsequent to hemorrhage of longer standing is often reddish brown as a result of hemolysis. In these samples a common cytological finding is evidence of erythrophagocytosis and there may be increased numbers of neutrophils because of an inflammatory reaction induced by the hemorrhage.

Chylous and pseudochylous effusions

Milky, pinkish or opalescent discoloration of PF is associated with chylous and pseudochylous effusions. The turbidity and color change in chylous effusions are the result of increased triglyceride content, with or without a concurrent increase in leukocytes. Pseudochylous effusions have a similar gross appearance because of high cellularity and cholesterol content. The cytological profile in both effusions is typified by increased proportions of small lymphocytes together with a mixed inflammatory profile.

Chylothorax is rare in horses, with most reports of this condition occurring in foals (DeHeer et al 2002). The underlying disorders associated with chylothorax include congenital diaphragmatic hernia, meconium impaction, and idiopathic causes. Pseudochylous effusions result from severe chronic inflammatory processes. Differentiation of chylous and pseudochylous effusions is best performed by determination of PF fluid triglyceride and cholesterol concentrations. Chylous effusions are characterized by triglyceride concentrations greater than, and cholesterol concentrations less than, paired serum values, whereas the converse is true for pseudochylous effusions.

Neoplasia

Thoracic neoplasia is a relatively common cause of pleural effusion in the horse, with more than one-third of effusions reportedly being neoplastic (DeHeer et al 2002). The most common neoplasm causing thoracic effusion is lymphoma, although mesothelioma, squamous cell

carcinomas, adenocarcinomas, and hemangiosarcomas also have been reported. Pleural fluids associated with thoracic neoplasms are usually classified as modified transudates (when lymphatic drainage is obstructed) or hemorrhagic exudates (when serosal surfaces and their blood vessels are involved, or when there is tissue necrosis).

Pleural fluid cytology may assist in diagnosis of thoracic neoplasia, although not all neoplasms exfoliate cells into the PF and therefore a lack of neoplastic cells does not preclude this diagnosis. In addition, necrosis or infection of a tumor may result in pleuritis and this inflammatory reaction may complicate the cytological diagnosis. Reactive mesothelial cells, which can be present in any type of pleural effusion, may be mistaken for neoplastic cells. The distinction between reactive and neoplastic mesothelial cells may not be easy to determine, particularly in the presence of concurrent inflammation. Therefore, a cytological diagnosis of mesothelioma, as with most other intrathoracic tumors, is best performed by a trained cytologist. There are a number of excellent texts which contain more detailed descriptions of thoracic neoplasms in the horse and which are recommended to the reader (Cowell & Tyler 2002).

REFERENCES

Beech J 1991 Tracheobronchial aspirates. In: Beech J (editor) Equine Respiratory Disorders. Lea & Febiger, Philadelphia, pp.41–53

Chapman PS, Green C, Main JPM et al 2000 Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. Veterinary Record 146: 91–95

Chiesa OA, Garcia F, Domingo M et al 1999 Cytological and microbiological results from equine guttural pouch lavages obtained percutaneously; correlation with histo-pathological findings. Veterinary Record 144: 618–621

Christley RM, Hodgson DR, Rose RJ et al 2001 A case-control study of respiratory disease in Thoroughbred race-horses in Sydney, Australia. Equine Veterinary Journal 33: 256–264

Clinkenbeard KD, MacAllister CG, Cowell RL et al 2002 Oral and nasal cavities, pharynx, guttural pouches, and paranasal sinuses. In: Cowell RL, Tyler RD (editors) Diagnostic Cytology and Haematology of the Horse, 2nd edn. Mosby, St Louis, pp.65–72

Cowell RL, Tyler RD 2002 Diagnostic Cytology and Haematology of the Horse, 2nd edn. Mosby, St Louis

Darien BJ 1994 Eosinophilic pneumonitis in foals and horses. Compendium on Continuing Education for the Practicing Veterinarian 16: 1210–1212

DeHeer HL, Parry BW, Grindem CB 2002 Pleural fluid. In: Cowell RL, Tyler RD (editors) Diagnostic Cytology and Haematology of the Horse, 2nd edn. Mosby, St Louis, pp.107–126

Derksen FJ, Brown CM, Sonea IM et al 1989 Comparison of transtracheal aspirate and bronchoalveolar lavage cytology in 50 horses with chronic lung disease. Equine Veterinary Journal 21: 23–26

Dixon PM 1997 Ancillary diagnostic techniques for the investigation of equine pulmonary disease. Equine Veterinary Education 9: 72–80

Freeman DE 1991 Guttural pouches. In: Beech J (editor) Equine Respiratory Disorders. Lea & Febiger, Philadelphia, pp.305–330

Freeman KP, Roszel JF 1997a Equine cytology patterns in respiratory conditions of noninfectious or unknown origin. Compendium on Continuing Education for the Practicing Veterinarian 19: 755–763

Freeman KP, Roszel JF 1997b Equine cytology patterns in respiratory conditions of probable or known infectious origin. Compendium on Continuing Education for the Practicing Veterinarian 19: 378–383

Hare JE, Viel L 1998 Pulmonary eosinophilia associated with increased airway responsiveness in young racing horses. Journal of Veterinary Internal Medicine 12: 163–170

Hewson J, Viel L 2002 Sampling, microbiology and cytology of the respiratory tract. In: Lekeux P (editor) Equine Respiratory Diseases. International Veterinary Information Service, Ithaca. Online. Available: http://www.ivis.org

Hodgson JL, Hodgson DR 2002a Inflammatory airway disease. In: Lekeux P (editor) Equine Respiratory Diseases. International Veterinary Information Service, Ithaca. Online. Available: http://www.ivis.org

Hodgson JL, Hodgson DR 2002b Tracheal aspirates: indications, technique and interpretation. In: Robinson NE (editor) Current Therapy in Equine Medicine V. WB Saunders, Philadelphia, pp.401–406

Hoffman AM 1999 Bronchoalveolar lavage technique and cytological diagnosis of small airway inflammatory disease. Equine Veterinary Education 11: 208–214

Hoffman AM, Viel L 1997 Techniques for sampling the respiratory tract of horses. Veterinary Clinics of North America; Equine Practice 13: 463–475

Hoffman AM, Mazan MR, Ellenberg S 1998 Association between bronchoalveolar lavage cytologic features and airway reactivity in horses with a history of exercise intolerance. American Journal of Veterinary Research 59: 176–181

Holcombe SJ, Robinson NE, Derksen FJ et al 2006 Tracheal mucus is associated with poor racing performance in thoroughbred horses. Equine Veterinary Journal (in press)

Hughes KJ, Malikides N, Hodgson DR et al 2003 Comparison of tracheal aspirates and bronchoalveolar lavage in racehorses. 1. Evaluation of cytological stains and the relative percentage of mast cells and eosinophils. Australian Veterinary Journal 81: 681–684

Leguillette R, Roy MF, Lavoie J-P 2002 Foal pneumonia. In: Lekeux P (editor) Equine Respiratory Diseases. International Veterinary Information Service, Ithaca. Online. Available: http://www.ivis.org

Mair TS 1987 Value of tracheal aspirates in the diagnosis of chronic pulmonary diseases in the horse. Equine Veterinary Journal 19: 463–465

Malikides N 2004 A longitudinal cohort study of inflammatory airway disease in racing thoroughbreds in Sydney, Australia. PhD thesis, University of Sydney, Australia

Malikides N, Hughes KJ, Hodgson DR et al 2003 Comparison of tracheal aspirates and bronchoalveolar lavage in racehorses. 2. Evaluation of the diagnostic significance of neutrophil percentages. Australian Veterinary Journal 81: 685–687

Martin BB, Beech J, Parente EJ 1999 Cytologic examination of specimens obtained by means of tracheal washes

performed before and after high-speed treadmill exercise in horses with a history of poor performance. Journal of the American Veterinary Medical Association 214: 673–677

McGorum BC, Dixon PM 1994 The analysis and interpretation of equine bronchoalveolar lavage fluid (BALF) cytology. Equine Veterinary Education 6: 203–209

McGorum BC, Dixon PM, Halliwell REW et al 1993a Comparison of cellular and molecular components of bronchoalveolar lavage fluid harvested from different segments of the equine lung. Research in Veterinary Science 55: 57–59

McGorum BC, Dixon PM, Halliwell REW et al 1993b Evaluation of urea and albumin as endogenous markers of dilution of equine bronchoalveolar lavage fluid. Research in Veterinary Science 55: 52–56

McKane SA, Canfield PJ, Rose RJ 1993 Equine bronchoalveolar lavage cytology: survey of thoroughbred racehorses in training. Australian Veterinary Journal 70: 401–404

Newton JR, Wood JLN, Dunn KA et al 1997 Naturally occurring persistent and asymptomatic infection of the guttural pouches of horses with *Streptococcus equi.* Veterinary Record 140: 84–90

Nicholls R, Pirie RS 2001 Preparation of bronchoalveolar lavage fluid cytology slices by cellular gravitation – method of preparation and comparison with cytospin preparations. Proceedings of the Word Equine Airway Symposium, Edinburgh, p.75

Pickles KJ, Pirie RS, Rhind S et al 2001 The effect of time, temperature and fixatives on cytological assessment of equine bronchoalveolar lavage fluid. Proceedings of the Word Equine Airway Symposium, Edinburgh, p.78

Raidal SL 1995 Equine pleuropneumonia. British Veterinary Journal 151: 233–261

Raidal SL, Love DN, Bailey GD 1995 Inflammation and increased numbers of bacteria in the lower respiratory tract of horses within 6 to 12 hours of confinement with the head elevated. Australian Veterinary Journal 72: 45–50

Robinson NE 2001 International workshop on Equine Chronic Airway Disease. Equine Veterinary Journal 33: 5–19

Robinson NE 2003 Inflammatory airway disease: defining the syndrome. Conclusions of the Havemeyer Workshop. Equine Veterinary Education 15: 61–63

Sweeney CR, Rossier Y, Ziemer EL et al 1992 Effects of lung site and fluid volume on results of bronchoalveolar lavage fluid analysis in horses. American Journal of Veterinary Research 53:1376–1379

Sweeney CR, Rossier Y, Ziemer EL et al 1994 Effect of prior lavage on bronchoalveolar lavage fluid cell population of lavaged and unlavaged lung segments in horses. American Journal of Veterinary Research 55: 1501–1504

Timoney JF 2004 The pathogenic equine streptococci. Veterinary Research 35: 397–409

Traub-Dargatz JL 1997 Field examination of the equine patient with nasal discharge. Veterinary Clinics of North America; Equine Practice 13: 561–588

Whitwell KE, Greet TRC 1984 Collection and evaluation of tracheobronchial washes in the horse. Equine Veterinary Journal 16: 499–508

Zinkl JG 2002 Lower respiratory tract. In: Cowell RL, Tyler RD (editors) Diagnostic Cytology and Haematology of the Horse, 2nd edn. Mosby, St Louis, pp.73–86

10 Radiography and Radiology of the Respiratory Tract

Safia Barakzai and Hester McAllister

Upper Respiratory Tract

Radiography is an extremely useful diagnostic tool for the evaluation of many parts of the equine upper respiratory tract (URT), in particular for those parts of the URT that cannot easily be evaluated endoscopically such as the paranasal sinuses. The techniques described in this chapter are applicable to all equine practices because portable radiography machines are adequate for obtaining diagnostic radiographs of the equine URT. Excellent quality radiographs can be obtained in the standing, heavily sedated horse, and consequently there is no requirement for general anesthesia.

Radiographic Techniques

The X-ray machine used must be capable of allowing both vertical and horizontal movements of the X-ray beam. Exposure requirements are not high for equine URT radiography, especially if cassettes with rare-earth intensifying screens are used. The use of large (35 × 43 cm) cassettes is often helpful when evaluating a complex structure such as the equine head because the position of any observed abnormality can be assessed in relation to obvious anatomical landmarks. The use of grids is discouraged for standing radiography because they are not required to obtain high-quality radiographs and their use causes increased risk of radiation exposure to personnel.

Radiation safety should be strictly adhered to when taking radiographs of the equine URT, as personnel holding the horse and the cassette are potentially close to the primary beam. The primary beam should be collimated to include only the areas of interest. All assisting personnel should wear lead aprons, lead gloves, and radiation exposure badges (dosimeters) and should maintain a distance of at least 2 m from the primary beam. Heavy sedation (such as with xylazine/detomidine/romifidine plus butorphanol) reduces head movement and thereby reduces the need for repeat exposures because of movement artifacts. Resting the nose of the horse on a stool may also help to minimize the swaying movements caused by heavy sedation. A fabric head collar without metal components should be used during radiography of the equine skull.

Lateral radiographs (Fig. 10.1)

These views are obtained with the cassette positioned in a vertical plane, as close as possible to the affected side of the head. The horizontal X-ray beam should be centered at the area of interest (Table 10.1). The principal disadvantage of the lateral view is that the structures on the left and right sides of the skull are superimposed, and therefore cannot be evaluated individually.
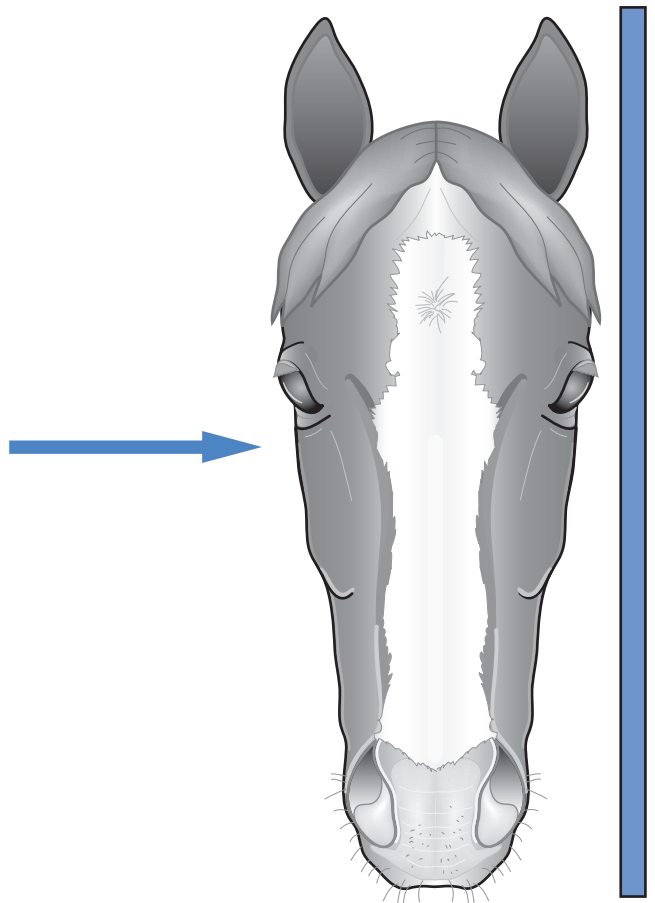
Fig. 10.1. Diagram showing direction of X-ray beam (arrow) and cassette position for lateral radiographs of the head.

Table 10.1. Center point of the X-ray beam for lateral radiographs of various structures in the equine upper respiratory tract	
Structure(s) being radiographed	**X-ray beam center point**
Maxillary sinuses and maxillary cheek teeth apices	Dorsal to the rostral aspect of the facial crest
Guttural pouches	Caudal aspect of the vertical ramus of the mandible
Nasopharynx, larynx and proximal trachea/esophagus	Caudoventral angle of the mandible
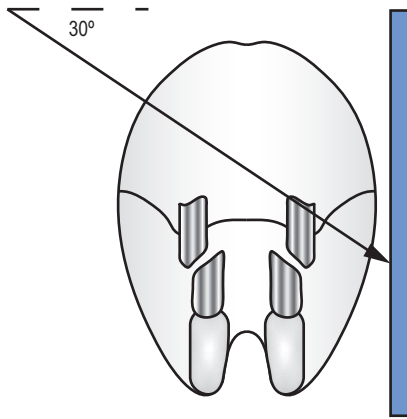
Fig. 10.2. Diagram showing direction of X-ray beam (arrow) and cassette position for 30° dorsolateral–lateral radiographs of the head to view the maxillary cheek teeth apices and sinuses.

Fig. 10.3. Diagram showing center point (×) and cassette position for dorsoventral radiograph of the head in the standing horse.

30° dorsolateral–lateral oblique radiographs (Fig. 10.2)

This oblique radiographic view separates the left and right sides of the maxillary and frontal sinuses and the maxillary cheek teeth rows, allowing evaluation of individual apices and the surrounding structures. The cassette is again positioned in a vertical plane, as close as possible to the affected side of the head. The X-ray tube is positioned at a higher level on the opposite side, with the beam directed 30° down from the horizontal and centered at the rostral aspect of the facial crest and collimated to include all six cheek teeth, or alternatively at the suspected tooth root apex. A higher exposure is required to optimally image the equine cheek teeth apices as compared to the paranasal sinuses. The presence of rostrocaudal angulation is a common technical fault associated with this projection and should be avoided because it can complicate radiographic interpretation.

Dorsoventral radiographs (Fig. 10.3)

This radiographic view is obtained by positioning the cassette underneath and parallel to the hemi-mandibles (the horse's head can be rested on the cassette). The X-ray beam is directed perpendicular to the plate, centered on the midline, at the level of the rostral aspect of the facial crests. Positioning is very important when taking this radiographic view, because any lateral deviation from a true dorsoventral will cause superimposition of the mandibular and maxillary cheek teeth rows on one side, and also obscure the ventral conchal sinus and nasal cavity on one side. This view is most useful for evaluating the areas of the ventral conchal sinus (medial compartment of the rostral maxillary sinus), nasal cavities, and nasal septum. Abnormalities of the cheek teeth such as laterally or medially displaced teeth, sagittal fractures, advanced caries and alveolar changes associated with advanced apical infections can also be observed using this radiographic view.

Additional radiographic techniques

The use of a small metal marker such as a paper clip, placed over an area of facial swelling can be useful when evaluating radiographic changes and assessing their likely significance, particularly if there is suspicion of an apical infection. If an external sinus tract is present, a blunt
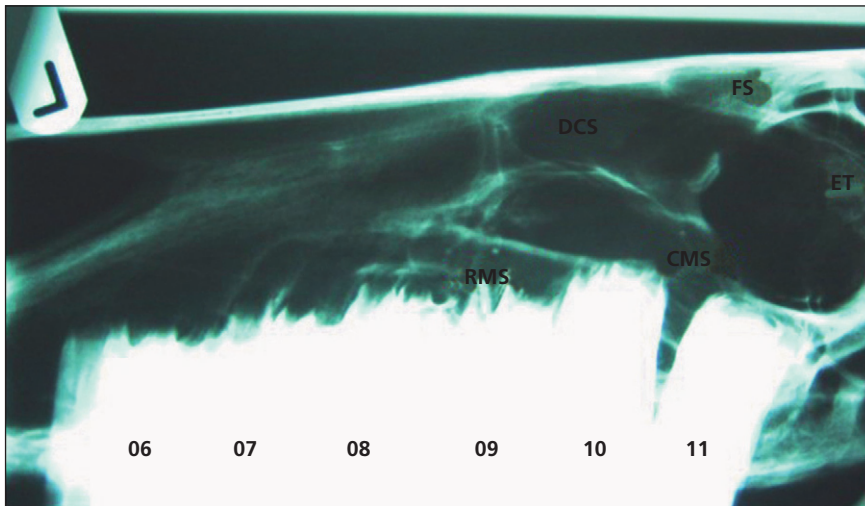
Fig. 10.4. Lateral small radiograph outlining the maxillary cheek teeth and paranasal sinuses of a normal horse. Note that the left and right rows of maxillary cheek teeth are superimposed, making it impossible to evaluate the apex of any individual cheek tooth. The apices and reserve crowns of the upper 08s and 09s (third and fourth maxillary cheek teeth) are within the rostral maxillary sinus (RMS), and the apices and reserve crowns of the upper 10s and 11s (fifth and sixth maxillary cheek teeth) are within the caudal maxillary sinus (CMS). The positions of the ethmoturbinates (ET), dorsal conchal sinus (DCS) and frontal sinus (FS) are also shown.

malleable metallic probe should be inserted into the tract and the appropriate lateral oblique radiograph should be taken. This technique can provide irrefutable evidence of dental disease, identify the affected apical area of the tooth, and provide a landmark for surgical procedures. The injection of contrast material such as Iohexol into a draining tract can similarly provide valuable diagnostic and spatial information.

The use of contrast paranasal sinusography has been described by Behrens et al (1991). The injection of contrast medium directly into the sinuses outlines solid masses that may be difficult to distinguish in survey radiographs because they closely conform to the contour of the sinuses, and also allows detection of interruption of normal gravitational flow of fluid through the sinuses. However, direct sinoscopy, or three-dimensional imaging modalities such as computed tomography or magnetic resonance imaging, are likely to be of greater diagnostic value for such abnormalities.

Radiography of the guttural pouches, temporomandibular joints and stylohyoid bones is occasionally necessary. To separate and thereby differentiate the left and right sides, these areas can be radiographed with the affected side next to the cassette, and the X-ray beam directed horizontally, angled at 10–15° in a rostrocaudal direction.

Nasal Cavity and Paranasal Sinuses

Normal anatomy and radiographic appearance (Figs 10.4 and 10.5)

The rostral maxillary sinus of the horse is usually positioned dorsal to the apices of the upper 08s and 09s (Triadan system, equivalent to the third and fourth maxillary cheek teeth) (Perkins 2002), and is separated from the caudal maxillary sinus by a complete bony septum. This septum is usually angled from rostrolateral to caudomedial, and therefore is not usually seen as a single
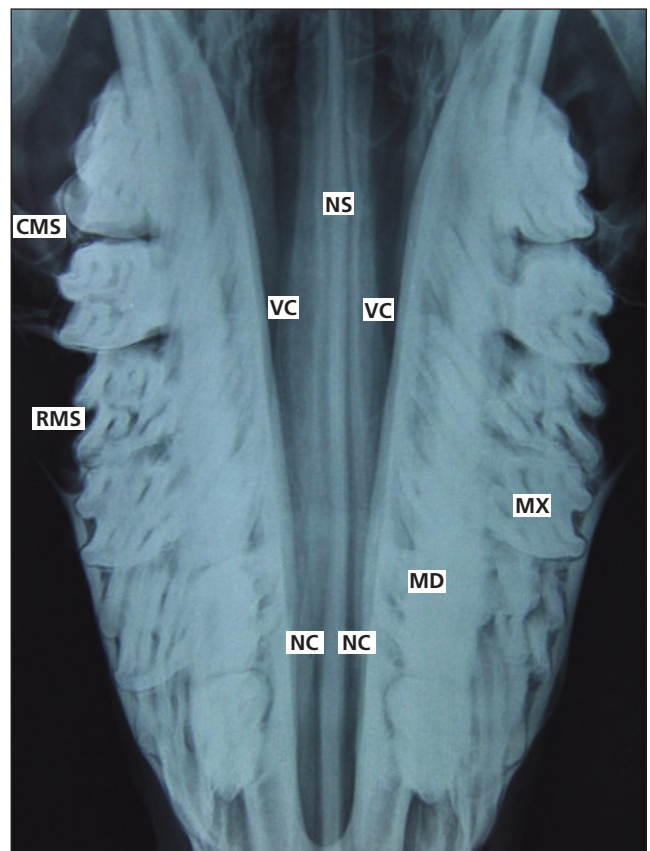
Fig. 10.5. Dorsoventral radiograph of the skull of a normal horse. The maxillary (MX) and mandibular (MD) cheek teeth rows are clearly evident. The nasal cavities (NC), nasal septum (NS) and ventral conchal sinuses (VC) can also be assessed on this view. The rostral (RMS) and caudal (CMS) maxillary sinuses, located lateral to the upper 08s–11s, may also be evaluated.

radio-opaque line in lateral radiographs. In horses less than 7 years of age, the reserve crowns of the upper 08s and 09s can almost completely fill the rostral maxillary sinus, and even in the older horse, with shorter cheek teeth reserve crowns, the rostral maxillary sinus often remains a
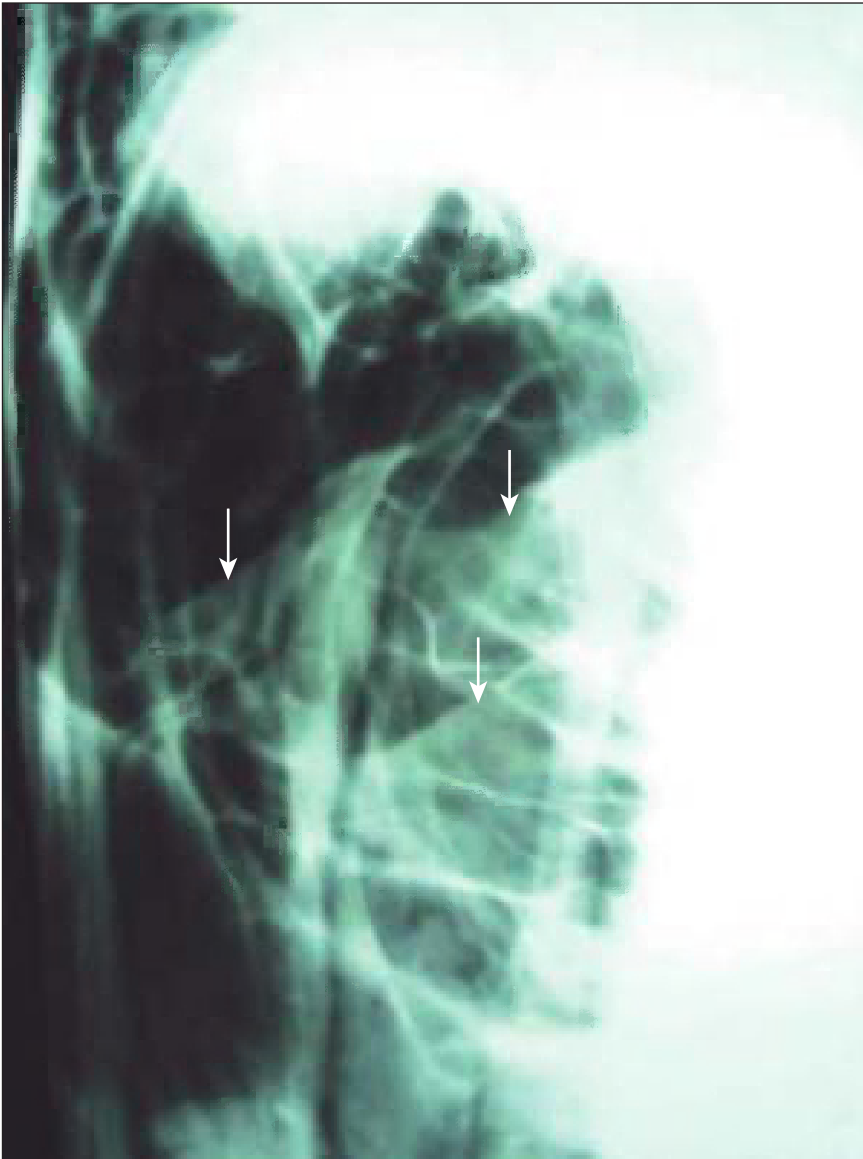
Fig. 10.6. Lateral radiograph of the sinuses of a horse with primary sinusitis. There are fluid lines present (arrows) in the rostral and caudal maxillary sinuses, and also in the conchofrontal sinus, which appear as horizontal lines with soft tissue opacities ventral to them.

small structure. The medial compartment of the rostral maxillary sinus is also known as the ventral conchal sinus, and communicates with the rostral maxillary sinus over the infra-orbital canal. The ventral conchal sinus often extends caudally (as the ventral conchal "bulla") to the level of 111/211 (sixth maxillary cheek teeth), and is best evaluated radiographically using a dorsoventral projection (see Figs 10.5 and 10.10).

The caudal maxillary sinus is usually positioned immediately dorsal to the apices of the upper 10s and 11s (fifth and sixth cheek teeth), and communicates with the dorsal conchal sinus via the large frontomaxillary aperture. The frontal sinus is the dorsocaudal extension of the dorsal conchal sinus, and appears as a triangular structure on lateral radiographs, positioned dorsal to the ethmoturbinates and rostral to the cranium. The frontal sinus, plus the dorsal conchal sinus together make up the conchofrontal sinus.

The nasal cavities are positioned medial and rostral to the ventral conchal sinus, and the left and right cavities are separated by the nasal septum, which runs in the midline. The septum, which can be seen on dorsoventral radiographs, should be evaluated for lateral deviation, most commonly as a result of expansive space-occupying lesions within the ventral conchal sinus or nasal cavity.

Primary sinusitis

Horses with primary sinusitis often have free fluid within the sinuses, which can be seen as one or more horizontal fluid lines (with increased opacity below the line) on lateral radiographs (Fig. 10.6). Other changes associated with
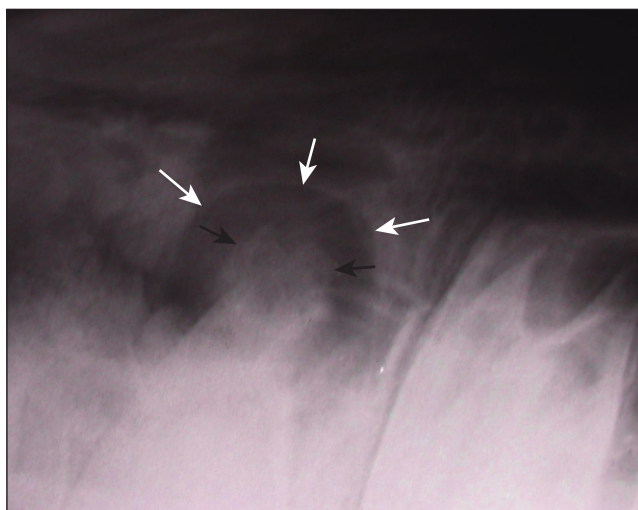
Fig. 10.7. A 30° dorsolateral oblique radiograph of a horse with periapical infection and dental sinusitis. The infected tooth (209) shows a soft tissue density (granuloma) around its rostral roots (black arrows), surrounded by a radiolucent halo (white arrows). The apex and reserve crown of this tooth lie within the rostral maxillary sinus.
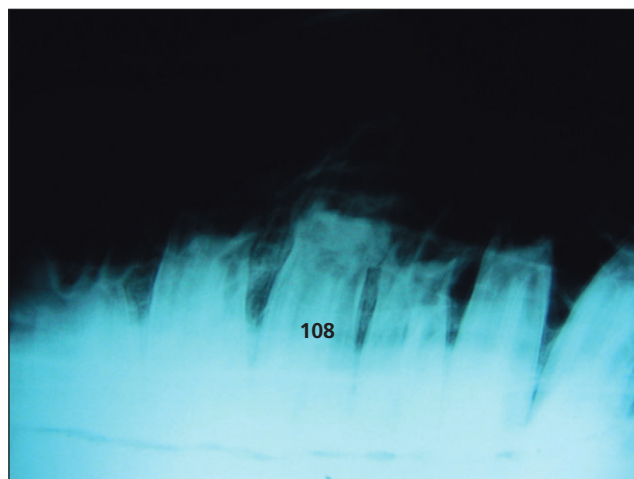
Fig. 10.8. A 30° dorsolateral–lateral oblique radiograph of the maxillary cheek teeth and maxillary sinuses of a horse with chronic apical infection of 108. Note the marked remodeling of the apex of this tooth, with loss of the normal pointed root outlines and deposition of radiodense material (cementoma formation) around all three roots.

primary sinusitis are localized, diffuse or delineated intrasinus radio-opacity, nasal septum deviation and mineralization of sinus walls in more chronic cases. In many cases of sinusitis, the increased soft tissue opacity within the sinuses may simply be the result of inflamed and hypertrophied sinus mucosa, which occurs with any chronic sinus infection (Gibbs & Lane 1987, Tremaine & Dixon 2001). It should be remembered that fluid or soft tissue opacities within the sinuses can also be seen secondary to other disorders that cause sinusitis (as detailed below). Trephination and lavage of the sinuses may decrease the amount of fluid accumulating within the sinuses and allow for more accurate radiographic evaluation of intrasinus structures such as the cheek teeth apices and ethmoturbinates.

Dental sinusitis

Periapical dental infections are a common cause of sinusitis in horses (Boulton 1985, Gibbs & Lane 1987, Tremaine & Dixon 2001). The apices of the upper 08s, 09s, 10s, and 11s (and variably the 07s) lie within the rostral and caudal maxillary sinuses, and abscesses which form around the dental apices can erode the thin alveolar bone, with resultant infection of the sinuses.

Radiographic changes consistent with early periapical infection include widening of the periodontal space and thinning of the lamina dura denta (the dense rim of cortical bone which lines the alveolus). When periapical infection has been present for many weeks, the affected apices develop lytic changes, especially in mature teeth where the true roots (non-enamel areas) are well formed.

These changes manifest as periapical radiolucent "haloes," and rounded or "clubbed" appearance of the tooth roots as a result of gross lysis/destruction of the root structures (Fig. 10.7). In more chronic periapical infections, a zone of radiodense sclerosis usually surrounds the periapical "halo", because of new bone deposition around the lytic infected area. More marked sclerosis develops around the apices of the first two maxillary cheek teeth than the caudal maxillary cheek teeth. This is because the apices of the first two maxillary cheek teeth are usually located in denser bone than those of the caudal four maxillary cheek teeth, which are situated in thin alveolar bone within the maxillary sinuses. Longstanding periapical infection may result in abnormal depositions of radio-opaque cementum at the tooth apex (Fig. 10.8). Dystrophic mineralization of the nasal conchae (coral formation) may also occur with chronic maxillary cheek tooth periapical infections (Gibbs & Lane 1987, Tremaine & Dixon 2001).

Soft tissue opacities may also be apparent in the sinuses if periapical infection of the caudal maxillary cheek teeth has occurred. These opacities may be caused by a rounded, soft tissue granuloma (Fig. 10.7) or later, an encapsulated abscess developing over the infected apex. Fluid lines may be apparent in straight lateral views of the sinuses, because of accumulation of liquid purulent material. In cases of dental sinusitis, as in other chronic sinusitis cases, inflamed and hypertrophied sinus mucosa may cause generalized increased soft tissue opacity within the sinuses (Gibbs & Lane 1987, Tremaine & Dixon 2001).

Although radiography has a good (95%) specificity for the diagnosis of periapical infections, it is not very sensitive (50%), particularly in early cases (Weller et al 2001).
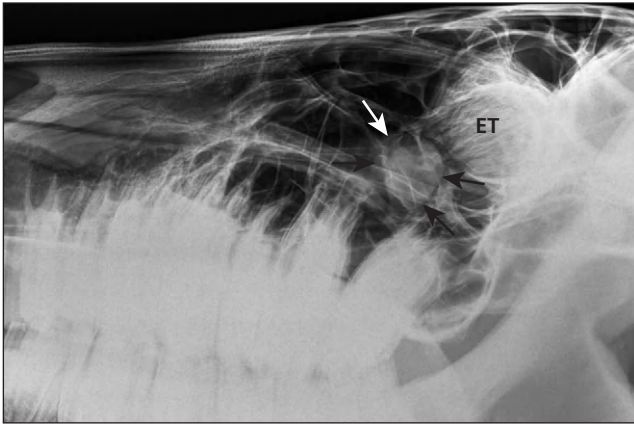
Fig. 10.9. Lateral radiograph of a horse wtih an ethmoidal hematoma (arrows) attached to the rostro-ventral aspects of the ethmotubinates (ET). The mass appears as a rounded soft tissue density.
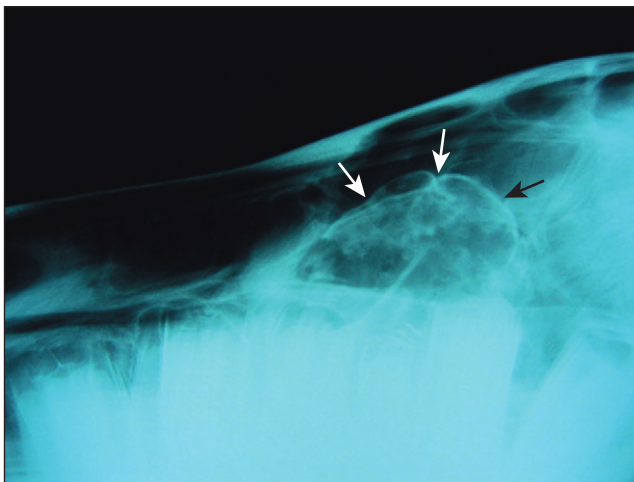
Fig. 10.10. Lateral radiograph of a horse with a large sinus cyst in its rostral and caudal maxillary sinuses. Note the calcified nature of the cyst wall (arrows).

Fig. 10.11. Dorsoventral radiograph of the same horse as Fig. 10.10. Note the soft tissue opacity mass causing enlargement of the left ventral conchal sinus, slight deviation of the nasal septum to the right and increased radio-opacity of the left rostral and caudal maxillary sinuses, lateral to the 09s–11s (fourth to sixth cheek teeth) and extending caudally.

Studies have shown that in cases of dental sinusitis, infected teeth can be recognized with confidence in only 50–57% of cases (Gibbs & Lane 1987, Tremaine & Dixon 2001). In part, this may be because the superimposition of opaque sinus contents and maxillary osteitis on underlying dental structures prevents recognition of subtle abnormalities. In such cases, scintigraphy is a useful adjunctive diagnostic technique that may provide evidence to allow differentiation between cases of primary and dental sinusitis (Weller et al 2001, Barakzai et al 2006).

Progressive ethmoidal hematoma (PEH)
(Fig. 10.9)

PEH lesions may be evident as discrete soft tissue opacity lesions on lateral skull radiographs; however, small PEH lesions can sometimes be missed because radiographic interpretation of the ethmoidal area is complicated by the superimposition of PEH lesions over the globes, orbits, and ethmoidal labyrinths. A well-circumscribed, round mass of soft tissue radio-opacity in the area of the ethmoid labyrinth or sphenopalatine sinus has been reported in 57% of horses with PEH (Tremaine & Dixon 2001).

Sinus cysts (Figs 10.10 and 10.11)

As with other causes of secondary sinusitis, sinus cysts are commonly associated with increased radio-opacity and fluid lines within the sinuses. Radiographic changes that are strongly suggestive of sinus cysts include soft tissue opacities, with a partially mineralized capsule containing irregular bony spicules; such changes are present in 35% of horses with sinus cysts (Tremaine & Dixon 2001). Distortion of the overlying facial bones, nasal septum deviation and secondary dental deformities are also commonly associated with sinus cysts (Lane et al 1987a,b, Tremaine & Dixon 2001).

Maxillary fractures

Maxillary fractures are commonly the result of kicks to the skull, and affected horses often present with epistaxis as a result of traumatic damage to the mucosa lining the maxillary or frontal sinuses. If skull fractures are suspected, oblique views taken at varying angles may be necessary to highlight the fracture lines. However, because of the complexity of bones within the skull, and particularly the sinus areas, non-displaced fractures are often difficult to detect radiographically. Superimposition of soft tissue opacities or fluid lines within the sinuses as a result of intrasinus hemorrhage and mucosal inflammation may further obscure fracture lines. Nasofrontal suture periostitis is a common delayed sequel to maxillary fracture, and is seen radiographically as proliferative new bone formation, most commonly at the nasofrontal suture lines (Tremaine & Dixon 2001).

Neoplasia

The most frequently occurring tumor of the equine sino-nasal area is squamous cell carcinoma (Priester & Mackay 1980, Head & Dixon 1999). Other reported tumor types include adenocarcinoma, fibrosarcoma, spindle cell sarcoma, myxoma, lymphosarcoma, hemangiosarcoma, and osteoma/osteosarcoma (Head & Dixon 1999). Neoplastic lesions within the sinus may appear radiographically as soft tissue opacities of variable size, and are often radiographically indistinguishable from other space-occupying masses. However, if gross destruction of bone is evident radiographically, this is strongly suggestive of neoplasia (Tremaine & Dixon 2001).

Guttural Pouch

The guttural pouches appear radiographically as large radiolucent structures located ventral to the base of the skull and atlas, dorsal to the nasopharynx, and mainly caudal to the vertical ramus of the mandible (Fig. 10.12). The guttural pouches are incompletely divided into medial and lateral compartments by the stylohoid bones, which articulate dorsally with the temporal bone at the temporohyoid joint.

Guttural pouch empyema

This disorder is most commonly associated with infection with *Streptococcus equi* var. *equi* (strangles), following abscessation and drainage of the retropharyngeal lymph nodes into the guttural pouches. Radiographically, fluid accumulation may be evident in one or both guttural pouches as an increased area of radio-opacity ventral to a horizontal fluid line. A dorsoventral radiograph of the caudal skull and cranial neck, taken with the head fully
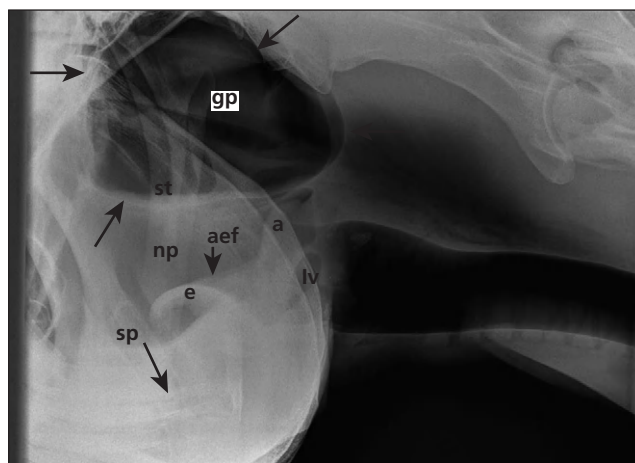
Fig. 10.12. Lateral radiograph of the nasopharynx (np), larynx and guttural pouches (gp). The borders of the guttural pouches are indicated with arrows. Note the stylohyoid bones (st), epiglottic cartilage (e), arytenoid cartilages (a), ary-epiglottic folds (aef), laryngeal ventricles (lv) and soft palate (sp).

extended, may allow easy differentiation of unilateral from bilateral disease, as would a slightly (10–15°) rostro-caudally angulated lateral view. Chronic empyema may lead to stagnation of pus within the pouch with its subsequent inspissation and chondroid formation. Chondroids may be seen as smooth, irregularly sized, discrete soft tissue opacities within the lumen of the guttural pouch.

Enlarged or abscessed retropharyngeal lymph nodes are often seen in the floor of the medial compartment of the guttural pouches in horses with strangles. These will be evident radiographically as an irregular outline of the ventral border of the pouch.

Guttural pouch mycosis

Mycotic lesions of the guttural pouches are not usually distinguishable on radiographs, unless they are very large and proliferative. However, horses that have had a recent episode of epistaxis as a result of guttural pouch mycosis may have a fluid line (blood) within the affected guttural pouch (Fig. 10.13). Radiographs may be useful in some cases to distinguish between guttural pouch mycosis and rupture of the rectus capitus ventralis muscles. This traumatic muscle rupture may occur in horses that have reared over backwards and sustained trauma to the base of the skull. In both disorders, endoscopic examination may reveal blood emerging from the ostia of one or both guttural pouches, and the guttural pouch may be blood-filled, obscuring the endoscopist's view, and preventing direct inspection of the structures within the guttural pouch. Radiography may reveal small avulsion fracture fragments of the ventral aspect of the occipital and basisphenoid bones if epistaxis is the result of rupture of the rectus capitus ventralis muscles.

Fig. 10.13. Lateral view of the guttural pouch of a horse that has had a recent episode of epistaxis from the internal carotid artery as a result of guttural pouch mycosis. Note the fluid line (arrows) within the guttural pouch, and mottled soft tissue densities visible dorsal to this which may represent large proliferative fungal fibrinous plaques.

More chronic cases of guttural pouch mycosis may have bony remodeling of the ventral aspect of the petrous temporal bone, and possibly also of the dorsal aspect of the stylohyoid bone. Pathological fractures of the stylohyoid bone as a result of chronic fungal osteitis are uncommon.

Guttural pouch tympany

This congenital condition is seen predominantly in filly foals and yearlings, and occurs when the ostium of the guttural pouch acts as a one-way valve, allowing air into but not out of one or both guttural pouches. The air-distended pouch is seen radiographically as a very large radiolucent structure extending much further caudally than normal. In severely affected animals, compression of the nasopharynx by the distended pouch may result in stridor and dysphagia. The condition is usually unilateral, and therefore the normal contour of the contralateral pouch may also be seen radiographically. Some degree of secondary empyema, caused by lack of drainage from the affected pouch, is common.

Masses within the guttural pouches

Tumors of the guttural pouch are uncommon. Melanoma is the most frequent (Fintl & Dixon 2000), but lympho-sarcoma, hemangiosarcoma, fibroma, and squamous cell carcinoma have also been reported. An abnormal outline of the pouch may be evident radiographically, with tumors occurring most commonly in the area of the retrophryn-geal lymph nodes in the floor of the pouch. Large tumors or secondary abscessation of the lymph nodes may cause nasopharyngeal compression and dyspnea or dysphagia.

Stylohyoid abnormalities

The temporohyoid articulation is most commonly affected as a result of temporohyoid osteopathy. In some cases extension of disease affecting the middle ear may result in osseous proliferation of the dorsocaudal aspect of the stylohyoid bone within the guttural pouch. Ossification of the normally cartilaginous temporohyoid articulation may occur, and normal tongue movements may then result in fracture of the mid-portion of the affected stylohyoid bone, or, more commonly, the petrous temporal bone (Blythe 1997). Fractures of the stylohyoid may also result from chronic guttural pouch mycosis if the fungal plaque is located over the stylohyoid bone.

Enlarged or fractured stylohyoid bones may be seen on lateral radiographs of the guttural pouch area, and slightly (10–15°) rostrocaudally angulated projections will allow more accurate evaluation by preventing superimposition of the left and right sides.

Nasopharynx/Larynx

Normal anatomy and radiographic appearance (Fig. 10.12)

The epiglottis is the most easily identifiable structure in radiographs of this area. It is positioned just rostral to the caudoventral angle of the mandible, and appears as a curved, dorsally convex soft tissue opacity, with the free tip pointing rostrally into the nasopharynx. It lies dorsal to the soft palate, and the caudal border of the soft palate should form a seal around the epiglottic base. The dorsal aspect of the soft palate can be followed rostrally, lying on the base of the tongue, until superimposition of the caudal cheek teeth obscures it from view. A small amount of air may be detected between the soft palate and the base of the tongue. Dorsal and rostral to the epiglottis is the radiolucent (air-filled) nasopharynx. The caudodorsal border of the nasopharynx is demarcated by the soft tissue opacities of the arytenoid cartilages. Depending on the radiographic exposure, the aryepiglottic folds may also be visible on lateral radiographs. The laryngeal ventricles are apparent as small rounded or elliptical radiolucent areas caudal to the base of the epiglottis. A degree of mineralization of the laryngeal cartilages may be considered normal even for some younger horses.

Arytenoid chondritis

Young male thoroughbreds are particularly predisposed to this condition which involves infection and inflamma-tion of one or both arytenoid cartilages. Radiography of the affected area in chronic cases may reveal abnormal mineralization of affected cartilages, and this is associated with a poor prognosis for return to athletic function.
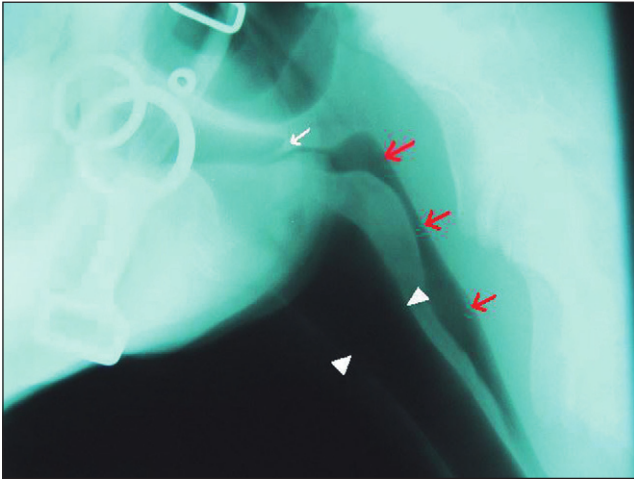
Fig. 10.14. Lateral view of the pharynx and larynx of a 3-month-old colt with fourth branchial arch defect showing an abnormal air column in the proximal esophagus (red arrows), and rostrally displaced palato-pharyngeal arch (white arrow). The normal radiolucent air column within the trachea is outlined with white arrowheads.

Fig. 10.15. Lateral radiograph of the pharynx and larynx of a horse with persistent DDSP. The soft palate (red outline) can be seen lying dorsal to the ventrally curved epiglottic cartilage (yellow outline). Note the artifacts caused by metal components of the headcollar.

Fourth branchial arch defect syndrome (cricopharyngeal–laryngeal dysplasia)

In 78% of animals affected by this condition, a continuous column of air extending from the nasopharynx into the lumen of the proximal esophagus (as a result of aplasia of the cricopharyngeus muscles) will be evident on lateral radiographs (Lane 2001) (Fig. 10.14). In contrast, normal horses may have only a small lucent linear air shadow, often less than 5 cm in length in the proximal esophagus. In some affected horses, rostral displacement of the palato-pharyngeal arch may be evident on radiographs as a soft tissue opacity "dew drop," intruding from the dorsal wall of the pharynx into the radiolucent nasopharynx. It is, however, imperative that these radiographs are taken with the horse unsedated, as rostral displacement of the palato-pharyngeal arch and air in the proximal esophagus may be observed in normal horses following sedation.

Epiglottic entrapment

The rostral free tip of the epiglottis can become trapped in a pouch of mucosa that develops from the mobile, sub-epiglottic mucosal folds. Epiglottal hypoplasia will predispose to this condition. Radiographically, the epiglottis appears shortened and less well defined than usual, and there may be concurrent dorsal displacement of the soft palate.

Epiglottic hypoplasia

In a horse with a truly hypoplastic epiglottis, lateral radiographs of the pharynx confirm that the epiglottal length is reduced from the normal length of 8.3–9.2 cm from tip to hyoid articulation in a thoroughbred-type horse,

to less than 6.5 cm (Linford et al 1983). When measuring epiglottic length using a lateral radiograph, the effect of magnification must be taken into account and the length corrected accordingly. Epiglottic hypoplasia may predispose affected animals to epiglottal entrapment. Although epiglottic hypoplasia was previously thought to predispose horses to intermittent dorsal displacement of the soft palate, this association is now in doubt.

Subepiglottic cysts

Subepiglottic cysts are sometimes not visible using an endoscope passed per nasum because the cyst is positioned ventral to the soft palate. Radiography is a particularly useful diagnostic aid for such cases to confirm the presence and size of these lesions. Lateral radiographs of horses with subepiglottic cysts show a well-demarcated, rounded soft tissue mass ventral to the epiglottis, which may appear to displace the epiglottic tip dorsally and caudally. The introduction of oropharyngeal contrast material may also be of value for delineating the cyst.

Persistent dorsal displacement of the soft palate (Fig. 10.15)

Radiography of horses affected with persistent dorsal displacement of the soft palate will reveal the soft palate lying dorsal to the epiglottic cartilage, and an unusually large amount of air between the base of the tongue and the soft palate. The epiglottis and subepiglottic area should be carefully checked for abnormalities such as subepiglottic cysts as these will not be visible endoscopically.
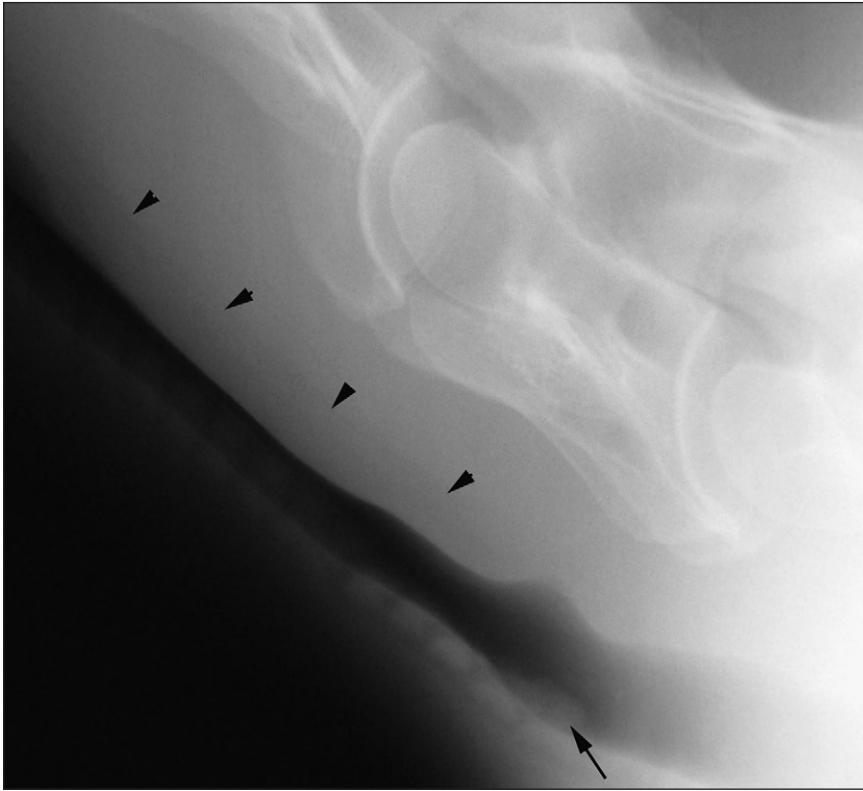
Fig. 10.16. Tracheal collapse. This 22-year-old pony had poor exercise tolerance, inspiratory difficulty, and coughing over the previous 12 months. This radiograph of the caudal cervical region (from the level of the fifth to seventh cervical vertebrae) shows the tracheal lumen is undulating and markedly narrowed. The black arrowheads outline the position of the normal tracheal lumen. A mucus plug (black arrow) is visible within the tracheal lumen.

Trachea

Normal anatomy and radiographic appearance

The cartilaginous tracheal rings are distinguishable on the dorsal and ventral aspect of the radiolucent air column in the tracheal lumen. This radiolucent column may be slightly wider at the most rostral aspect of the trachea, but narrows to a uniform width within three or four tracheal rings. The cartilage rings may become mineralized with age. The intrathoracic trachea runs in a horizontal direction and then dips ventrally at the heart base. The trachea divides at the heart base into left and right mainstem bronchi at the level of the fifth or sixth intercostal space. The terminal trachea may be displaced dorsally as a result of hilar lymphadenopathy or cardiomegaly particularly involving the left atrium.

Tracheal trauma/perforation

Horses that have sustained traumatic injury to the ventral neck may have tracheal perforations that are evident radiographically as radiolucent lines of air tracking up and down the soft tissues of the neck. Rupture of the trachea causes diffuse subcutaneous emphysema, which may track into the thoracic cavity and cause pneumomediastinum and pneumothorax (Fubini et al 1985).

Tracheal collapse

Lateral radiographs of the trachea of ponies with this disorder may appear normal if the radiograph is taken during expiration, but inspiratory radiographs may show marked narrowing of the air column within the cervical trachea at the site of obstruction (Fig. 10.16) because the trachea collapses as a result of the subatmospheric pressures within the trachea at this stage in the respiratory cycle. Developmental rotation of a section of a collapsed trachea can also be radiographically misleading. Similar radiographic features may be obtained after removal of a tracheotomy tube, if the tracheal rings were completely transected to allow placement of the tube, because the remaining part of the ring may become unstable and tend to collapse inwards during inspiration.

Intra/extraluminal tracheal masses

Primary neoplastic conditions of the trachea are rare but include lymphoma and squamous cell carcinoma. Excessive granulation tissue or chondroma-type masses affecting the adjacent cartilages may develop following removal of a temporary or permanent tracheostomy tube. Intraluminal masses may be seen radiographically as soft tissue opacities impinging on the radiolucent air column within the trachea.
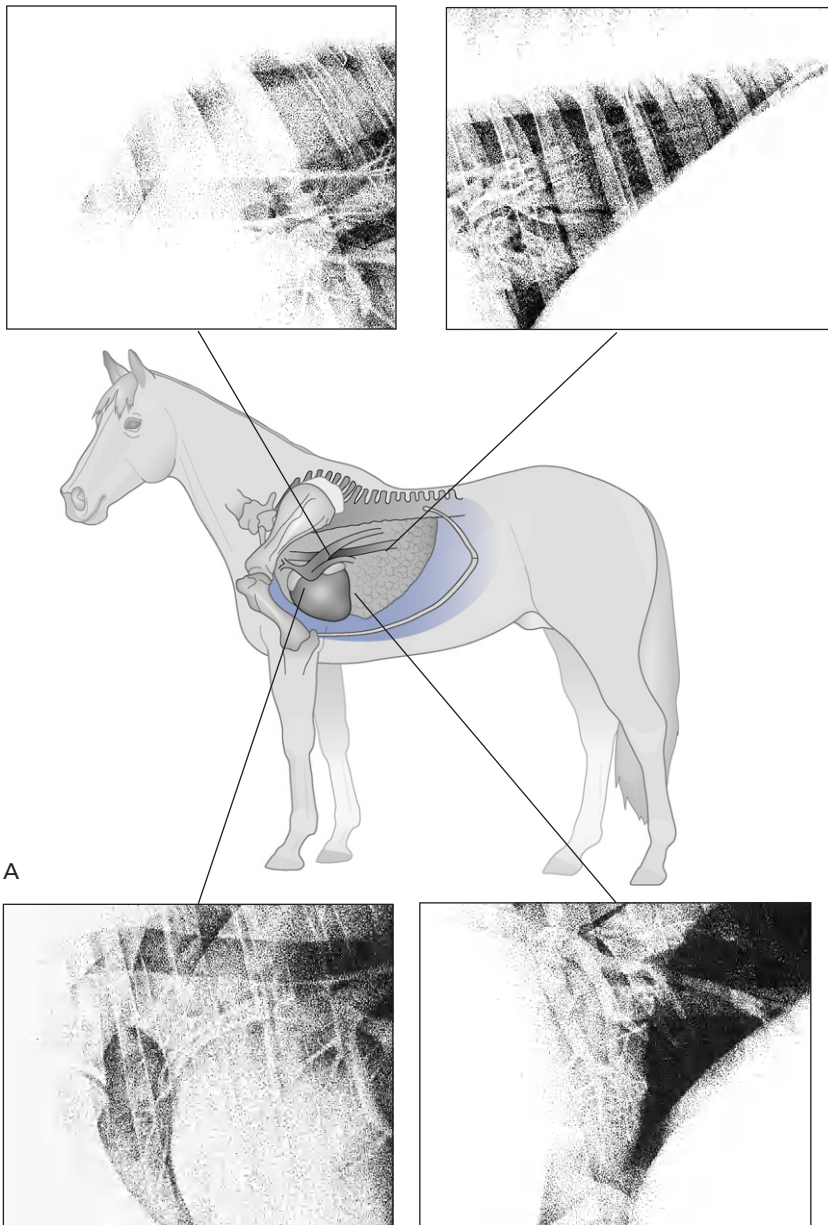
Fig. 10.17. (A) Diagram indicating the centering points for a complete radiographic examination of the adult equine thorax. The radiographic images related to each centering point are indicated in pictures 1–4. Redrawn from Farrow 1981b, with permission.

Continued

Extraluminal tracheal masses, such as abscesses of the caudal cervical lymph nodes as a result of strangles, or tumors, may cause tracheal compression, thereby narrowing the intraluminal air column, which may be seen radiographically.

Thoracic Radiography

Radiographic technique

Radiography of the thorax in adult horses presents a particular challenge because of the size of the chest. In addition, the muscles of the forelimb overly the cranial thorax and increase the tissue depth in this area. In adult horses, thoracic radiographs are taken in the standing position with the cassette located on the side of interest. The horse should stand level, with its weight evenly distributed on all four limbs. The largest cassette size (35 × 43 cm) should be used. For thoracic radiography in adult horses, the thorax is divided into at least three, and often four, overlapping radiographic areas (Fig. 10.17A), namely craniodorsal, caudodorsal, cranioventral and caudoventral (Farrow 1981a). If the horse stands with the forelimbs extended forwards, the cranioventral thorax can be more clearly seen on the radiograph. Chemical restraint ensures a cooperative patient and provides maximum
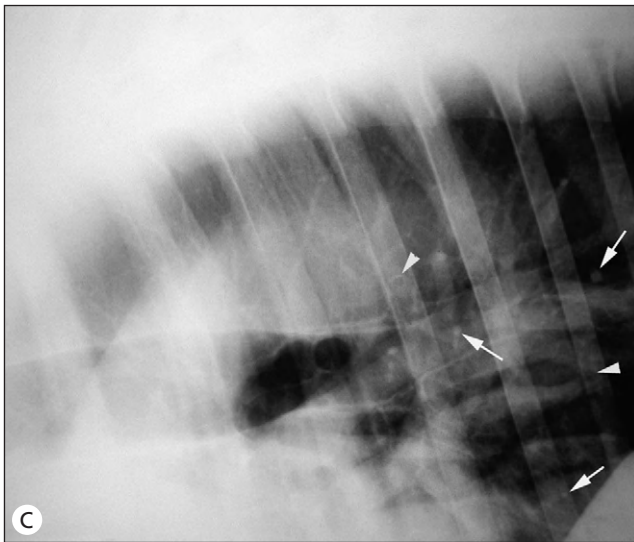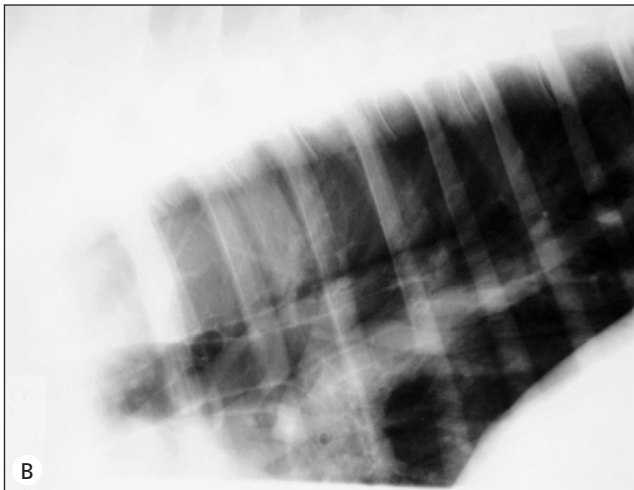
Fig. 10.17, cont'd. (B) Lateral radiograph of the normal caudodorsal lung field of an adult horse. The vessels are clearly seen tapering towards the periphery. The thin, linear, radio-opaque bronchial walls are visible in the hilar region. The background lung opacity is the normal interstitial component of the lung field. (C) End-on blood vessels (white arrows) are relatively radio-opaque, particularly when superimposed on rib shadows. They reduce in size towards the lung periphery, which helps to differentiate them from nodular infiltrates. Bronchi are seen in cross-section (arrowheads). (D) The esophagus, containing a small volume of fluid, is seen in the caudodorsal thorax (arrows) running horizontally towards the diaphragm.
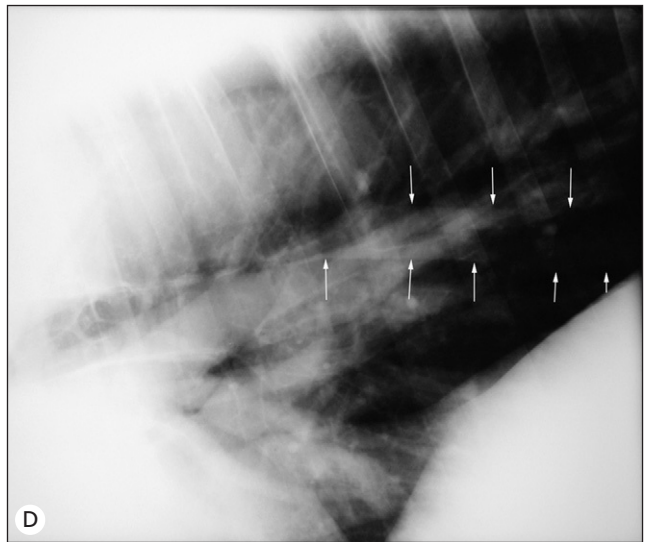
radiation safety for the personnel but may not be required if the animal is ill and depressed. Assisting personnel should be kept to the minimum and they should not stand close to the X-ray tube or cassette.

Breathing movement artifact is one of the main limiting factors in the production of diagnostic radiographs using low and medium output X-ray machines. Fast, rare-earth intensifying screens, in combination with fast film, enable exposure times to be kept to a minimum and reduce movement blur. The use of a grid improves the quality of the final image but the requirement for increased exposure factors increases the risk of motion artifact. The cassette and grid must be properly aligned with the X-ray tube to avoid grid artifacts. Accurate centering is optimal if there is a gantry system to link the cassette, grid and X-ray tube, although use of a drip stand and a large shoulder bag to hold the cassette and grid may suffice. If a grid is not used, scattered radiation from the animal can be reduced by employing the "air gap" technique, whereby the cassette

is placed 15–30 cm (depending on the animal's size) away from the chest wall (King et al 1981). This technique has the disadvantage of increasing magnification of the thoracic structures.

Exposure factors vary depending on the area of interest, size of animal and the nature of the pathological changes present. If the exposure is made in the expiratory phase, which is generally longer than the inspiratory phase, there is less likelihood of movement blur. However, expiratory studies reduce the air/tissue contrast and the resultant increased lung opacity may be misconstrued as being abnormal. Inspiratory images provide more tissue contrast, but the incidence of movement blur is increased. Overexposure results in the lung fields appearing excessively radiolucent, resulting in failure to identify subtle pulmonary changes. Conversely, underexposure emphasizes soft tissues and simulates a generalized pulmonary infiltration and pulmonary overcirculation (Koblik & Hornof 1985). Gross pathological abnormalities that are

restricted to one hemithorax will be evident on either right or left lateral radiographs. However, subtle changes affecting only one hemithorax may be missed unless both left and right lateral radiographs are obtained. Thus, in larger horses, both sides of the thorax should be radiographed. Lesions in the hemithorax that is closer to the cassette will be more sharply defined.

Foals may be radiographed standing or in lateral recumbency; high output machines and grids are usually unnecessary (Lamb & O'Callaghan 1989, Mair & Gibbs 1989). A ventrodorsal view may be obtained in very young foals (Jean et al 1999). However, the potential benefit of obtaining such a view must be weighed against the positional stress inflicted on the foal.

Indications for thoracic radiography

Indications for thoracic radiography in horses include abnormal findings on thoracic auscultation, chronic respiratory disease, cough, nasal discharge, exercise intolerance, tachypnea, dyspnea, and dysphagia. Radiography is the diagnostic method of choice for evaluating pulmonary parenchymal disorders, especially those involving the axial or deep lung, and mediastinal disorders (Lamb & O'Callaghan 1989). Radiography is, however, less valuable than ultrasonography for detection and assessment of pleural and peripheral lung disorders (Ainsworth & Hackett 2004). While thoracic radiographs are useful for monitoring the progression of pulmonary disease processes, the radiographic appearance does not correlate well with the severity of the disease process and the resolution of radiographic lesions often lags behind the progression of the clinical syndrome (Sweeney 1991). Thus radiological observations must be interpreted in the light of the clinical findings and ancillary laboratory test results.

Normal radiographic anatomy

The thoracic structures that are usually visible on radiographs are the trachea, lungs, main-stem bronchi, cardiac silhouette, aorta, pulmonary vasculature, caudal vena cava, mediastinum, dorsal margin of the diaphragm, ribs, vertebrae, and sternum.

Lungs

The lungs differ from those of other domestic species in that they lack distinct lung lobes and deep interlobar fissures. However, they are subdivided, somewhat arbitrarily, into left cranial, left caudal, right cranial, right caudal, and right intermediate or accessory lobes (Farrow 1981b). The right accessory lobe is separated from the rest of the right lung by the caudal vena cava and the phrenic nerve. The right accessory lobe overlies the caudal vena cava on a lateral radiograph, and its bronchovascular system may be visible in this region. The poorly defined interlobar divisions are not evident on radiographs of normal horses. The interstitium, which surrounds the airways and pulmonary vessels, forms the normal background opacity of the lung field. It appears as a network or honeycomb of ill-defined, non-linear radio-opaque strands (Fig. 10.17B). Aerated radiolucent lung is imaged cranial, dorsal, and caudal to the cardiac silhouette. The lung fields of newborn foals are more opaque than those of older foals and adults; this is exacerbated when foals are radiographed in lateral recumbency as a result of postural atelectasis.

Bronchi

On the lateral view, bronchi are seen as a series of diverging air-filled tubes with gradually narrowing lumina as they course from the tracheal bifurcation towards the lung periphery. The bronchial walls are clearly defined as thin, radio-opaque, linear shadows in the central and middle lung-fields. In cross-section, bronchi are identified as thin-walled radio-opaque rings that gradually reduce in diameter towards the periphery.

Pulmonary vasculature

The pulmonary vessels are visible as a series of linear, tapering soft tissue opacities running in tandem with the bronchi to the lung periphery. In cross-section they are identified as circular soft tissue opacities lying adjacent to the bronchi. When superimposed on the ribs, they appear particularly radio-opaque and may mimic intrapulmonary nodules (Fig. 10.17C). The branches of the pulmonary artery are seen dorsal to the caudal heart base as they emerge from the main pulmonary artery. Pulmonary veins are visible as they enter the left atrium at the caudodorsal aspect of the cardiac silhouette, dorsal to the caudal vena cava. As the pulmonary veins course towards the caudodorsal aspect of the cardiac silhouette they diverge ventrally, away from the pulmonary arteries and bronchi. Pulmonary arteries and veins cannot be distinguished from each other in the lung periphery.

Mediastinum

The air-filled trachea is usually the only structure visible in the soft tissue region of the cranial mediastinum. The heart base, aorta, and pulmonary vasculature are profiled in the middle mediastinal region. The aorta, esophagus and caudal vena cava are seen in the caudal mediastinum. The esophagus is not normally visible in the thorax unless it contains air, fluid or food (Fig. 10.17D).

Diaphragm

The diaphragmatic outline is identified as a gently curved structure in the caudal thorax. The caudal vena cava passes through it roughly at the mid-thoracic level. In adult horses the ventral half of the diaphragm is often obscured by the cardiac silhouette.

Interpretation of abnormal radiographic appearance

Assessment of thoracic radiographs requires a complete examination of all the radiographs, evaluating the positioning, exposure factors, and recognition of the normal and abnormal anatomical structures. In addition, the location (perihilar, mid or caudal; peripheral or central), distribution (discrete, widespread or localized), and type of radiographic pattern all provide valuable information (Sande & Tucker 1997).

Radiographic patterns

Pulmonary disease may cause a variety of radiological changes that are broadly divided into four different patterns, namely alveolar, bronchial, interstitial, and vascular (Butler et al 2000). While the use of these patterns in describing lung diseases is somewhat controversial, it gives clinicians a method of assessing and classifying the radiographic abnormalities. The location and distribution of the pattern(s) may help the clinician to formulate a differential diagnosis. However, radiographic patterns rarely occur individually, but commonly develop as mixed patterns which are dynamic and which change rapidly. Importantly, they are not pathognomonic for any specific condition and each pattern may result from a variety of clinical conditions.

Alveolar pattern

An alveolar pattern occurs as a result of flooding of the normally air-filled alveoli with fluids such as blood, transudate or exudate, or as a result of a lack of aeration caused by atelectasis or consolidation. This increases radio-opacity in the affected area, thereby reducing radiographic contrast and obscuring the margins of the pulmonary blood vessels. Air-filled bronchi, termed air bronchograms (Fig. 10.18A,B), are usually visible as a series of radio-lucent branching tubes surrounded by an ill-defined and poorly marginated, fluffy soft tissue opacity. This pattern is identified most easily in the ventral and caudoventral lung regions. Air bronchograms not only place the disease within the air spaces but also indicate the patency of the proximal bronchus. If the alveolar infiltrate is severe, large areas of lung may be affected.

Bronchial pattern

The primary, secondary, and often the tertiary bronchi are usually visible on radiographs of normal adult horses. The recognition of an abnormal bronchial pattern is often appreciated only when severe pathological changes are present. Thickened bronchial walls appear as prominent, paired, tapering radio-opaque lines, which extend further into the periphery than usual. In cross-section they appear as distinct radio-opaque rings with radiolucent centers (Fig. 10.18C,D). A bronchial pattern may be seen in horses with chronic airway inflammatory disorders such as recurrent airway obstruction. Bronchiectasis, remodeling and dilatation of the terminal bronchi in response to chronic bronchial diseases such as recurrent airway obstruction, may appear radiographically as cylindrical or saccular dilatations of the bronchial walls (Butler et al 2000). A bronchial pattern can result from inflammatory and allergic airway disease, and is often seen in combination with interstitial and alveolar infiltrates. The bronchial walls of older horses may be more radio-opaque because of mineralization, although the clinical significance of this is questionable.

Interstitial pattern

The normal equine lung has a large interstitial component and it is important not to misconstrue the normal radiographic appearance of the interstitium as evidence of interstitial disease. The interstitial pattern is used to describe the radiographic appearance of pathological changes affecting the interstitium. Radiographically, interstitial patterns can be subdivided into nodular and generalized (King 1981). A nodular interstitial pattern is associated with infectious bacterial or mycotic diseases causing abscesses and granulomas, silicosis (Berry et al 1991), and neoplasia. The generalized pattern may appear linear, as a pronounced honeycomb-like network, or ill-defined and hazy. The resultant generalized increased opacity of the lung background is interspersed with radiolucent lung tissue. The generalized pattern is caused by fibrosis or cellular or fluid infiltrate in the interstitial tissue, such as occurs with viral and bacterial pneumonia, tuberculosis, allergic lung disease, early pulmonary edema and pulmonary fibrosis. The margins of the pulmonary vessels and bronchial walls are less distinct (Fig. 10.18E,F). An interstitial pattern is rarely seen as a distinct entity.

Fig. 10.18. (A,B) Alveolar pattern. (A) This 1-week-old foal has a severe alveolar infiltrate in the ventral lung that obscures the cardiac silhouette and ventral diaphragmatic outline. (B) A close-up of (A). Air bronchograms (arrows) are evident. Diagnosis: aspiration pneumonia. (C,D) Bronchial pattern. (C) A 4-month-old foal showing a widespread interstitial infiltration. Peribronchial thickening is evident. (D) A close up of (C). The bronchial walls are seen in longitudinal (black arrows) and cross-section (white arrow). Diagnosis: pneumonia. (E,F) Interstitial pattern. (E) A 1-year-old colt with a widespread interstitial pattern. There is an overall increase in lung radio-opacity and the pulmonary vessels are indistinct. (F) A close up of (E) showing a honeycomb pattern in the lung region just dorsal to the caudal vena cava.
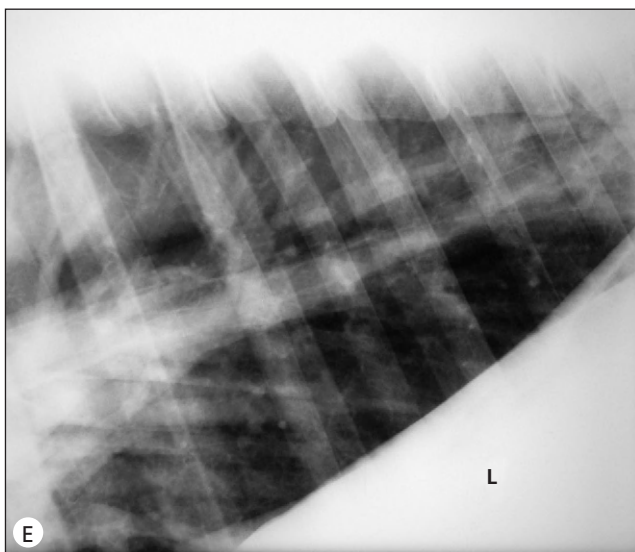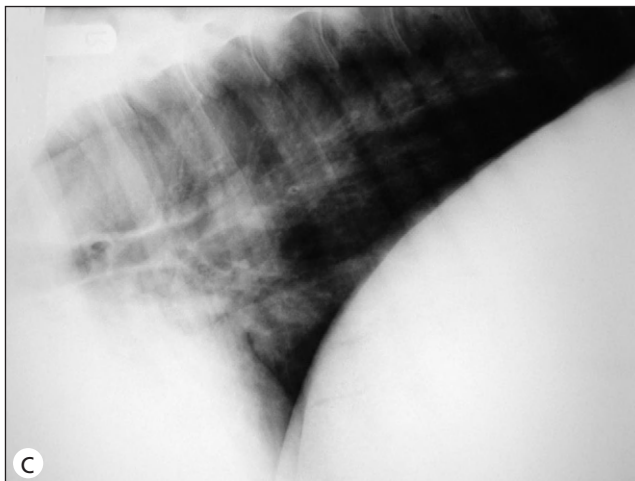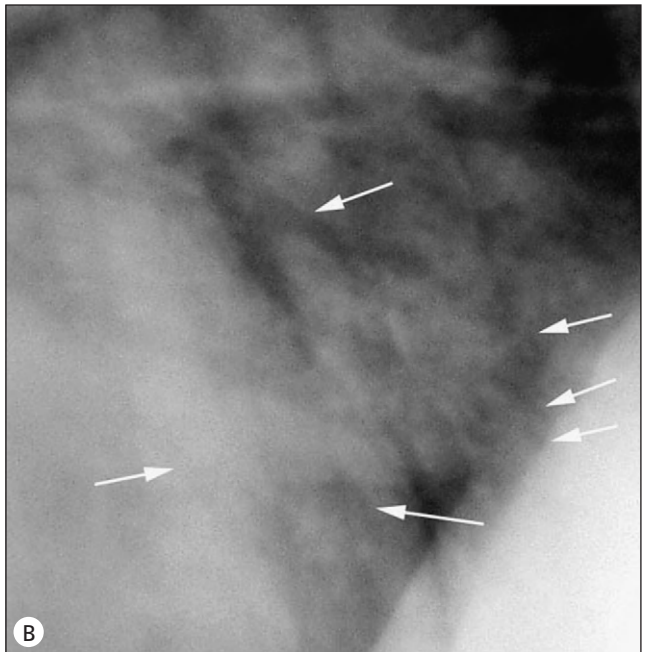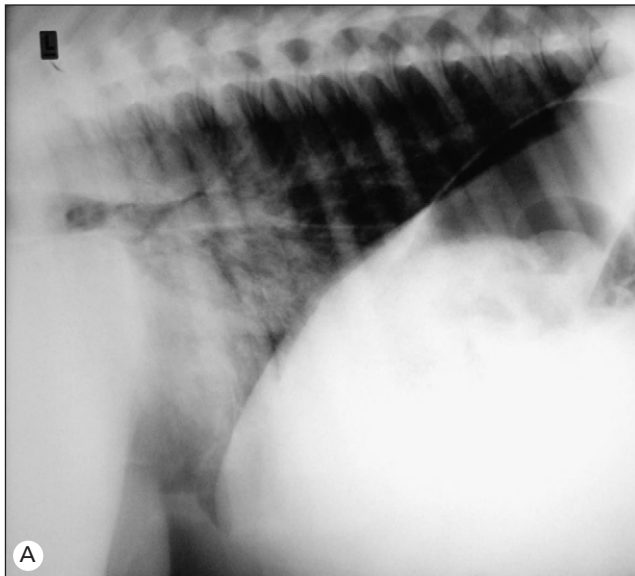
Vascular pattern

The vascular pattern is rarely seen in horses. Exposure factors must be correct because overexposure or underexposure will obliterate or accentuate the pulmonary vessels, respectively. Furthermore, assessment of the vascular structures is subjective. An increased vascular pattern may be seen in severe left-sided cardiac failure and in foals with left to right cardiovascular shunts. In contrast, hypovolemia and right to left sided cardiac shunts may cause an apparent decreased vascularity. Animals with severe emphysema or lung hyperinflation appear to have smaller blood vessels (Lamb & O'Callaghan 1989).

Pneumonia

The radiographic signs of pneumonia are diverse and depend on the nature, stage, and severity of the disease (Barr 2003). Pneumonia caused by infectious agents may result in an interstitial, bronchial or mixed pattern, with or without a concomitant alveolar pattern forming patchy lung infiltrates. Consolidation may lead to large coalescing soft tissue opacities. Other additional radiographic features that can be associated with pneumonia are pulmonary abscesses, pleural effusion, pneumothorax, and hilar lymphadenopathy. Pneumonia often affects the cranioventral or ventral regions of the lungs, which are difficult to image in adult horses because they are obscured by the cardiac silhouette.

- Viral pneumonias are rarely diagnosed radiographically but they cause radiographic signs of interstitial lung disease. More commonly, a secondary bacterial infection has occurred by the time the animal is radiographed (Sande & Tucker 1997) (Fig. 10.19A,B).
- Pneumonia in foals caused by *Rhodococcus equi* is usually located in the perihilar region and pulmonary abscessation is a common observation (Fig. 10.19C,D).
- Aspiration pneumonia usually affects the cranial and ventral lung lobes (Fig. 10.19E).
- Mycotic pneumonia, which is rare in horses, causes a nodular interstitial infiltrate that is best seen in the peripheral lung fields. Mycotic pneumonia must be differentiated from other granulomatous diseases and from metastatic neoplasia.
- Hematogenous pneumonia in adults often has a caudodorsal distribution (Rush & Mair 2004).

Lung abscesses

Lung abscesses generally occur secondary to bacterial pneumonia. They are evident as circular soft tissue opacities of varying sizes (Fig. 10.20). They may be cavitated with thick walls and contain a discernible horizontal fluid–gas interface, indicating gas-forming anaerobes, or a bronchial fistula (Mair & Lane 1989).

Acute respiratory distress syndrome

This complex syndrome is associated with severe alveolar and interstitial damage. Consequently, radiographic changes include an initial interstitial pattern progressing to a severe bronchointerstitial infiltration affecting the caudodorsal lung fields with a subsequent alveolar pattern (Lester & Lester 2001).

Recurrent airway obstruction

Radiography of horses with recurrent airway obstruction is rarely diagnostically rewarding, except for the purpose of eliminating other differential diagnoses. Acutely affected cases may be normal radiographically. In chronic cases there may be a generalized increase in the background opacity of the lung fields and a widespread interstitial infiltration with a variable bronchial component. Flattening of the diaphragm as a result of pulmonary hyperinflation may be noted. Comparative studies taken at maximum inspiration and expiration are sometimes useful in horses with suspected recurrent airway obstruction. Air trapping will result in little radiological difference between the inspiratory and expiratory radiographs (Farrow 2002).

Atelectasis

Atelectasis, which refers to absence of air in the alveoli, may be evident radiographically as a severe alveolar pattern, usually in the caudodorsal and/or caudoventral lung fields. Quite marked alveolar infiltration is seen in foals that have been recumbent for prolonged periods (Farrow 2002).

Exercise-induced pulmonary hemorrhage (EIPH)

The typical radiographic presentation of EIPH is a generalized interstitial pattern. A triangular-shaped soft tissue opacity with an alveolar pulmonary pattern is often seen in the caudodorsal lung, merging with the diaphragmatic shadow (O'Callaghan et al 1987a,b). This soft tissue infiltrate gradually resolves with time, leading to a mixed pulmonary pattern (Fig. 10.21). Pulmonary infarcts may appear similar radiographically to EIPH, but the lesion does not regress over time (Butler et al 2000).
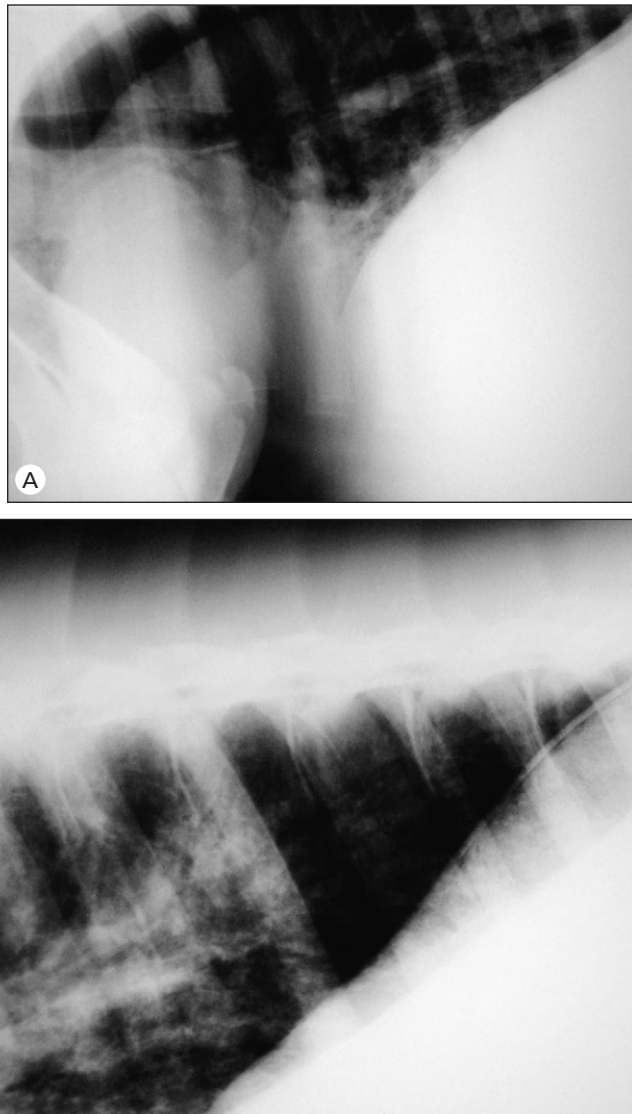
Fig. 10.19. (A,B) Pneumonia in an 8-month-old foal. There is a widespread interstitial infiltration with a focal alveolar pattern in the caudoventral (A) and caudodorsal (B) lung fields.

Continued

Pulmonary edema

Pulmonary edema results in a mixed pattern of interstitial and alveolar infiltrates with air bronchograms, usually affecting primarily the hilar and caudodorsal regions.

Pulmonary bullae

Pulmonary bullae are air-filled cavities within the lung tissue. Their thin walls, and homogeneous radiolucent contents, enable differentiation from pulmonary abscesses.

Pleural effusion

The pleural cavities are not normally identified on thoracic radiographs. A minimum of 500 ml pleural fluid must be present before it produces radiographic signs in horses (Koblik & Hornof 1985). On standing radiographs, pleural effusion causes an increased homogeneous opacity in the ventral thorax, with an uneven horizontal border. The cardiac silhouette is obscured and the fluid opacity merges with the diaphragmatic outline (Fig. 10.22). A sharply defined horizontal fluid line is only evident if there is concomitant pneumothorax. In other species, including
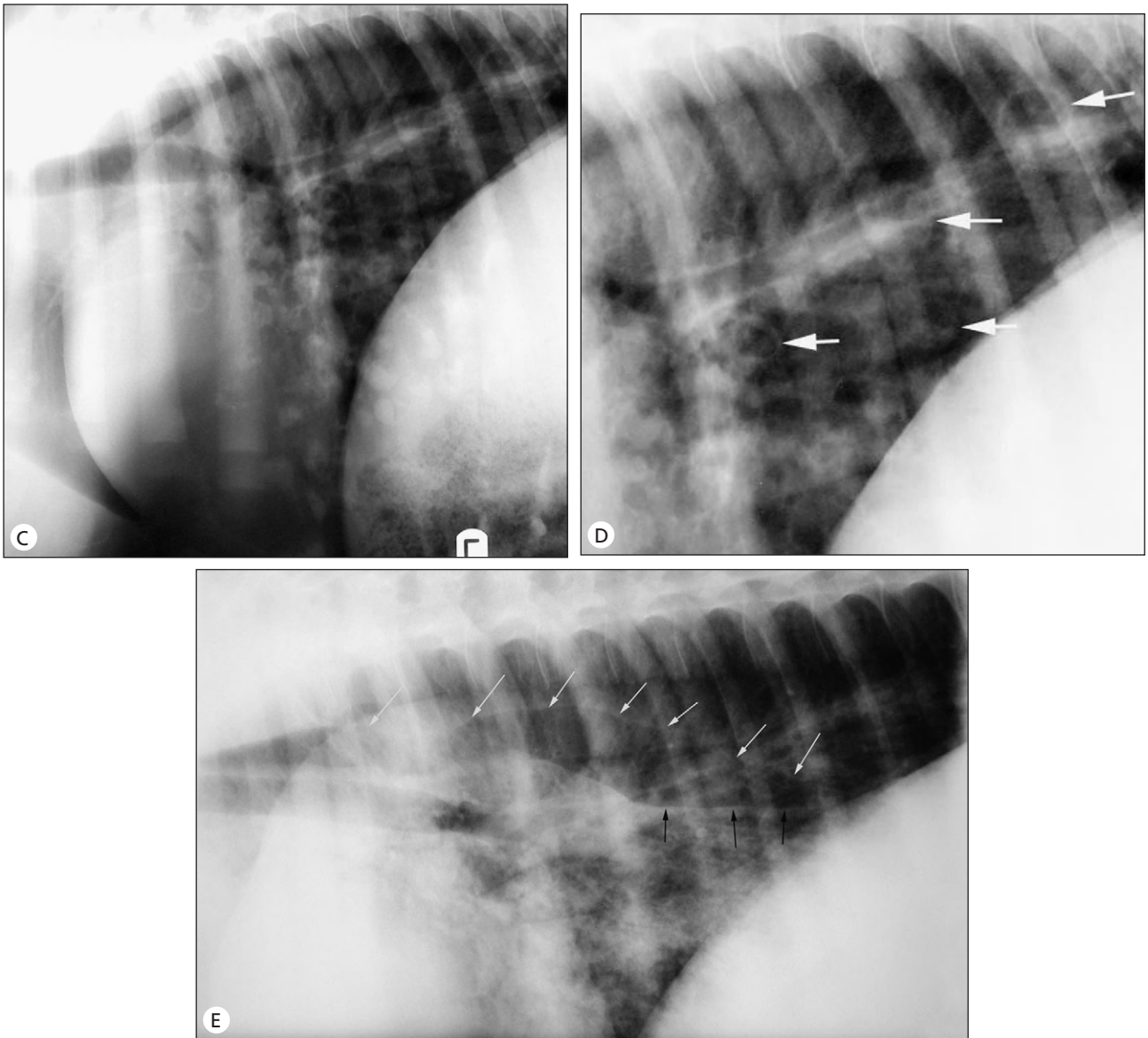
Fig. 10.19, cont'd. (C,D) A 6-month-old foal with *Rhodococcus equi* infection. Multiple cavitated abscesses (arrows) are seen throughout the lung fields. (E) Six-week-old foal with aspiration pneumonia and mega-esophagus. Air outlines the distended esophagus (white arrows) and a fluid–air interface (black arrows) indicating intra-esophageal fluid is also visible. There is a widespread mixed pulmonary infiltrate and the diaphragm is flattened because of hyperinflation.

humans and dogs, an obvious radiographic feature of pleural effusion is separation of the individual lung lobes by fluid. This feature is less obvious in horses with pleural effusion because the equine lung is separated only into left and right lungs and the accessory lobe of the right lung. However, one of the first signs of pleural effusion in horses is visualization of the ventral margin of the accessory lobe of the right lung when it is separated from the surrounding organs by the effusion. Pleural effusion causes compression atelectasis, leading to increased opacity of the lung fields

that are visible dorsal to the effusion. This may give the impression of an increased interstitial pattern, which may be misinterpreted as evidence of pulmonary pathology.

Some authors suggest that radiography is best performed following pleural drainage to visualize the ventral thorax and lung field more clearly (Rush & Mair 2004). However, the iatrogenic pneumothorax that may occur following this procedure may confuse radiographic interpretation. When thoracic radiographs are taken of foals in the recumbent lateral position pleural effusion may be
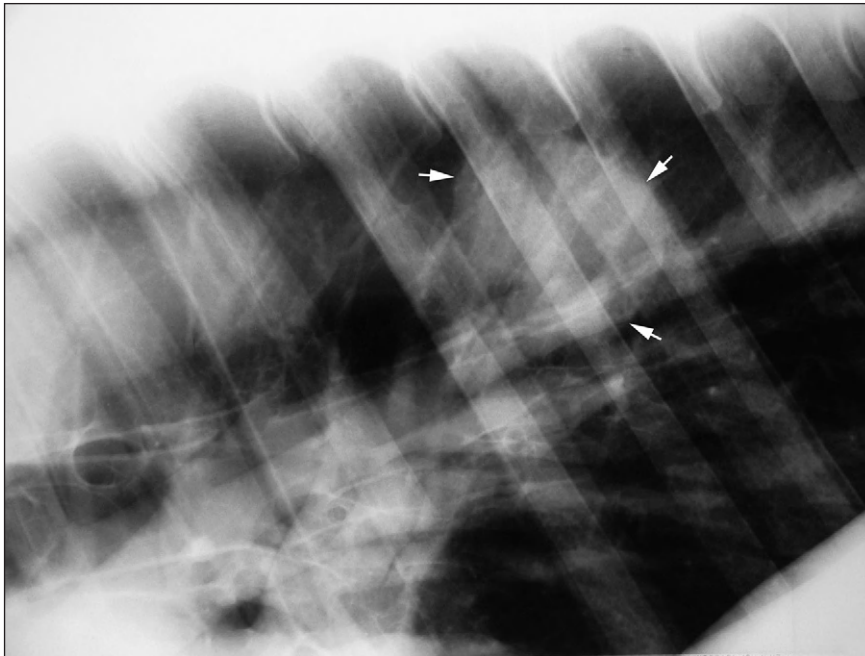
Fig. 10.20. Pyogranulomatous pulmonary abscess. A large well-defined circular soft tissue opacity (arrows) is visible in the dorsal lung field.
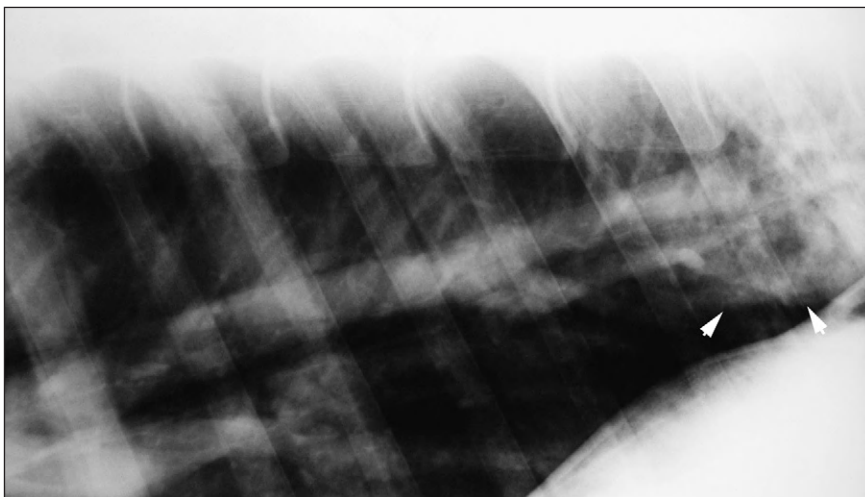
Fig. 10.21. Exercise-induced pulmonary hemorrhage. There is a widespread interstitial infiltrate. A focal mixed pattern, representing a resolving intrapulmonary hemorrhage, is seen in the caudodorsal region of the lung between the diaphragm and the vertebrae. The blood vessels in the affected area are obscured by the pulmonary infiltration. The ventral margin of the aorta is indicated by the arrowheads.

seen surrounding and outlining the lungs and separating them from the adjacent thoracic wall. Care should be taken to differentiate between cavitated extrapleural abscesses and pleural effusion (Fig. 10.23). Ultrasonography is more sensitive than radiography for confirming the presence of pleural fluid.

Pneumothorax

The dorsal edge of the partially collapsed lung is retracted ventrally from the vertebrae and is evident radiographically as a curved radio-opaque shadow, running horizontally across the thoracic cavity (Fig. 10.24). If the pneumo-

thorax is unilateral, pulmonary vessels in the normal lung may still be seen in the caudodorsal thorax, partially obscuring the collapsed lung edge. The outline of the aorta is accentuated in horses with pneumothorax because it is surrounded by air (Boy & Sweeney 2000).

Pneumomediastinum

Air in the mediastinum outlines structures that are not normally visible radiographically, including the mediastinal blood vessels and the esophagus. In contrast, free air makes the trachea more difficult to identify. Air in the esophagus may simulate a pneumomediastinum.

Fig. 10.22. Pleural effusion in a horse with lymphosarcoma. There is an increased soft tissue opacity obscuring the cardiac silhouette. The edge of the opacity has an irregular horizontal margin (arrow).
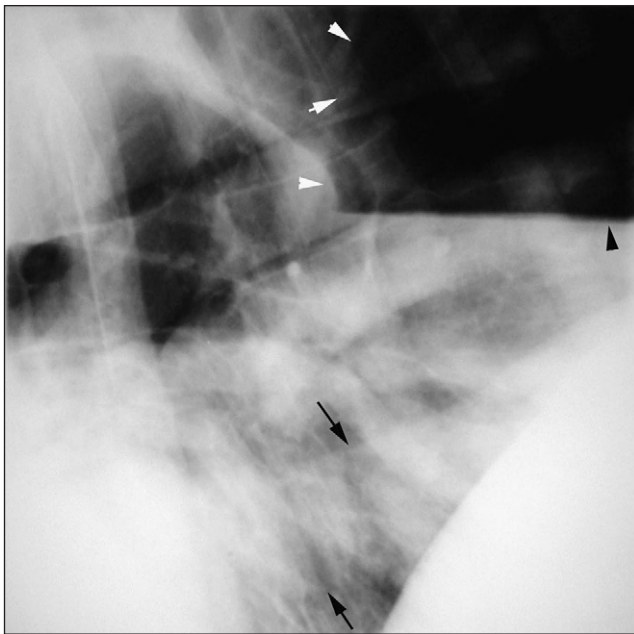
Fig. 10.23. A 6-year-old mare with a large, cavitating, gas-filled extrapleural abscess at the level of the mid-dorsal thorax (white arrowheads). A fluid line (black arrowhead) is visible in the ventral part of the abscess. The pulmonary vessels and caudal vena cava (black arrows) are seen superimposed on the fluid opacity indicating that the underlying lung is in a normal position.

Mediastinal masses

Mediastinal masses, including neoplasms, abscesses, lymphadenopathy and granulomas, may be seen as soft tissue opacities which displace the trachea or are seen in the perihilar region.

Thoracic neoplasia

Primary lung tumors are rare. They are seen as soft tissue nodules or masses within the pulmonary parenchyma (Fig. 10.25) that may be difficult to differentiate radiographically from intrapulmonary abscesses or cysts. Metastatic lung disease may be seen as diffuse infiltrates, multiple nodules or masses of varying sizes scattered throughout the pulmonary parenchyma with or without pleural fluid.

Diaphragmatic hernias

Diaphragmatic hernias may result in interruption of the normal curved diaphragmatic outline, and the gas cap of the stomach being located more cranially than usual. Rarely, intestines may be evident within the thoracic cavity as gas-filled tubular shadows.

Fractured ribs

Rib fractures are easily missed on survey radiographs unless the fracture ends are grossly displaced or callus formation is evident (Fig. 10.26). In foals, ventrodorsal views can aid detection of rib fractures and will reveal the
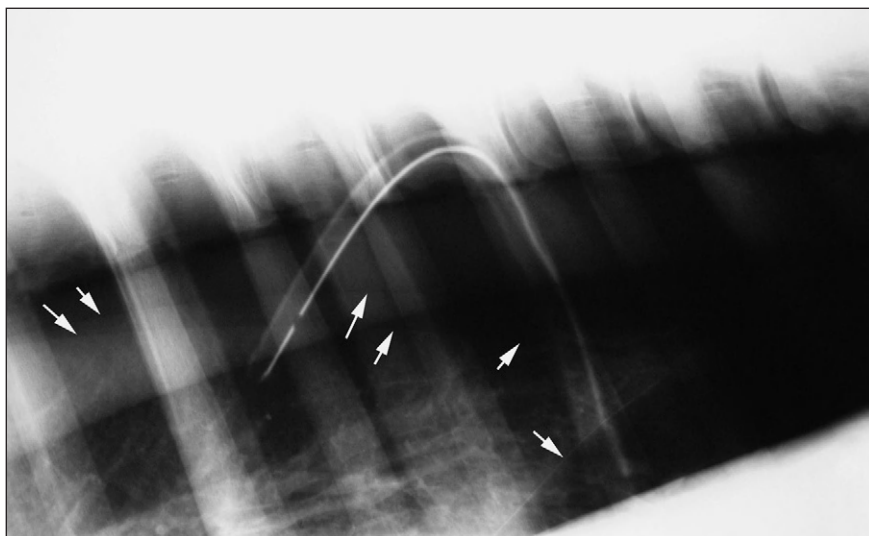
Fig. 10.24. Pneumothorax in a 1-year-old filly. The lateral radiograph shows an intrapleural drain placed in the right pleural cavity. Air is present in the dorsal thorax and the partially collapsed lung margins (arrows) are clearly seen.
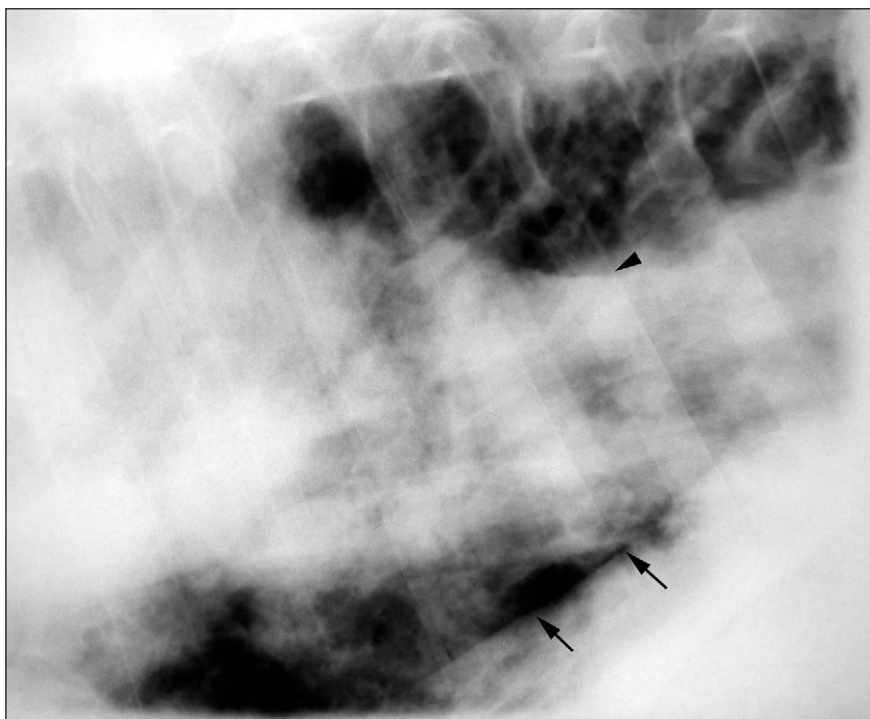
Fig. 10.25. This 12-year-old thoroughbred mare has an ill-defined widespread mixed pulmonary pattern with indistinct soft tissue opacities overlying the pulmonary vasculature. A fluid line is visible within the mass (arrowhead). Post-mortem examination identified a granular cell tumor. (Black arrows indicate the cranial edge of the diaphragm.)

associated asymmetry of the thoracic wall (Jean et al 1999). Ultrasonographic examination may be more useful in defining the presence of rib fractures than radiography.

Sternal fractures

Sternal fractures and the associated pleural or subpleural hemorrhage may be evident radiographically, particularly if they are displaced (Fig. 10.27).

Hypertrophic osteopathy

Chronic pulmonary disease may result in hypertrophic osteopathy, which leads to an irregular periosteal proliferative reaction affecting the diaphyses of the long bones, and occasionally the bones of the head, but not joints (Mair et al 1996). The distribution is often, but not invariably, bilaterally symmetrical (Fig. 10.28).
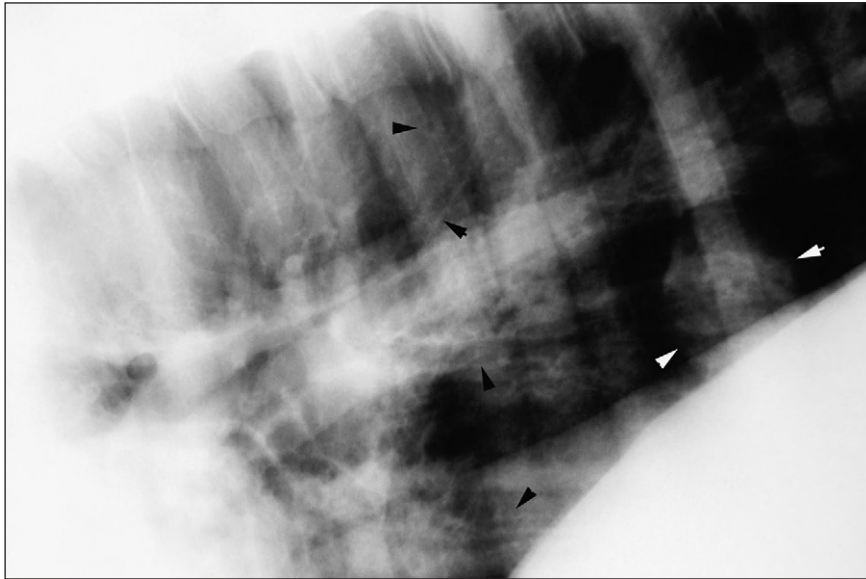
Fig. 10.26. Rib fracture and pneumonia. This 6-month-old foal had a history of *Rhodococcus equi* pneumonia. This lateral radiograph taken with the right side against the cassette (a right lateral view) shows a healed rib fracture with a large smooth callus (white arrowheads) dorsal to the caudal vena cava. The fracture involves a rib on the left side as the sharper, less-magnified right rib is superimposed on the fractured rib. The lung markings are superimposed on the callus. There is a widespread interstitial infiltrate and prominent bronchial lines (black arrowheads).

Fig. 10.27. A 3-year-old pony with a sternal fracture. The lateral radiograph shows a rectangular separated fragment of bone (black arrow) surrounded by a radiolucent cavity (white arrow). A discharging sinus tract is seen as a radiolucent channel exiting ventrally from the sequestered fragment (white arrowhead). Sclerotic bone encircles the lesion.
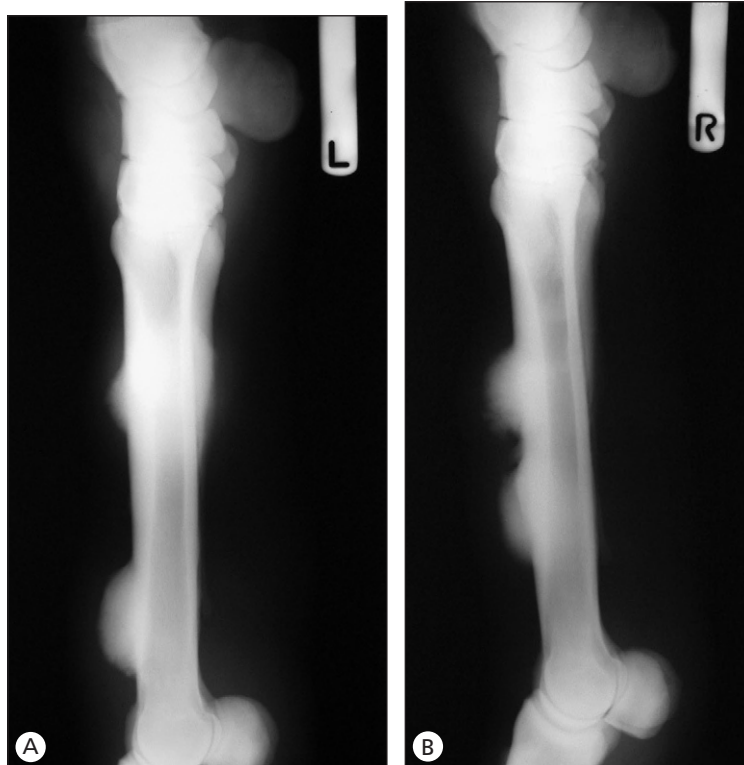
Fig. 10.28. A 6-year-old mare with hypertrophic osteopathy. There are asymmetric bony exostoses on the left (A) and right (B) third metacarpal bones.

REFERENCES

Ainsworth DM, Hackett RP 2004 Disorders of the respiratory system. In: Reed SM, Bayly WM, DC Sellon (editors) Equine Internal Medicine, 2nd edn. WB Saunders, Philadelphia, pp.289–353

Barakzai SZ, Tremaine WH, Dixon PM 2006 Use of scintigraphy for diagnosis of equine paranasal sinus disorders. Veterinary Surgery 35: 94–101

Barr BS 2003 Pneumonia in weanlings. Veterinary Clinics of North America; Equine Practice 19: 35–49

Behrens E, Schumacher J, Morris E 1991 Contrast paranasal sinusography for evaluation of disease of the paranasal sinuses of five horses. Veterinary Radiology and Ultrasound 32: 105–109

Berry CR, O'Brien TR, Madigan JE et al 1991 Thoracic radiographic features of silicosis in 19 horses. Journal of Veterinary Internal Medicine 5: 248–256

Blythe LL 1997 Otitis media and interna and temporohyoid osteoarthropathy. Veterinary Clinics of North America; Equine Practice 13: 21–42

Boulton CH 1985 Equine nasal cavity and paranasal sinus disease: a review of 85 cases. Journal of Equine Veterinary Science 5: 268–275

Boy MG, Sweeney CR 2000 Pneumothorax in horses: 40 cases (1980–1997). Journal of the American Veterinary Medical Association 216: 1955–1959

Butler JA, Colles CM, Dyson SJ et al 2000 Clinical Radiology of the Horse, 2nd edn. Blackwell Scientific, Oxford

Farrow CS 1981a Radiography of the equine thorax – anatomy and technique. Veterinary Radiology 22: 62–68

Farrow CS 1981b Equine thoracic radiology. Journal of the American Veterinary Medical Association 179: 776–781

Farrow CS 2002 The equine lung. In: Thrall DE (editor) Textbook of Veterinary Diagnostic Radiology, 4th edn. WB Saunders, Philadelphia pp.463–475

Fintl C, Dixon PM 2001 A review of five cases of parotid melanoma in the horse. Equine Veterinary Education 13: 17–24

Fubini SL, Todhunter RJ, Vivrette SL et al 1985 Tracheal rupture in two horses. Journal of the American Veterinary Medical Association 187: 69–70

Gibbs C, Lane JG 1987 Radiographic examination of the nasal and paranasal sinus regions of the horse. II: Radiological findings. Equine Veterinary Journal 19: 474–482

Head KW, Dixon PM 1999 Equine nasal and paranasal sinus tumours. Part 1: Review of the literature and tumour classification. Veterinary Journal 157: 261–278

Jean D, Laverty S, Halley J et al 1999 Thoracic trauma in newborn foals. Equine Veterinary Journal 31: 149–152

King GK 1981 Equine thoracic radiography. Part II. Air gap rare-earth radiography of the normal equine thorax. Compendium on Continuing Education for Practicing Veterinarians 3: S283–S288

King GK, Martens RJ, Clark DR 1981 Equine thoracic radiography. Part I. Air gap rare-earth radiography of the normal equine thorax. Compendium on Continuing Education for Practicing Veterinarians 3: S278–S282

Koblik PD, Hornof WJ 1985 Diagnostic radiology and nuclear cardiology: their use in assessment of equine cardiovascular disease. Veterinary Clinics of North America; Equine Practice 1: 289–309

Lamb CR, O'Callaghan MW 1989 Diagnostic imaging of equine pulmonary disease. Compendium on Continuing Education for Practicing Veterinarians 11: 1110–1119

Lane JG 2001 Fourth branchial arch defects in thoroughbred horses: a review of 60 cases. Proceedings of the 2001 World Equine Airways Society Conference, World Equine Airways Society, Edinburgh, Scotland, CD-ROM

Lane JG, Gibbs C, Meynink S et al 1987a Radiographic examination of the facial, nasal and paranasal sinus regions of the horse: I. Indications and procedures in 235 cases. Equine Veterinary Journal 19: 466–473

Lane JG, Longstaffe JA, Gibbs C 1987b Equine paranasal sinus cysts: a report of 15 cases. Equine Veterinary Journal 19: 534–544

Lester GD, Lester NV 2001 Abdominal and thoracic radiography in the neonate. Veterinary Clinics of North America; Equine Practice 17: 19–46

Linford RL, O'Brien TR, Wheat JD et al 1983 Radiographic assessment of epiglottic length and pharyngeal and laryngeal diameters in the Thoroughbred. American Journal of Veterinary Research 44: 1660–1666

Mair TS, Gibbs C 1989 Thoracic radiography in the foal. Equine Veterinary Education 1: 56–61

Mair TS, Lane JG 1989 Pneumonia, lung abscesses and pleuritis in adult horses: a review of 51 cases. Equine Veterinary Journal 21: 175–180

Mair TS, Dyson SJ, Fraser JA et al 1996 Hypertrophic osteopathy (Marie's disease) in Equidae: a review of twenty-four cases. Equine Veterinary Journal 28: 256–262

O'Callaghan MW, Pascoe JR, O'Brien TR et al 1987a Exercise-induced pulmonary haemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. VI. Radiological/pathological correlations. Equine Veterinary Journal 19: 419–422

O'Callaghan MW, Pascoe JR, Tyler WS et al 1987b Exercise-induced pulmonary haemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. VIII. Conclusions and implications. Equine Veterinary Journal 19: 419–422

Perkins J 2002 Quantitative and qualitative anatomy of the equine maxillary and conchal sinuses with particular reference to the nasomaxillary aperture. MSc Thesis, University of Edinburgh

Priester WA, Mackay FW 1980 The occurrence of tumors in domestic animals. National Cancer Institute Monograph 54: 59–99

Rush BR, Mair TS 2004 Equine Respiratory Disorders. Blackwell Science, UK

Sande RD, Tucker RL 1997 Radiology of the equine lungs and thorax. In: Rantanen NW, Hauser ML (editors) The Diagnosis and Treatment of Respiratory Disease. Proceedings of the 1997 Dubai International Equine Symposium. Rantanen Design, USA pp.139–157

Sweeney CR 1991 Exercise induced pulmonary hemorrhage. Veterinary Clinics of North America; Equine Practice 7: 93–104

Tremaine WH, Dixon PM 2001 A long term study of 277 cases of equine sinonasal disease. Part 1: Details of horses, historical, clinical and ancillary diagnostic findings. Equine Veterinary Journal 33: 274–282

Weller Y, Livesey L, Maierl J et al 2001 Comparison of radiography and scintigraphy in the diagnosis of dental disorders of the horse. Equine Veterinary Journal 33: 49–58

11 Ultrasonography of the Respiratory Tract

Diana S Rosenstein

What is Diagnostic Ultrasound?

Energy in the form of sound is used to create images of internal organs based on the reflection of sound waves at tissue interfaces. The ultrasound machine uses electricity to stimulate piezoelectric crystals in the probe to emit sound waves. These waves enter the patient and interact with the tissues. Some of these waves reflect off the tissues and are detected by the probe to create an electrical signal that is displayed on a monitor. The appearance of the final image is determined by many characteristics of the sound waves, the tissues, and the scanning technique.

Ultrasonography is useful in evaluating the equine thorax as a complementary modality to radiology. The absence of radiation exposure, the reasonable expense, the portability of an ultrasound machine, and the sensitivity of ultrasound imaging for peripheral lesions are qualities that make this modality an asset in the evaluation of the respiratory system. Pathological changes including pleural effusion, pleural adhesions and thickening, pulmonary masses, pulmonary consolidation, diaphragmatic hernia, and mediastinal diseases may be well visualized sonographically with greater sensitivity than with thoracic radiography. Lateralization of a pleural or pulmonary lesion may also be accomplished more readily using ultrasound than with radiographs. However, sonographic artifacts and natural attenuation of the sound beam restrict the diagnostic utility of this modality in the thorax to peripheral tissues. Scanning from various locations around the thorax maximizes the access to these structures. Transesophageal ultrasound takes advantage of the digestive tract for access to intrathoracic, particularly mediastinal, structures. This equipment, however, is not yet readily available beyond large institutions.

Basic Physics of Diagnostic Ultrasound and Interactions of Sound with Matter

Artifacts associated with sound transmission have a tremendous impact on the image, and therefore an understanding of sound propagation and interaction of sound with matter is essential. This form of energy moves as a wave by causing compression and expansion of the molecules that comprise the medium in which it is traveling. This wave of energy has a wavelength, a frequency, and a direction. Frequency is the unit for cycles of compression and expansion per second and is commonly measured in units of Hertz (Hz). Most diagnostic ultrasound imaging is performed in the 2.5–10 megaHertz (MHz) range.

If a sound wave is traveling through a uniform medium, the wave continues to be propagated at a constant velocity (frequency × wavelength) and direction. When the wave reaches an interface of different media, such as an organ parenchyma and capsule, or the edges of two adjacent organs, interactions occur that affect the sound wave's velocity and direction. There are two basic categories of interactions that occur at these interfaces, namely attenuation and refraction.

Attenuation comprises several interactions that decrease the amount of energy remaining in the sound wave as it continues further into the patient. Reflection, absorption, and scatter are all types of attenuation.

- **Reflection** is the redirection of the sound wave or creation of an echo (Fig. 11.1). If the probe detects this echo, it generates a bright spot, contributing to the final image. As in the game of pool, angle of incidence equals
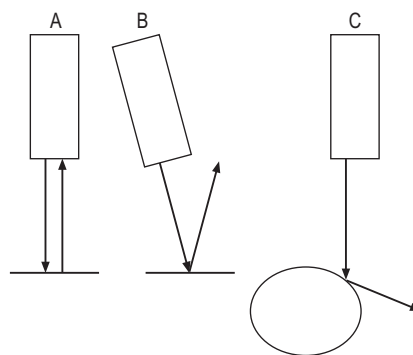
Fig. 11.1. Angle of incident sound beam determines the angle of reflected sound. Sound traveling perpendicular to the tissue surface is reflected back to the transducer (A). Sound traveling at an acute angle to the tissue surface is reflected away from the transducer (B). Sound reflected from the margin of a round structure is directed away from the transducer (C).

angle of reflection, meaning that the angle at which the original sound wave strikes the tissue interface is equal to the angle at which the echo is reflected. This concept is important in explaining why the angle at which a probe is held and the shape of a structure both have a significant effect on the sonographic appearance of a structure. A tissue interface is most likely to reflect echoes back to the probe when the sound wave strikes a flat surface at a perpendicular angle. Positioning of the probe at an acute angle to the tissue surface or imaging of a rounded structure will create reflected echoes that travel in a direction that will not be received by the probe and therefore no image of that interface will be generated on the monitor (Fig. 11.1). Acoustic impedance is a fundamental property of each structure that is determined by its physical density and velocity of sound propagation. The difference in acoustic impedance between two adjacent tissues determines the amount of reflection that occurs at this interface. The large difference in acoustic impedance between soft tissue and air causes reflection of almost the entire sound wave, while most of the energy is reflected at a soft tissue–bone interface. Two adjacent structures of similar acoustic impedance, such as muscle layers, are more likely to reflect less energy and refract the beam, allowing it to penetrate into deeper tissues.

- **Absorption** is the transfer of sound energy into heat that is retained within the tissue. This type of interaction is intentionally used for therapeutic ultrasound but is also a component of diagnostic ultrasound. It does not contribute to creation of the image but does contribute to the darkening of the far field. A higher frequency sound wave is more likely to be absorbed than a lower frequency sound wave; therefore use of a high-frequency transducer is limited to imaging of superficial structures because the beam cannot penetrate into deeper tissues.
- **Scatter** is the random distribution of echoes when they are reflected from a rough surface. The resulting image does not clearly delineate the margins of the original structure.
- **Refraction** is the continuation of a sound wave beyond an interface, into deeper structures at a new velocity and wavelength. This type of interaction is necessary to visualize deeper structures.

Some combination of all these interactions ultimately creates the final image with characteristic patterns for organs, pathological changes, and interfering artifacts.

Reverberation is a common artifact in thoracic sonography. Since most of the beam is reflected off the soft tissue–air interface, it returns to the probe with enough energy to generate another echo off the surface of the transducer, directed back into the body. This repeating cycle of returning echoes generates multiple lines on the image at equidistant intervals. This reverberation artifact is seen in the normal thorax as it occurs at the interface of the pleural surface and aerated lung (Fig. 11.2). The complete reflection of the sound beam at the soft tissue–air interface and the reverberation artifact obscure visualization of deeper structures; therefore, only peripheral pulmonary lesions can be visualized. Consequently, pulmonary lesions that are separated from the visceral pleura by aerated lung will not be visualized.

At a soft tissue–bone interface, most of the energy is reflected and the remaining energy is absorbed, so the image appears dark from the surface of the bone towards the far field. This dark region is called an acoustic shadow, and it is commonly seen on thoracic images deep to the ribs. The artifact is obvious in a transverse image with the transducer held across two ribs and the intercostal space (Fig. 11.2). Although all regions of the thorax
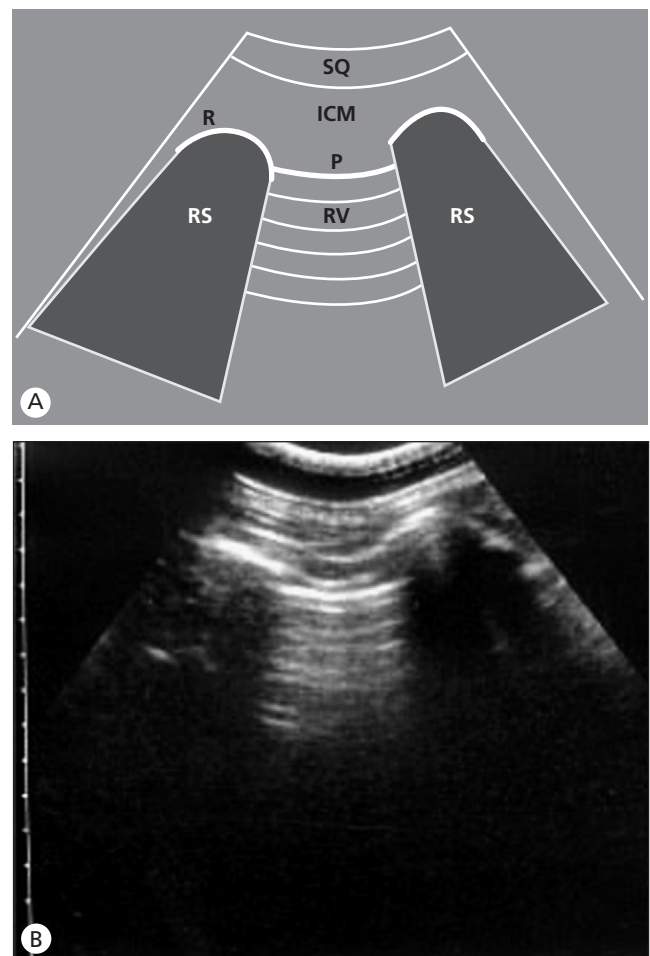
Fig. 11.2. Transverse plane sonographic image of a normal equine thorax – drawing (A) and sonogram (B). SQ = subcutaneous tissue, ICM = intercostal muscles, R = rib, RS = rib shadow, P = pleura, RV = reverberation artifact.

should be imaged in multiple planes, the shadow artifact from the ribs may be avoided by scanning in the longitudinal plane, with the transducer aligned along an intercostal space (Fig. 11.3).

Sound waves propagate efficiently through a liquid medium because the molecules within the liquid are readily compressed and expanded. The sound wave continues to move forward, so no echoes are generated (anechoic areas) and the representative region of the image is black. Tissues with a more rigid architecture, such as connective tissue, are more resistant to compression and rarefaction of the molecules; therefore acoustic impedance of these tissues is higher. The larger the difference in acoustic impedance of adjacent tissues, the more reflective the interface is to sound (echogenic). Descriptive prefixes are used to compare the relative echogenicity of structures, including hyper- (more echogenic), hypo- (less echogenic) and iso- (equally echogenic).
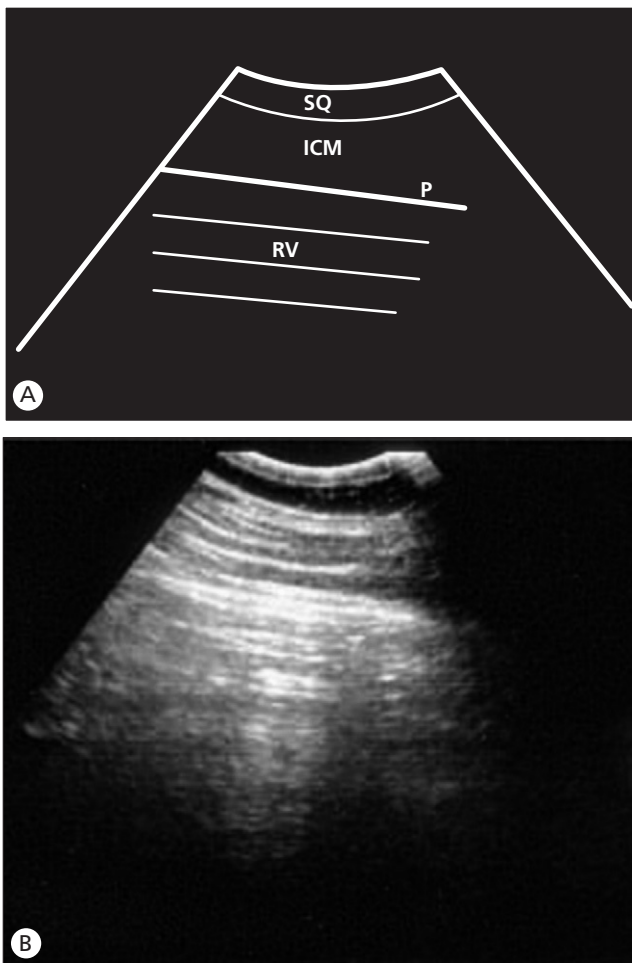
Technique

Since gas–soft tissue interfaces are highly reflective, the air trapped in the horse's haircoat must be removed to transmit the sound waves from the transducer into the patient. The standard protocol for patient preparation includes clipping the hair from the entire area to be scanned and cleansing the skin with a moist towel. External landmarks for the thoracic cavity are lines connecting points from the 17th intercostal space (ICS) at the height of the tuber coxae, extending cranioventrally to the point of the elbow, continuing dorsally to the caudoventral margin of the scapula then extending caudally to the point of origin in the 17th ICS (Fig. 11.4). If the haircoat is thin, it may not be necessary to clip the hair; however, the probe must be moved in the direction of hair growth to avoid disturbance of the moistened hair and the development of air pockets. Acoustic coupling gel is applied to the skin and massaged to further displace air from the skin surface. Dry or soiled skin will cause poor contact artifacts. A hyperechoic region in the extreme near field of the image with distant reverberation or an overall dark image may be the result of trapped air and poor contact between the probe and the skin (Fig. 11.5). Further cleansing, reapplication of more gel or firmer pressure of the probe against the horse's body will help to resolve this artifact.

A variety of transducers are appropriate for scanning the thorax. Superficial structures (within 10 cm of the skin) may be examined with a higher frequency probe (7.5–10 MHz) for good image resolution. To examine deeper structures (>15 cm), a lower frequency probe (2.5–3.5 MHz) should be used to improve sound-beam

Fig. 11.3. Longitudinal plane image of a normal equine thorax – drawing (A) and sonogram (B). SQ = subcutaneous tissue, ICM = intercostal muscles, P = pleura, RV = reverberation artifact.
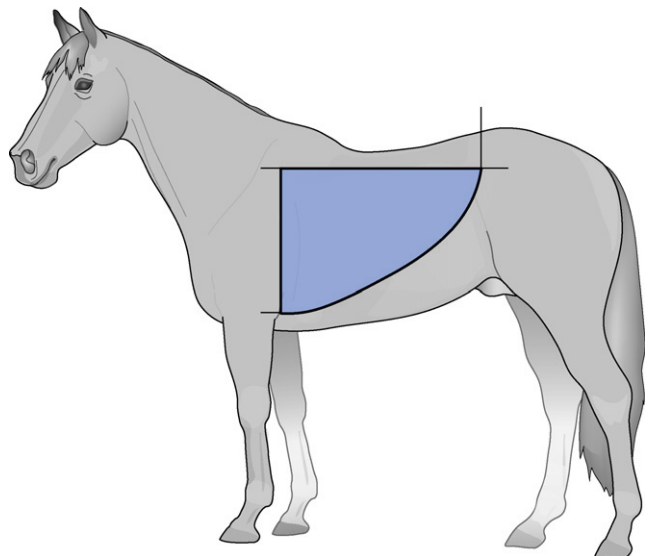
Fig. 11.4. External landmarks for preparation of the equine thorax for a sonographic examination.
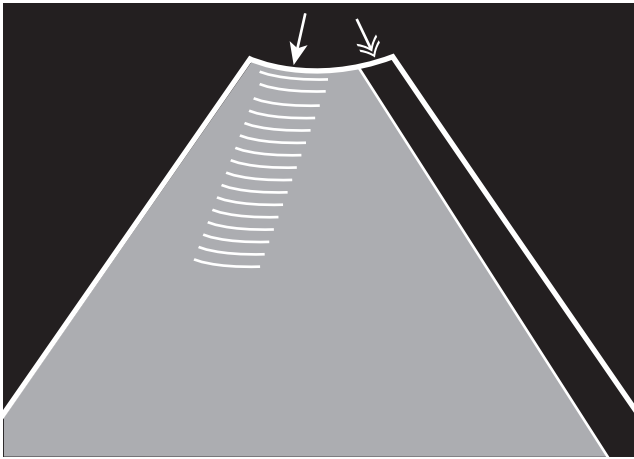
Fig. 11.5. Diagram illustrating two types of poor contact artifacts. Gas, hair or debris causes reverberation that originates at the body surface–probe interface (single arrow). Weak contact between the probe and body wall or dry skin cause a dark image (double arrow).

penetration, albeit at the sacrifice of image resolution. An excellent compromise between these two frequencies is a 5-MHz probe, which will provide good quality resolution and adequate penetration for most peripheral pleural and pulmonary lesions.

A linear transducer with a flat surface will provide good contact with the body wall. If the cord extends from one end of the transducer, such as the shape of a transrectal probe, it will be awkward to hold against the thoracic body wall. If the cord extends from the back of the transducer, it will be more convenient to manipulate. The image is rectangular and is usually limited in size to a narrow field of view. A curvilinear probe produces a wedge-shaped image with a wider field of view; however, the curved surface provides less contact with the flat or convex surface of the thoracic body wall and therefore the periphery of the image may be dark and non-diagnostic. Application of additional acoustic gel or more manual pressure of the probe against the body may alleviate this artifact. A round transducer is convenient for imaging in several planes in small spaces. This type of probe is ideal for cardiac sonography as it may be easily rotated to acquire longitudinal and transverse cardiac images between ribs.

A systematic plan for imaging the entire thorax is essential to perform a complete examination. Imaging along the ICS from dorsal to ventral, progressing from caudal to cranial across the thorax, is a recommended protocol. At each ICS the structures must be scanned in a transverse plane (transducer crosses adjacent ribs with the ICS in the center of the field) and in the longitudinal plane (transducer is aligned along the ICS, between two ribs). Movement of the transducer must be slow enough to allow for examination of the thorax in each location through inspiratory and expiratory phases of respiration.

The right and left sides of the thorax should be completely scanned at each examination. Abnormalities should be labeled and recorded as paper printed images, stored still or video clips on CD or tape, and written comments in the medical record.

Normal Anatomy

Normally inflated lungs contain air and are therefore highly echogenic. The reverberation artifact cast by the echogenic interface of pleura and aerated lung obscures the visualization of deeper structures. The adjacent pleural surfaces glide smoothly against each other as the lungs change in size with inhalation and exhalation. These pleural layers appear as one thin echogenic line, as the two surfaces are in contact. Ribs are echogenic at the surface, smoothly marginated, convexly curved and cast a dark distant shadow (Fig. 11.2). Structures deep to the ribs cannot be visualized through these skeletal structures. To examine the underlying lung, the probe must be angled cranially or caudally from an adjacent ICS. In the longitudinal plane, the probe is positioned lengthwise between ribs so that this artifact is eliminated (Fig. 11.3). In the caudal thorax, the diaphragm may be visualized as a thin, smooth echogenic line of demarcation between the aerated lungs and the cranial border of the liver.

Pleural Effusion

A small volume of pleural fluid may be imaged in healthy horses. Separation of the parietal and visceral pleura by fluid is seen as a band of hypoechoic material deep to the intercostal muscles and superficial to the lung (Fig. 11.6). The pleural layers may be visualized as echogenic lines bordering this fluid but they are not always discernible. The echogenicity of the fluid varies with its contents. A fluid of lower cellularity or protein content is lower in echogenicity while a highly cellular or proteinaceous fluid is more echogenic. Most equine thoracic sonographic studies are performed with the patient in the standing position. Gravity will cause the fluid to accumulate ventrally; therefore the ventral thorax is an important region to examine for pleural effusion. In the recumbent horse (usually neonates) it is crucial to examine the lower region of the thorax for effusion. A complete examination must include scanning of both sides of the chest at every evaluation, because the mediastinum may be a barrier to the spread of hemithoracic disease to the contralateral side, and because the nature and volume of pleural effusions may differ greatly between two sides of the same patient. Measuring the dorsal extent of the fluid at each examination will provide useful information regarding disease progression. If thoracocentesis is performed, then the thorax should be scanned following the removal of pleural fluid to document residual fluid levels. Fibrinous or fibrous
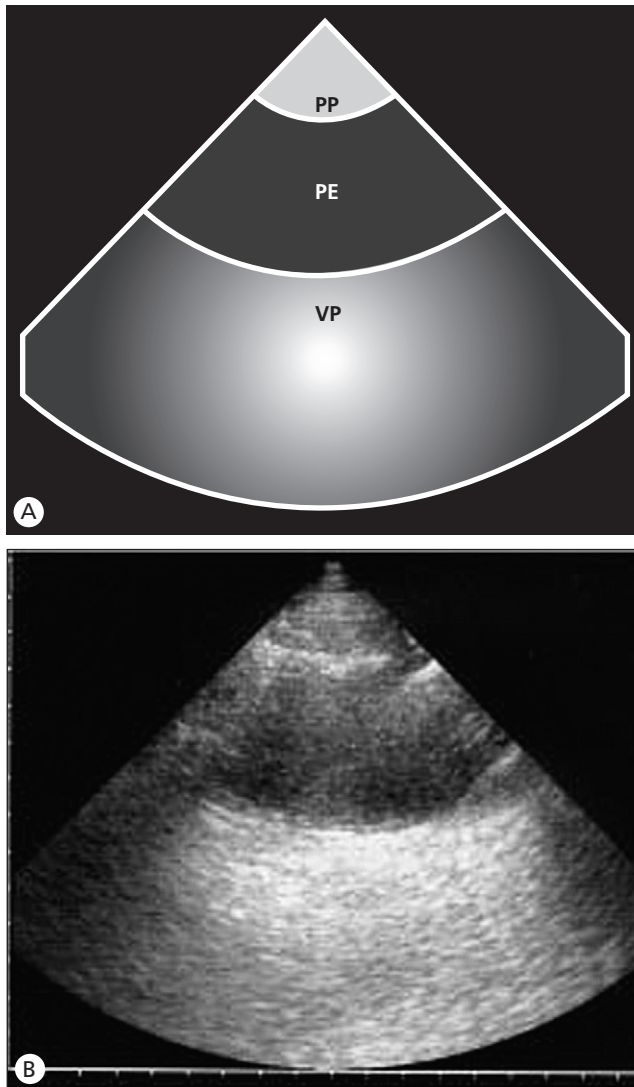
Fig. 11.6. Sonographic image of pleural effusion in the equine thorax – drawing (A) and sonogram (B). Pleural effusion (PE) separates the parietal pleura (PP) and the visceral pleura (VP).

Fig. 11.7. Sonographic image of pleuritis in the equine thorax – drawing (A) and sonogram (B). Comet tail artifacts (C) originate from the irregular pleural surface (P). Reproduced with the permission of Dr Matthew Davis, Merritt & Associates Equine Hospital, Wauconda, IL, USA.

bands of tissue may cause loculation of pleural fluid; therefore ultrasound guidance may be beneficial for placement of the needle to maximize access to the fluid.

Pleuritis

The shape of a tissue interface affects the appearance of the reflected echoes. A roughened texture to the pleural surface causes narrow streaks of reverberation artifacts, commonly named comet tails (Fig. 11.7). Comet tails may be identified in some healthy equine lungs, especially in the ventral margins of the lung field at the end of expiration. Thus their significance should be assessed in relation to other clinical data. However, numerous widespread comet tails should arouse a suspicion of lung or pleural disease. During respiratory movement, these artifacts appear to move across the image as the irregular pleural surfaces change in position. The underlying lung may be well aerated, resulting in the usual reverberation; however, the pleural surface is not a long, continuously smooth edge, so the echogenic lines are narrower. In cases of chronic pleuritis, adhesions between the visceral and parietal pleura may restrict the gliding motion during inhalation and exhalation. The lung appears stuck to the parietal pleura and fails to move during respiration (Fig. 11.8). This lesion is, however, not always recognizable on a still image and requires patient, slow scanning to identify in real-time scanning.
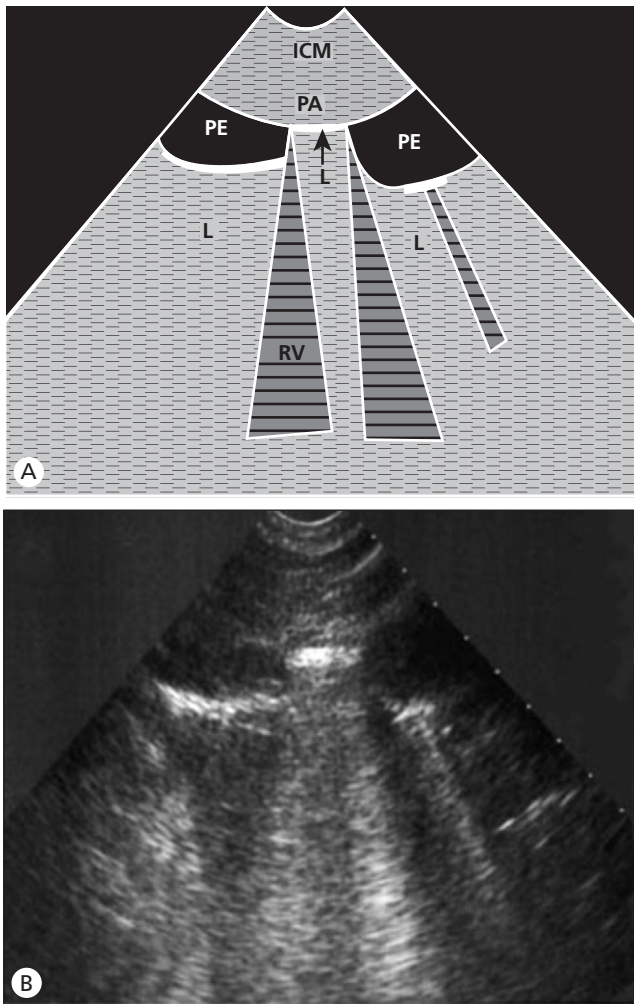
Fig. 11.8. Sonographic image of pleural effusion (PE) and pleural adhesion (PA) in the equine thorax – drawing (A) and sonogram (B). Adhesion of the visceral and pleural surfaces (arrow) restricts movement of the lung (L). ICM = intercostal muscles, RV = reverberation artifact. Reproduced with the permission of Bruce McGorum.

Fig. 11.9. Sonographic image of fibrinous pleuritis in the equine thorax – drawing (A) and sonogram (B). PP = parietal pleura, FT = fibrinous tags, PE = pleural effusion, VP = visceral pleura, HL = hepatized lung.

Effusion with Fibrinous Tags

Fibrinous and fibrous bands of tissue may extend from a pleural surface into a region of pleural effusion. These bands may appear as floating or waving linear echogenicities that sway in the pleural fluid during breathing (Fig. 11.9). The mediastinum (pericardial diaphragmatic ligament) should not be mistaken for a fibrinous band as it is recognized in the caudal right thorax, extending from the caudal margin of the lung to the diaphragm in the far field (Fig. 11.10). The mediastinum is evident as a long convoluted single echogenic fold that undulates within the pleural fluid, while fibrin generally occurs as multiple shorter strands. This section of the mediastinum is not visualized in the normal thorax

because of the presence of aerated lung between the body wall and the central thoracic structures. However, in the presence of pleural effusion and hypoinflation of the lungs, the mediastinal structures may be visualized.

Atelectasis and Consolidation

Atelectasis is incomplete aeration of the lung, which may be a primary or secondary disorder. Compression atelectasis occurs when increased external pressure inhibits lung expansion. Pulmonary atelectasis is a common finding in the presence of pleural effusion because the lung is unable to fully expand. Bronchial or bronchiolar obstruction may cause regions of alveolar atelectasis. The normal reverberation artifact is absent because the soft
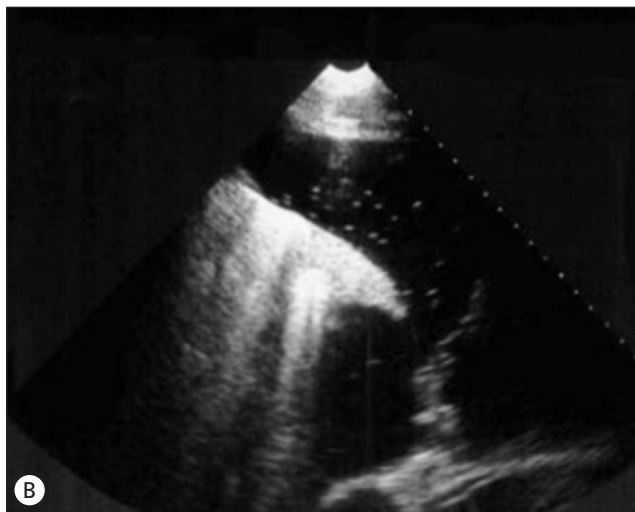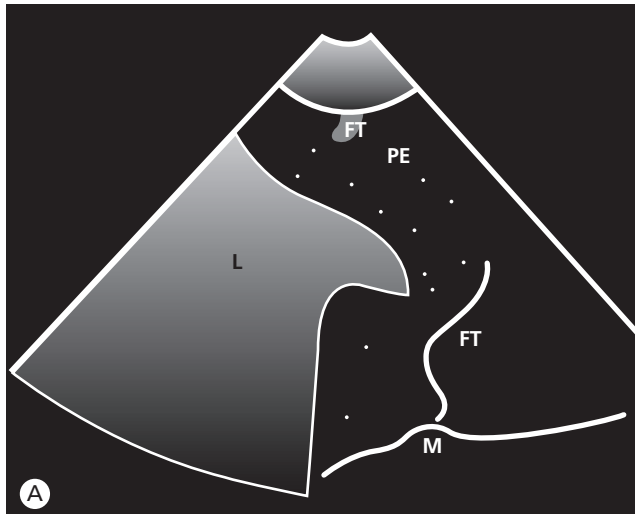
Fig. 11.10. Sonographic image of pleural effusion (PE) with fibrinous tags (FT) and partial lung atelectasis (L) in the equine thorax – drawing (A) and sonogram (B). Pleural effusion allows for visualization of the mediastinum (M).
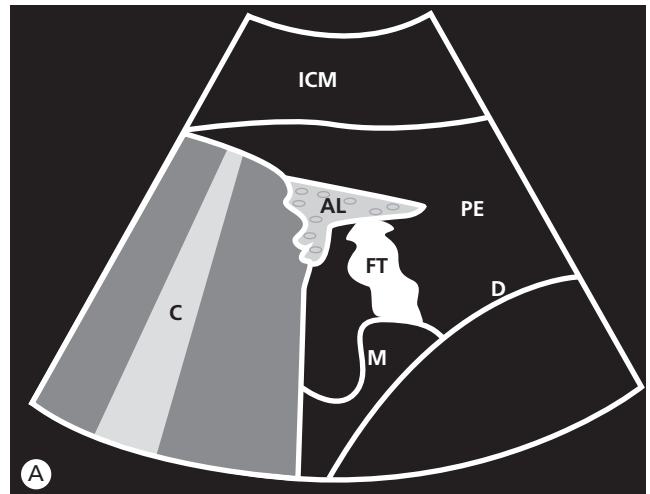
Fig. 11.11. Sonographic image of pulmonary atelectasis and fibrinous pleuritis in the equine thorax – drawing (A) and sonogram (B). ICM = intercostal muscle, PE = pleural effusion, AL = atelectatic lung, FT = fibrinous tag, C = comet tail artifact, M = mediastinum, D = diaphragm. Reproduced with the permission of Dr Matthew Davis, Merritt & Associates Equine Hospital, Wauconda, IL, USA.

tissue–gas interface is not present. Hypoinflated lung tissue appears similar to liver tissue and may be termed "hepatized lung". The lung parenchyma is moderately echogenic with tubular, hypoechoic, vascular structures in a branching pattern. Air may be present in the larger bronchi and bronchioles, depending on the severity of the atelectasis. These air-filled bronchi will appear highly echogenic and circular or linear in shape. Pleural effusion will enhance visualization of the visceral pleura, which should be smooth along the flat surface of the atelectatic lung and sharply marginated at the lobar edges (Figs 11.10 and 11.11).

Consolidation is the process of becoming firm. In the lung, this may occur when air in the alveoli is replaced with liquid or solid material. The displacement of gas will eliminate the normal reverberation artifact from the affected lung. The sonographic pattern of consolidated lung varies with the underlying pathology. If a pulmonary mass is present adjacent to the visceral pleura, a nodule with defined margins may be visualized (Fig. 11.12). If the disease is more diffuse, such as an exudative pneumonia, then the lung may appear similar to the hepatized lung described with atelectasis. Consolidated lung may have a rounded or irregular margin, in contrast to the smooth margin with sharp corners of atelectatic lung. The presence of pleural effusion may enhance visualization of the visceral pleura for differentiation between atelectatic and consolidated lung. A sonographic pattern of
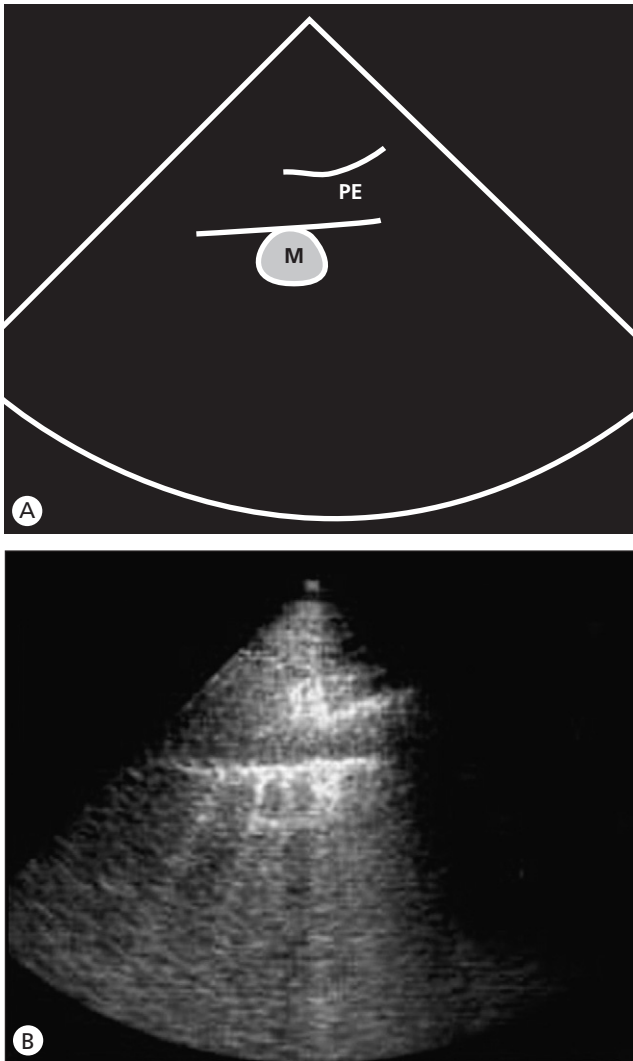
Fig. 11.12. Sonographic image of focal pulmonary consolidation (M) and pleural effusion (PE) in the equine thorax – drawing (A) and sonogram (B).

Fig. 11.13. Sonographic image of diffuse pulmonary consolidation in the equine thorax – drawing (A) and sonogram (B). Note the indistinct demarcation between the consolidated lung (CL) and the adjacent aerated lung (arrow).

non-inflated lung that is heterogeneous in echogenicity and disorganized in architecture is suggestive of pulmonary consolidation (Figs 11.13 and 11.14).

Pulmonary Mass

Ultrasonography is more sensitive than radiography at detecting small pulmonary masses, but only if they contact the visceral pleura (Fig. 11.12). The presence of aerated lung between the pleural margin and a deeper mass will create the normal reverberation artifact, obscuring visualization of a deeper lesion. A distinct, echogenic peripheral margin to a mass with hypoechoic or echogenic central material is suggestive of an abscess (Fig. 11.15). A mass of soft tissue echogenicity with internal vascular structures may be a neoplasm, other focal consolidation or

focal atelectasis. Sonographic guidance for transthoracic fine-needle aspiration or biopsy of a peripheral pulmonary mass may provide samples for cytology, histopathology, and bacteriology.

Pneumothorax

Free gas in the pleural space is difficult to identify via ultrasound examination. The reverberation artifact of normally aerated lung appears similar to the artifact from free pleural gas (Fig. 11.16). Critical assessment of the gliding pleural surfaces may be helpful to identify pneumothorax. In pneumothorax, the normal breath-induced gliding movement of the visceral pleura over the parietal pleura is absent, and consequently the gas-filled pleural space remains stagnant throughout
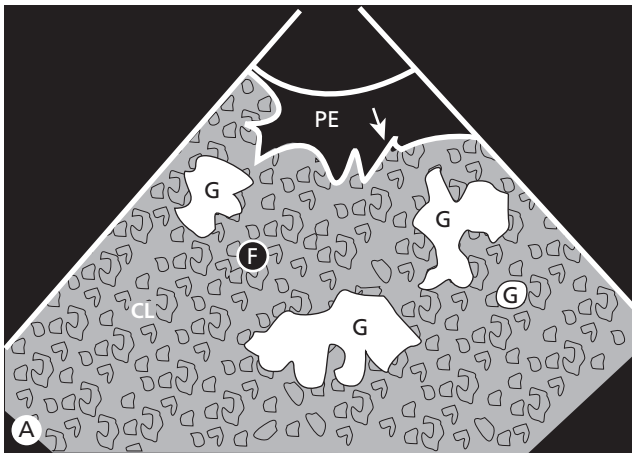
Fig. 11.14. Sonographic image of diffuse pulmonary consolidation in the equine thorax – drawing (A) and sonogram (B). Pleural effusion (PE) enhances visualization of the irregular lung margin (arrow). Foci of gas (G) and fluid (F) are present in the consolidated lung (CL).
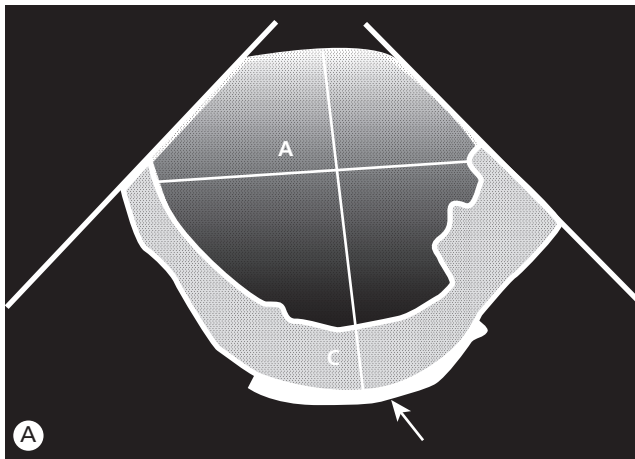
Fig. 11.15. Sonographic image of a pulmonary abscess in an equine lung. The hypoechoic center of the abscess (A) is surrounded by a thick echogenic capsule (C). The interface of this mass with aerated lung (arrow) is present in the far field of the image.
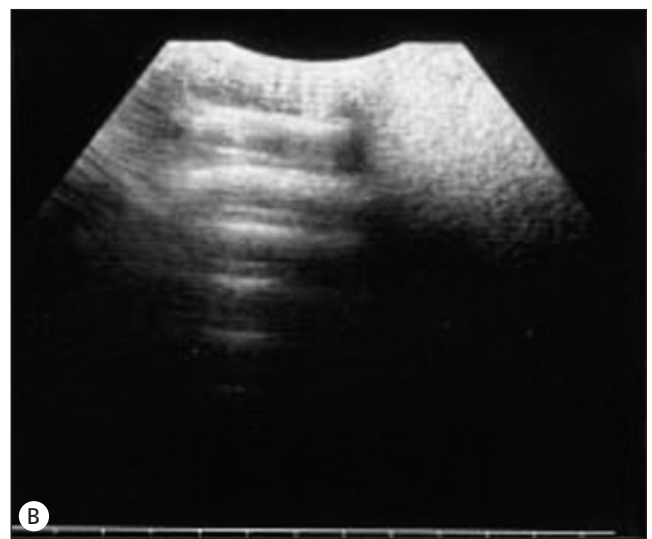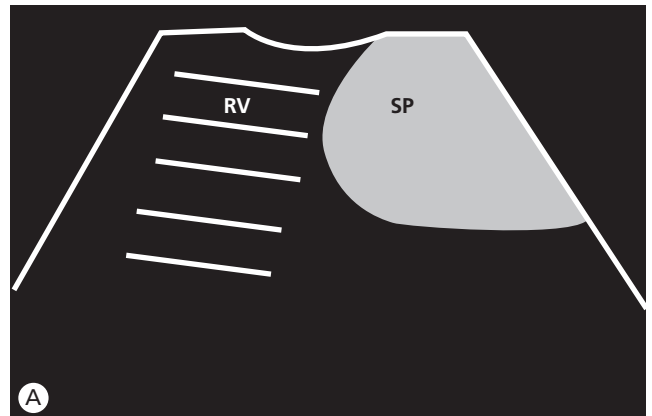
Fig. 11.16. Sonographic image of pneumothorax in an equine thorax – drawing (A) and sonogram (B). Reverberation artifact (RV) originates from the parietal pleural interface with the free pleural gas. SP = spleen.

the respiratory cycle. Dynamic imaging is essential to appreciate this lesion, as the still image appears similar to that of normal lung. In suspected cases of pneumothorax, it is likely to be more efficient to radiograph the horse first, identify the presence of a pneumothorax, then ultrasound the horse to determine lateralization of any pathological findings. The radiographs will provide a base on which to guide the ultrasound examination.

FURTHER READING

Curry TS, Dowdy JE, Murry RC 1990 Christensen's Physics of Diagnostic Radiology, 4th edn. Lea & Febiger, Philadelphia, pp.323–340
Nyland TG, Mattoon JS 1995 Veterinary Diagnostic Ultrasound. WB Saunders, Philadelphia
Reef VB 1998 Equine Diagnostic Ultrasound. WB Saunders, Philadelphia

12 Scintigraphy of the Equine Upper Respiratory Tract

Safia Barakzai

Introduction

With the increasing availability of radioisotopes, scintigraphy (nuclear medicine) began to emerge as a diagnostic and occasionally therapeutic discipline in human medicine after the Second World War (Weaver 1995). It is unique among the imaging modalities because the images reflect active physiological processes rather than the structural features portrayed by radiography, ultrasonography, computed tomography or magnetic resonance imaging. Scintigraphy involves the intravenous administration of a gamma-ray-emitting radioisotope, which is bound to a tissue-seeking molecule. Technetium-99m (^{99m}Tc), currently the most commonly used radioisotope in the equine field, is a meta-stable radionuclide which emits a gamma-ray of 140 keV, with a physical half-life of 6 h (Lamb & Koblik 1988). If bone is to be imaged, ^{99m}Tc is linked to a phosphate or diphosphonate-containing molecule (e.g. methylene diphosphonate, MDP), which has an affinity for the hydroxyapatite component of bone (Lamb 1991, Weaver 1995). The radioisotope is cleared at a fast rate from the blood and soft tissues, and is incorporated selectively into bone in areas of resorption or formation (Lamb & Koblik 1988). Patterns of uptake are registered in a sodium iodide crystal within a gamma camera or a hand-held probe, 2–4 h after injection (Lamb 1991, Javer et al 1996, Seeherman 1998).

Scintigraphy using ^{99m}Tc-MDP was first used as a diagnostic tool in equine orthopedics by Professor Ueltschi in Switzerland in 1975 and since then its use has become widespread in equine hospitals and referral centers for the diagnosis of lameness. However, only recently have reports of the use of scintigraphy for the detection of disorders of the equine skull in significant numbers of horses begun to emerge (Weller et al 2001, Archer et al 2003a,b, Barakzai et al 2006). The ability of scintigraphy, using ^{99m}Tc bound to phosphates, to detect changes in bone before they become radiographically apparent (because increased bone turnover usually precedes structural change) is one of the key advantages of this technique in the equine patient.

Disadvantages of scintigraphy include the expense of acquiring and setting up a gamma camera and appropriate software programs, licensing for the use, storage and disposal of radioactive waste, appropriate stabling facilities which comply with radiation protection legislation, and the risks of radiation exposure to personnel. There is also a requirement for technical expertise when reading scintigraphic images. Additionally, in most centers, horses are considered "radioactive" and must be isolated for 24–48 h post injection, thereby delaying further diagnostic procedures or treatment.

The predominant application of scintigraphy in the equine upper respiratory tract is the investigation of potential periapical infection of the cheek teeth, which is often associated with secondary sinusitis if the caudal four maxillary cheek teeth are involved, or facial swelling with or without a cutaneous draining tract and/or unilateral nasal discharge if the rostral two maxillary cheek teeth are involved. It is particularly useful for differentiation of dental sinusitis from primary sinusitis or sinusitis caused by other lesions such as encapsulated abscesses or sinus cysts. This differentiation is extremely important for appropriate treatment of these disorders, as the consequences of extracting a non-diseased tooth are serious.

The complex structure of the equine skull can make radiographic images of this area difficult to assess because of numerous superimposed structures, and early periapical infections are particularly difficult to detect radiographically (Gibbs & Lane 1987, Metcalf et al 1989). In the more caudally positioned cheek teeth, where secondary sinusitis is common, infected teeth can be recognized with confidence in only 50–57% of cases (Lane et al 1987a, Tremaine & Dixon 2001, Weller et al 2001). Lane et al (1987a) suggested that the increase in bone density associated with maxillary osteitis superimposed on opaque sinus contents obscures the underlying dental structures and prevents detailed inspection so that subtle abnormalities may be missed. In many cases of sinusitis, the increased soft tissue opacity within the sinuses may be the result of inflamed and hypertrophied sinus mucosa, which occurs secondary to any type of chronic sinus infection (Lane et al 1987a, Tremaine & Dixon 2001). In contrast to radiography, scintigraphy has been shown to have excellent sensitivity (95–96%) and moderate specificity (79–86%) for detecting dental disease in the horse (Weller et al 2001, Barakzai et at 2006). Scintigraphy also has a high specificity (92%) and moderate sensitivity (79%) for detection of equine paranasal sinus disorders (Barakzai et al 2006).

Scintigraphic Protocol

Most equine upper respiratory tract scintigraphy is performed using the bone marker ^{99m}Tc-MDP. A dose of 1–1.5 MBq/100 kg bodyweight is administered to the horse via an indwelling jugular catheter. Typically, only bone-phase images are acquired at 2–4 h post injection, as pool or soft tissue phase images do not usually provide any additional useful information and considerably increase the radiation exposure of personnel (Gayle et al 1999, Weller et al 2001).

The use of ^{99m}Tc-hexamethylpropyleneamine oxime (HMPAO)-radiolabeled leukocytes has also been described for equine dental scintigraphy (Weller et al 2001) but it does not facilitate positive identification of apical infections because of a lack of anatomical resolution, and it also incurred considerable extra cost. A less expensive alternative to Indium-111 or ^{99}Tc-HMPAO-labeled leukocytes may be the use of a ^{99}Tc-labeled antigranulocyte murine antibody Fab′ fragment (Leukoscan™), which has been shown to be valuable for the detection of human bone infection. This antibody fragment is mixed with Tc-pertechnetate, and injected intravenously, thus removing the requirement to bleed the patient, separate leukocytes, label them with the radionuclide and then re-infuse them (as is required for the ^{99m}Tc-HMPAO method). Although the antibody fragment has been used successfully in equine clinical cases (Barakzai, unpublished observations 2002), the results have not been documented in the literature to date.

Heavy sedation is usually required to allow close positioning of the camera next to the horse, and is achieved using a combination of α_2-agonist (e.g. xylazine, detomodine or romifidine) and butorphanol. Some horses are very sensitive to the sounds generated by movement of the camera, and "plugging" the ears of such horses with cotton wool may reduce auditory stimuli. Blinkers may also be used to prevent the horse moving away from the camera as it is brought into position, as long as they do not contain metallic components that will attenuate the gamma rays. Similarly, a rope head collar should be used because buckles and rings on a standard head collar can create artifactual "cold spots."

The head of the heavily sedated horse should be rested on a stool or similar object (Fig. 12.1), to minimize movement induced by sedation and minimize rotation in the sagittal plane. Images should be acquired using dynamic studies (e.g. 30 consecutive 2-second frames), because there will be inevitable movements of the horse's head during the acquisition period which, even if small in magnitude, may cause "blurring" of lesions on a static study. Dynamic studies should then be motion corrected using a computerized algorithm applied to each dynamic frame to find the best fit and superimpose the individual frames on each other, resulting in a single corrected

Fig. 12.1. Positioning of the patient (with head resting on a stool) and gamma camera to obtain lateral images of the skull.

summated image. A 128×128 matrix is best for acquiring clear scintigraphic images of the equine head. Counts should ideally be over 200,000 for each image, but this will depend on the dose of Tc administered, the uptake by the tissues, and the interval between injection and image acquisition.

Scintigraphic views

Lateral images are obtained with the gamma camera perpendicular to the floor and parallel to the sagittal plane of the head (Fig. 12.1). Depending on the diameter of the gamma camera and the size of the patient's head, two lateral views centered at different postitions may be necessary to view the entire row of cheek teeth and all of the paranasal sinuses. Dorsal views are obtained by positioning the camera parallel to the frontal bones (Fig. 12.2). Image quality may be enhanced when acquiring dorsal images by positioning a lead shield under the head to attenuate gamma-rays arising from the neck and chest (Ramzan 2003). Oblique views may be useful for assisting lesion localization (Ramzan 2003). In horses suspected of having disorders affecting the

Table 12.1. Normal scintigraphic appearance of the cheek teeth in relation to age of horse

Age of horse (years)	Scintigraphic appearance of cheek teeth
<1	No patterns of uptake that allow differentiation of the CT
2	Irregular scintigraphic activity associated with the CT, but often the apices of the caudal maxillary CT have IRU
3	Focal areas of intense IRU over all CT apices and in the interdental bone of 06s–08s
4	More uniform activity over upper 06s–10s, IRU over upper 11s
5–10	Uniform activity over all CT apices, and clear delineation of interdental bone. CT appear as "cold spots" with relatively less uptake than surrounding bone. Upper 09s have shorter reserve crown than others
>10	Clear delineation of CT gradually disappears, replaced by diffuse pattern of activity as a result of diminishing length of reserve crowns and depth of alveoli

Reproduced from Ramzan 2003, with permission.
CT = cheek teeth; IRU = increased radionuclide uptake.

Fig. 12.2. Positioning of the patient (with head resting on a stool) and gamma camera to obtain dorsal images of the skull.

hemi-mandibles, a ventral view may be obtained by positioning the camera underneath and parallel to the horizontal mandibular rami.

Normal Scintigraphic Anatomy

Age-related variations

Normal patterns of ^{99m}Tc-MDP uptake in the equine head will vary markedly between different age groups because of the development and eruption of the permanent dentition and the accompanying active apical bone remodeling. The scintigraphic changes associated with eruption of the cheek teeth are shown in Table 12.1. It is important to remember when assessing scintigrams of young horses that areas of increased radionuclide uptake (IRU), which are the result of normal eruption of cheek teeth, are bilaterally symmetrical, whereas disorders involving cheek tooth apices are most commonly unilateral.

Lateral views (Fig. 12.3)

Lateral views are often the most useful for identification and localization of periapical infections of individual cheek teeth. The reserve crowns of the cheek teeth appear as "cold spots" of reduced uptake of radionuclide and are

Fig. 12.3. Right lateral scintigram of a normal 5-year-old horse showing cheek teeth (CT), interdental bone (ID), ethmoturbinates (ET), temporomandibular joint (TMJ), zygomatic arch (ZYG), and vertical ramus of the mandible (VR).

surrounded by zones of IRU corresponding to the alveolar bone and interdental (interproximal) bone. The erupted crown is represented by an area of absent radionuclide uptake. The ethmoturbinates can be identified as a region of IRU positioned dorsally and caudally to the sixth maxillary cheek tooth, and are located within the frontal sinuses. The normal temporomandibular joints are also associated with focal areas of marked IRU, as are the atlanto-occipital joints. The ventral and caudal edges of the mandible and the zygomatic arch can be clearly identified as areas of high metabolic activity (Weller et al 2001). Care should be taken when evaluating areas of IRU on lateral views, as IRU on one side of the head may also be seen on the contralateral image (termed "strike-through", Archer et al 2003b). However, the affected side should have higher counts and, additionally, a dorsal or ventral view of the skull should confirm that the lesion is unilateral and will indicate clearly which side is affected.

The areas of the rostral and caudal maxillary sinuses (dorsal to the caudal four cheek teeth) and the concho-frontal and frontal sinuses (dorsal and caudal to the caudal maxillary sinus) are not clearly demarcated in scintigrams of the normal horse.

Dorsal views (Fig. 12.4)

IRU is seen in the alveolar and interdental bone associated with the cheek teeth but it is often impossible to identify individual teeth apices with accuracy in dorsal scintigrams. The ethmoturbinates are also clearly seen as areas of IRU immediately caudal and axial to the sixth maxillary cheek

Fig. 12.4. Dorsal scintigram of a normal 7-year-old horse showing maxillary cheek tooth row (MCT), area of the maxillary sinuses (MS), ethmoturbinates (ET), zygomatic arch (ZYG), and temporomandibular joint (TMJ).

teeth. A moderate amount of IRU may also be seen in the normal zygomatic arch and temporomandibular joints. The premaxilla, frontal, and maxillary bones have diffuse mild radionuclide uptake, and the regions of the rostral and caudal maxillary sinuses (lateral to the caudal four cheek teeth) and the ventral conchal sinus (medial to the caudal four cheek teeth) are not clearly demarcated in the normal horse. It should be noted that slight rotation of the camera or the horse's head can result in distortion of the image and loss of symmetry (Archer et al 2003a).

Scintigraphic Imaging of Disorders of the Equine Upper Respiratory Tract

Dental disorders

Scintigraphy using ^{99m}Tc-MDP has been used for the investigation of human dental disease that cannot be diagnosed radiographically, such as pulpitis, caries, and periodontitis (Tow et al 1978, O'Mara 1985). In humans and dogs, scintigraphic abnormalities have been documented before clinical or radiographic evidence of dental disease is present, indeed as early as 7 days after initiation of periapical infection in experimental models (Garcia et al 1974, Tow et al 1978). Similarly, horses with evidence of pulpar exposure on oral examination of the occlusal surface of the cheek teeth, but with no clinical signs of periapical infection, have been shown to have areas of IRU in the periapical regions of the affected teeth using scintigraphy (Barakzai 2005). Therefore, scintigraphy can be a very useful technique for identification of early periapical infection in horses, particularly in cases that have equivocal radiographic changes. Nonetheless, scintigraphy is most useful for the diagnosis of periapical infection when used in combination with other diagnostic techniques such as radiography and, of course, clinical examination (Metcalf et al 1989, Seeherman 1998, Boswell et al 1999, Weller et al 2001).

Uptake of ^{99m}Tc-MDP that is associated with periapical infection is typically focal and intense, with IRU located over the apical region of the affected tooth (Fig. 12.5). Region of interest studies performed on cases of periapical infection have shown IRU of 24–259% greater than the same region on the contralateral side when using right and left lateral views (Archer et al 2003b, Barakzai 2005). Because "strike-through" may occur when comparing two lateral views, region of interest studies taken from the left and right sides on a dorsal (or ventral) view can show an even greater quantitative IRU (as high as 700%, Barakzai 2005) on the affected side. If periapical infection is accompanied by secondary dental sinusitis, the focal intense uptake over the affected apex will be surrounded by a diffuse region of moderately increased activity over the affected sinus(es) (Fig. 12.6).

After dental extraction, areas of IRU can be present for up to 24 months postoperatively, presumably as a
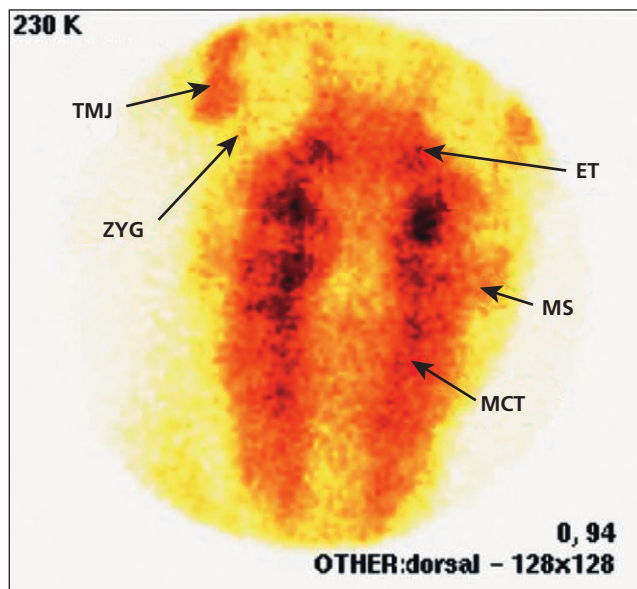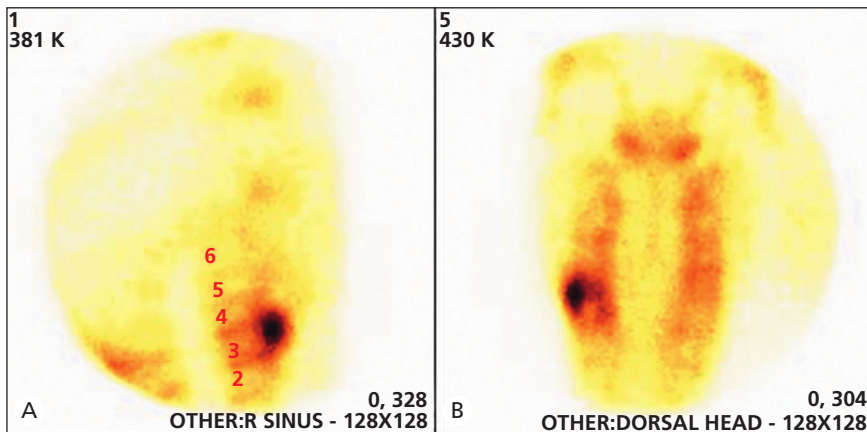
Fig. 12.5. Right lateral (A) and dorsal (B) scintigrams of a 7-year-old horse with peri-apical infection of 108 (third right maxillary cheek tooth) showing focal area of intense IRU over the apex of this tooth on both views. The affected tooth can be accurately identified as 108 on the lateral view (the cheek teeth numbers are marked in red), but it is less clear which tooth is affected on the dorsal view.

result of continued remodeling of the dental alveolus (Barakzai 2005).

Periodontal disease can cause areas of mild to moderate IRU on scintigrams of the equine skull (Weller et al 2001, Archer et al 2003b, Barakzai 2005). However, because this disorder is often bilateral, multifocal and commonly affects older horses where the cheek teeth are not clearly delineated, it can be difficult to definitively diagnose this disorder using scintigraphy. Periodontal disease should be clinically evident from a thorough examination of the oral cavity, and is usually associated with diastemata, displacements or major dental overgrowths. Therefore there is little additional benefit from the use of scintigraphy in its diagnosis.

Primary sinusitis

Experimental and clinical evidence in the human and veterinary fields indicate that bony changes occur in cases of primary sinusitis (Bolger et al 1997, Perloff et al 2000). Additionally, bony changes associated with sinusitis are reported to extend not only to bone adjacent to the infected sinus and those which communicate with it, but may also affect the bone of the contralateral sinonasal complex (Perloff et al 2000). Human facial bone scintigraphy with ^{99m}Tc-diphosphonate is of diagnostic value in neoplastic and inflammatory paranasal sinus lesions, including purulent sinusitis (Bergstedt & Lind 1978, Bergstedt et al 1978). When compared to computed tomography (CT), bone scintigraphy was more sensitive in detecting osteitis in human patients with chronic sinusitis, because bony changes can only be detected on CT when the disease is severe enough to destroy bone, or when there is substantial bony thickening (Jang et al 2002).

Horses with primary sinusitis may show variable patterns of IRU within the affected paranasal sinuses, but generally IRU is more diffuse and less marked (Archer et al 2003b, Barakzai 2005) than occurs with periapical infection (Fig. 12.7). It should be possible to identify the

rostral and caudal maxillary and frontal sinuses individually on scintigrams based on their anatomical relationship to the cheek teeth and ethmoturbinates.

In one equine scintigraphic study that included 15 cases of primary sinusitis, nine of these cases had a focal area of moderate or marked IRU within the sinuses (26–496% increase compared to contralateral side, Barakzai et al 2006; Fig. 12.8). One of these horses had an encapsulated abscess at surgery, which corresponded to the area of IRU seen on the scintigrams, but there was no explanation for the focal areas of IRU seen in the other horses. This is an important finding, because if these focal areas of IRU that are observed
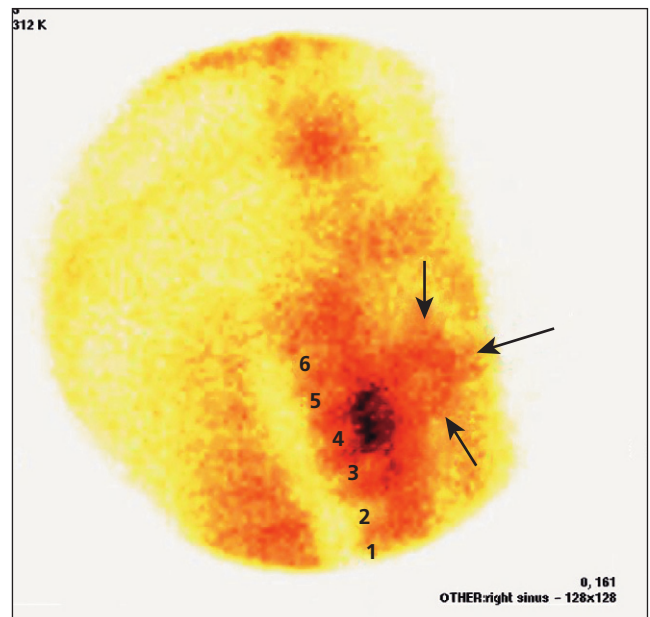
Fig. 12.6. Right lateral scintigram of an 18-year-old horse with periapical infection of 109 (fourth right maxillary cheek tooth) and secondary rostral maxillary sinusitis. There is a focal area of marked IRU above the affected tooth, and a more diffuse, moderately increased uptake in the area of the rostral maxillary sinus, dorsal to this tooth (arrows). The cheek tooth numbers are marked in black.
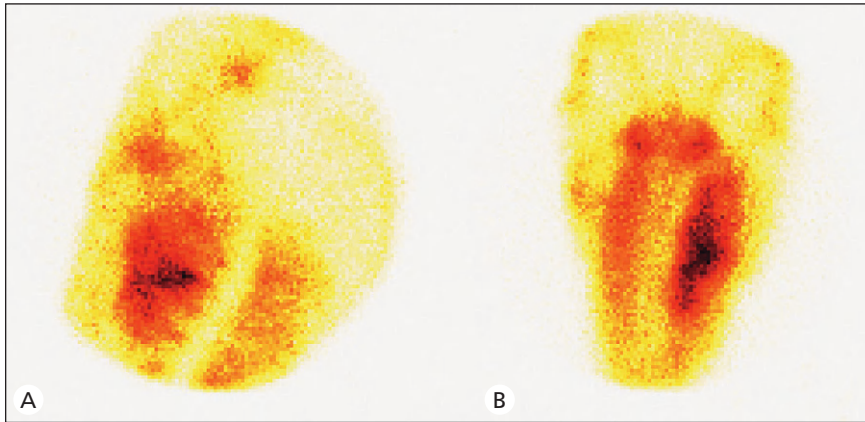
Fig. 12.7. Left lateral (A) and dorsal (B) scintigrams of a horse with primary sinusitis. The area of IRU is more diffuse (spanning the rostral and caudal maxillary sinuses), and is less marked than occurs with periapical infection of a cheek tooth.
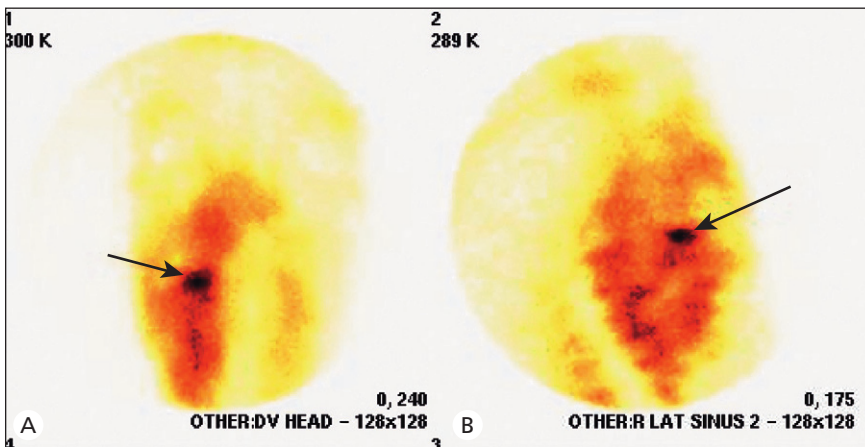
Fig. 12.8. Dorsal (A) and right lateral (B) scintigrams of a horse with primary sinusitis. Note the focal area of marked IRU (arrow) in the caudal maxillary sinus, which, in the lateral view, is positioned too far dorsal to be associated with an apical infection of the cheek teeth. No specific lesion was found within the caudal maxillary sinus during sinus surgery, which correlated with this focal area of IRU.

in cases of primary sinusitis happen to be positioned over the apex of a cheek tooth, a false diagnosis of periapical infection may be made. Careful three-dimensional localization of the focal area of IRU (as seen in the case in Fig. 12.8) should help prevent such false diagnoses.

Sinus trephination

Trephination of the frontal sinuses is often used to facilitate direct sinus endoscopy as an auxiliary diagnostic technique in some cases of equine sinusitis. Trephination of a bone would be expected to result in a region of IRU (as a result of exposure of hydroxyapatite crystals and instigation of remodeling and callus formation) if the horse later underwent scintigraphy to further investigate the cause of the sinusitis. However, trephination of the frontal sinus performed between 1 day and 6 weeks before scintigraphic examination has been found to have no significant effect on radionuclide uptake in the area of the trephine hole (Archer 2003b, Barakzai & Dixon 2003).

Sinus cysts

Sinus cysts are expansive fluid-filled space-occupying lesions that occur uncommonly in the equine sinuses and frequently cause bone remodeling or even destruction, accompanied by a low-grade inflammatory response (Dixon 1985, Gibbs & Lane 1987, Lane et al 1987b, Tremaine et al 1999). Histologically, the cyst wall is lined by a layer of secretory respiratory-type pseudostratified epithelium, with plates or spicules of bone frequently present (Lane et al 1987b). The association of these lesions with bony remodeling and new bone formation (within the cyst wall) may give a typical appearance on scintigraphic examination, with areas of IRU around the margin of the lesion attributable to uptake of radionuclide by calcified sections of the cyst wall (Fig. 12.9). However, sinus cysts can usually be accurately diagnosed with a combination of radiography, endoscopy, direct sinocentesis and sinoscopy, and scintigraphic examination is rarely indicated for the diagnosis of these lesions.
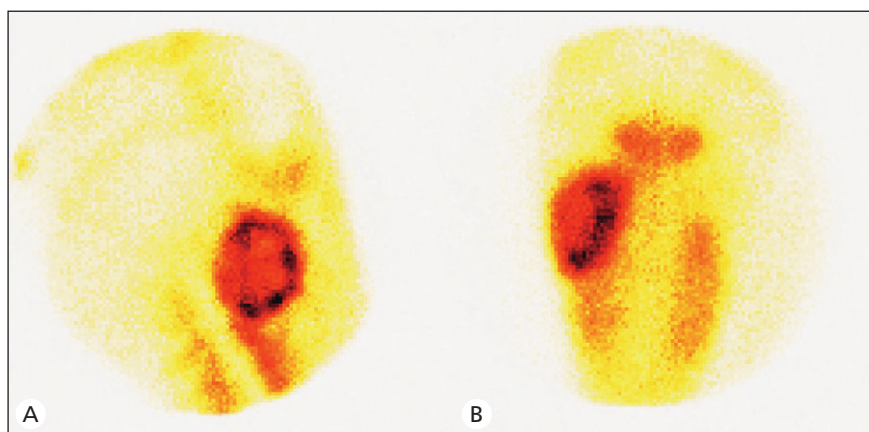
Fig. 12.9. Right lateral (A) and dorsal (B) scintigrams of a horse with a circular sinus cyst in the right caudal maxillary sinus. Note the areas of marked IRU around the margin of the lesion, associated with uptake of ^{99m}Tc-MDP by calcified sections of the cyst wall.
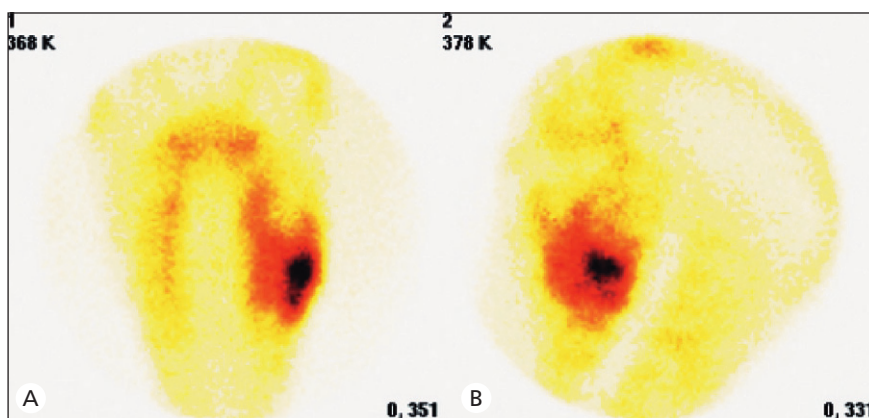
Fig. 12.10. Dorsal (A) and lateral (B) scintigrams of a horse with a maxillary fracture that presented with facial swelling and no known history of trauma. Scintigraphy allowed differentiation between periapical infection of a cheek tooth and fracture of the left lateral maxilla, because the focal area of marked IRU is positioned lateral to the left cheek tooth row on the dorsal view.

Progressive ethmoidal hematomas

The scintigraphic appearance of progressive ethmoidal hematomas is not well documented, but a focal intense IRU is usually seen adjacent to the ethmoturbinates, which is sometimes accompanied by a more diffuse IRU as a result of secondary sinusitis (Barakzai 2005). However, progressive ethmoidal hematomas can usually be accurately diagnosed by a characteristic history of low-grade epistaxis, radiography, endoscopy, and sinoscopy, and scintigraphic examinations are rarely indicated for the diagnosis of these lesions.

Skull fractures (Fig. 12.10)

Fractured bones often have a focal intense IRU within hours of the fracture occurring, as a result of exposure of hydroxyapatite crystals, followed by instigation of bone remodeling and callus formation (Lamb & Koblik 1988, Lamb 1991). IRU is variable with equine skull fractures. Stable fractures, such as those caused iatrogenically when creating sinus flaps or trephine holes, appear to be associated with little or no IRU (Barakzai & Dixon 2003, Barakzai 2005). In contrast, skull fractures which are traumatic in origin are often associated with intense IRU, as is nasofrontal suturitis, a common sequel of equine skull trauma (Tremaine & Dixon 2001, Barakzai 2003).

Neoplasia of the head

The use of scintigraphy in the evaluation of malignant disease of the human facial bones and sinuses has been limited. CT is usually performed as part of a staging procedure, and will usually demonstrate bony involvement in malignancies. However, scintigraphy remains an extremely sensitive method for demonstration of malignant involvement of bones, and a normal bone scan virtually excludes tumor involvement of bone (O'Mara 1985). It has been suggested that gamma scintigraphy may be a useful additional imaging modality for the early detection of sinonasal neoplasia in the horse (Archer et al 2003b).

REFERENCES

Archer DC, Blake CL, Singer ER et al 2003a The normal scintigraphic appearance of the equine head. Equine Veterinary Education 15: 243–249

Archer DC, Blake CL, Singer ER et al 2003b Scintigraphic appearance of selected diseases of the equine head. Equine Veterinary Education 15: 305–313

Barakzai SZ 2005 Use of scintigraphy for the diagnosis of suspected equine dental and paranasal sinus disorders. MSc Thesis, University of Edinburgh

Barakzai SZ, Dixon PM 2003 Effect of sinus trephination on scintigraphy of the equine skull. Veterinary Record 152: 629–630

Barakzai SZ, Tremaine WH, Dixon PM 2006 Use of scintigraphy for diagnosis of equine paranasal sinus disorders. Veterinary Surgery 35: 94–101

Bergstedt HF, Carenfelt C, Lind MG 1978 Facial bone scintigraphy. IV. Diagnosis of bone involvement by purulent sinusitis. Acta Radiologica: Diagnosis 20: 379–384

Bergstedt HF, Lind MG 1978 Facial bone scintigraphy. II. Diagnostic potential in neoplastic and inflammatory lesions. Acta Radiologica: Diagnosis 19: 993–999

Bolger WE, Leonard D, Dick EJ et al 1997 Gram negative sinusitis: a bacteriologic and histological study in rabbits. American Journal of Rhinology 11: 15–25

Boswell JC, Schramme MC, Livesey LC et al 1999 Use of scintigraphy in the diagnosis of dental disease in four horses. Equine Veterinary Education 11: 294–298

Dixon PM 1985 Equine maxillary cysts. Equine Practice 71: 25–33

Garcia DA, Entine G, Tow DE 1974 Detection of small bone abscesses with a high resolution cadmium telluride probe. Journal of Nuclear Medicine 15: 892–895

Gayle JM, Redding WR, Vacek JR et al 1999 Diagnosis and surgical treatment of periapical infection of the third mandibular molar in five horses. Journal of the American Veterinary Medical Association 215: 829–832

Gibbs C, Lane JG 1987 Radiographic examination of the nasal and paranasal sinus regions of the horse. Part 2: Radiological findings. Equine Veterinary Journal 19: 474–482

Jang YJ, Koo TW, Chung SY et al 2002 Bone involvement in chronic rhinosinusitis assessed by ^{99m}Tc-MDP bone SPECT. Clinical Otolaryngology and Allied Sciences 27: 156–161

Javer AR, Stevens HE, Stillwell M et al 1996 Efficacy of nuclear scintigraphy in the diagnosis and management of sinusitis. Journal of Otolaryngology 25: 375–382

Lamb CR 1991 The principles and practice of bone scintigraphy in small animals. Seminars in Veterinary Medicine and Surgery (Small Animal) 6: 140–153

Lamb CR, Koblik PD 1988 Scintigraphic evaluation of skeletal disease and its application to the horse. Veterinary Radiology 29: 16–27

Lane JG, Gibbs C, Meynik SE et al 1987a Radiographic examination of the facial, nasal and paranasal sinus regions of the horse: I: indications and procedures in 235 cases. Equine Veterinary Journal 19: 466–473

Lane JG, Longstaffe JA, Gibbs C 1987b Equine paranasal sinus cysts: a report of 15 cases. Equine Veterinary Journal 19: 534–544

Metcalf MR, Tate LP, Sellett LC 1989 Clinical use of ^{99m}Tc-MDP scintigraphy in the equine mandible and maxilla. Veterinary Radiology 30: 80–87

O'Mara RE 1985 Role of bone scanning in dental and maxillofacial disorders. In: Freeman LM, Weissman HS (editors) Nuclear Medicine Annual. Raven Press, New York, pp.78–91

Perloff JR, Gannon MD, Bolger WE et al DW 2000 Bone involvement in sinusitis: an apparent pathway for the spread of disease. Laryngoscope 110: 2095–2099

Ramzan PHL 2003 The head. In: Dyson SJ, Pilsworth RC, Twardock AR, Martinelli MJ (editors) Equine Scintigraphy. Equine Veterinary Journal, Newmarket, pp.225–226

Seeherman HJ 1998 Clinical applications of bone scanning. In: White NA, Moore JN (editors) Current Techniques in Equine Surgery and Lameness, 2nd edn. WB Saunders, Philadelphia, pp.592–605

Tow DE, Garcia DA, Jansons D et al 1978 Bone scan in dental diseases. Journal of Nuclear Medicine 19: 845–895

Tremaine WH, Dixon PM 2001 A long term study of 277 cases of equine sinonasal disease. Part 1: Details of horses, historical, clinical and ancillary diagnostic findings. Equine Veterinary Journal 33: 274–282

Tremaine WH, Clarke CJ, Dixon PM 1999 Histopathological findings in equine sinonasal disorders. Equine Veterinary Journal 31: 296–303

Weaver MP 1995 Twenty years of equine scintigraphy – a coming of age? Equine Veterinary Journal 27: 163–165

Weller R, Livesey L, Maierl J et al 2001 Comparison of radiography and scintigraphy in the diagnosis of dental disorders in the horse. Equine Veterinary Journal 33: 49–58

13 Pulmonary Scintigraphy

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Introduction

As a complementary investigation tool, scintigraphy provides a unique method for studying regional lung function. The numerous potential applications of scintigraphic tests in the study and diagnosis of pulmonary disorders are listed in Table 13.1. The purpose of this chapter is to assist nuclear medicine practitioners in performing and interpreting the results of lung scintigraphy in horses, primarily for:

- assessing regional ventilation
- determining regional ventilation to perfusion ratios
- measuring alveolar clearance.

Assessment of mucociliary clearance, detection of pulmonary bleeding sites, the study of aerosol deposition within the equine lung, and imaging of pulmonary infection and inflammation will be covered briefly as these techniques have primarily a research application or they are inadequately validated in horses.

Imaging of Regional Lung Function

The properties of the radiopharmaceuticals that are used in lung function imaging are summarized in Table 13.2.

Lateral images of the lungs are usually obtained by positioning the horse with the thorax against the face of the gamma camera. Because of the large surface area of the equine lungs, one cranial and one caudal view per lung must be acquired. The computer then integrates the cranial and caudal views to form a single lateral view for each lung. This integration requires placement of reference radioactive marks at fixed positions on the chest wall (Votion et al 1997). Multiple sequential imaging may also be performed, whereby a series of images is recorded for a selected time period.

Digital images are recorded in matrices of either 64×64, 128×128 or 256×256 pixels, and images are stored on the computer for subsequent analysis. When image acquisition is completed, a horse that has received technetium-99m (^{99m}Tc) radiopharmaceuticals must be quarantined for 48 h, by which time the ^{99m}Tc (half-life 6 h) has significantly decayed.

Scintigraphic images may be displayed in a color-coded fashion according to the relative concentration of radio-

Table 13.1. The potential applications of scintigraphic tests in the study and diagnosis of equine pulmonary disorders
Evaluation of regional ventilation and perfusion and their relationship Definition of functional changes induced by pulmonary disorders Diagnosis of an unknown disorder Assessment of the extent of a pulmonary disease Monitoring the course of a pulmonary disease Assessment of the response to therapy
Measurement of alveolar clearance Detection of subclinical RAO Evaluation of management procedures in horses suffering from RAO Assessment of the severity of pulmonary diseases Monitoring the course of pulmonary diseases Assessment of the response to therapy
Assessment of mucociliary clearance Assessment of changes in mucociliary clearance induced by respiratory disorders Evaluation of drugs such as mucolytics, expectorants or mucokinetics
Detection and quantification of pulmonary bleeding sites Diagnosis of EIPH Assessment of the severity of EIPH Monitoring the site and time course of EIPH Assessment of the response to therapy
Study of aerosol deposition within the lung Evaluation of aerosol delivery systems and techniques Determination of inhaled drug dosage requirements Study of inhaled drug deposition
Imaging sites of pulmonary infection and inflammation Detection and diagnosis of occult pulmonary infections and inflammation Assessment of the severity of pulmonary diseases Monitoring the course of pulmonary diseases Assessment of the response to therapy Study of inflammatory cell recruitment in lung disorders

EIPH = exercise-induced pulmonary hemorrhage; RAO = recurrent airway obstruction.

activity in each pulmonary zone. These images can reveal, almost at a glance, pulmonary dysfunction (e.g. a mismatch in ventilation and perfusion distribution) or an abnormal deposition pattern in aerosol studies, although appropriate image processing yields additional information.

Table 13.2. Radiopharmaceuticals used in lung function imaging				
Radiopharmaceutical	Study	Dosage	Advantage(s)	Disadvantage(s)
Radioactive gas				
^{81m}Kr	Ventilation	1.8 liter/min*	Easy to administer No risk of environmental contamination Low radiation dose High-quality images True ventilation images On-line monitoring of functional changes Ventilation scanning may be repeated No horse quarantine required Dual acquisition with ^{99m}Tc-albumin aggregates or ^{99m}Tc-microspheres is feasible	Limited availability Expensive
	Perfusion	25 ml/min**	On-line monitoring of functional changes	Time consuming More expensive than the labeled albumin aggregate and microsphere methods Artifact due to circulating activity
Technetium-labeled compounds				
^{99m}Tc-DTPA	Ventilation	7.4 MBq/kg	The most commercially available	Lung clearance (poor quality of the ventilation image in horses with lung disorders)
	Alveolar clearance	7.4 MBq/kg	Measures alveolar epithelium injury Detects subclinical inflammation	
^{99m}Tc-Nanocolloid	Ventilation	7.4 MBq/kg	Negligible lung clearance	
^{99m}Tc-Carbon (Technegas)	Ventilation	to be defined	Probably better ventilation imaging agent than liquid aerosols Requires only a few breaths to be administered	Risk of environmental contamination High cost of Technegas generator device
^{99m}Tc-albumin aggregates or -microspheres	Perfusion	1.11 MBq/kg	The easiest method Imaging of the whole lung without artifact due to circulating activity Images of perfusion at exercise may be obtained	

* The elution rate suggested is for a generator containing approximately 925 MBq (25 mCi) of its parent isotope.
** Elution of the generator with 5% dextrose in water.

Assessment of Regional Ventilation

Radiopharmaceuticals

Regional ventilation imaging in horses may be performed using radioactive krypton-81m (^{81m}Kr) gas or radiolabeled aerosols. Scintigraphy using ^{81m}Kr gas allows assessment of ventilation in a time scale of seconds, whereas radiolabeled aerosol scintigraphy allows static imaging of the lungs but does not provide information about airflow.

The ^{81m}Kr is a radioactive noble gas that has a very short half-life (13 seconds). It is obtained by eluting a rubidium-81 (^{81}Rb)/^{81m}Kr generator with air. The rate of

elution is 1.8 liter/min for a generator containing approximately 925 MBq ^{81}Rb. The horse breathes the eluted ^{81m}Kr from the generator continuously, through a tube connected to a facemask. The ^{81m}Kr may also be delivered directly into the trachea via an intratracheal catheter. Owing to the short half-life of ^{81m}Kr, there is no safety requirement to collect exhaled air. Unfortunately, the short half-life of ^{81}Rb (4.58 h) limits ^{81m}Kr availability in clinical practice.

As an alternative to ^{81m}Kr, inhaled radiolabeled aerosols may be used. Ventilation imaging with aerosols assumes that the deposition of small inhaled particles follows the distribution of inhaled air, and that areas of the lungs in which no radioactive aerosol particles are deposited

are likely to be unventilated. However, other features of respiratory disorders, such as high respiratory rate and airway obstruction, reduce penetration of radiolabeled aerosols into the peripheral lung and favor their deposition in central conducting airways. Consequently, the deposition pattern of radiolabeled aerosols does not necessarily match the ventilation distribution. On the other hand, the image obtained using radiolabeled aerosols is highly sensitive to ventilation restriction and airflow modification. The radiolabeled aerosols used in equine scintigraphy comprise compounds labeled with ^{99m}Tc. The ^{99m}Tc radionuclide readily labels a variety of compounds and is both easily and cheaply obtainable via a molybdenum-99 (^{99}Mo)/^{99m}Tc generator. Most scintigraphic assessments of ventilation in horses have used ^{99m}Tc-diethylene triamine pentaacetic acid (DTPA) as the ^{99m}Tc-labeled compound. However, DTPA is absorbed from the lungs by passive diffusion through the alveolar–capillary barrier. In a number of diseases, the permeability of this barrier is considerably increased and thus ^{99m}Tc-DTPA molecules are absorbed more rapidly from the lungs. This property may be useful in the measurement of alveolar clearance, but in ventilation studies a significant quantity of radiopharmaceutical may have already been cleared from the lungs during the time required to acquire the aerosol deposition images. A non-diffusible compound such as nanocolloid of human albumin, that shows negligible absorption over time, is therefore preferable to DTPA.

Radiolabeled aerosols are prepared by introducing sodium pertechnetate [Na$^+$(^{99m}TcO$_4$)$^-$] to lyophilized reaction vials. The radioactive solution (7.4 MBq/kg body weight) is then aerosolized using an inhalation device which must produce aerosol droplets that have an aerodynamic particle size of between 0.5 and 2 μm. Droplets that are smaller than 0.5 μm tend to be exhaled, whereas droplets exceeding 2 μm tend to impact in the larger conducting airway rather than reaching the alveoli. The horse breathes the radioactive aerosol for several minutes, i.e. the time required to aerosolize and deliver the entire dose. Because of potential atmospheric radioactive contamination, the radiolabeled aerosol must be administered to the horse via an airtight delivery system, and exhaled air must be collected through a filter to remove exhaled radioactive particles.

An ultrafine dry aerosol called Technegas, which so far has not been used in equine medicine, could potentially be of use in horses. This ventilation imaging agent is produced by evaporating sodium pertechnetate at 2500°C on a carbon surface (Burch et al 1986). The ^{99m}Tc-carbon particles produced have gas-like penetrating characteristics but, despite their small size (below 0.2 μm), the dry aerosol particles adhere to the alveolar walls. In calves, a dose of 20 MBq/kg body weight of sodium pertechnetate yielded excellent images of regional ventilation (Coghe et al 2000). Technegas has the advantage over radiolabeled aerosols

of not being diffusible through the alveolar–capillary barrier (Isawa et al 1996). However, as the entire dose of Technegas is administered in a few seconds, severe atmospheric contamination with Technegas may occur if the animal accidentally disconnects from the delivery system during administration. During labeled aerosol administration (i.e. liquid aerosols or Technegas), it is preferable to sedate the horse to minimize the risks of accidental disconnection from the delivery system.

Imaging of regional ventilation

Ventilation images obtained using ^{81m}Kr must be acquired during administration of the radioactive gas because of the short half-life of the radiopharmaceutical. However, a great advantage of this short half-life is that studies may be repeated without carry over of radioactivity from preceding studies. This can facilitate on-line visualization of the course of alterations in ventilation induced by specific conditions and interventions, such as following administration of bronchodilators.

In contrast, static images of radiolabeled aerosol deposition are acquired after administration of the radiopharmaceutical. Approximately 30–60 min is required to obtain all views of the lungs.

Interpretation of regional ventilation scintigrams

In healthy horses, ventilation is evenly distributed throughout the lung field. Functional modifications in regional ventilation result from airway obstruction caused by bronchospasm, excess mucus in the airway, and thickening of the bronchial airway wall by edema and inflammation. When ventilation is imaged with radiolabeled aerosols, these abnormalities lead to deposition of aerosol droplets predominantly in the large airways, and reduced deposition in the terminal airways and alveoli. Consequently, aerosol images are considered abnormal when the ventilation distribution is uneven, with the degree of irregularity in aerosol distribution being directly proportional to the severity of functional disturbance (Fig. 13.1). Image analysis may then provide quantitative assessment of ventilation, which allows comparison of subjects or groups of subjects to reference values and overcomes problems with subjective interpretation of images.

The extent to which the aerosol has penetrated into the smaller airways and alveoli may be assessed by calculating the penetration index (PI). The PI has been defined as the ratio of radioactivity recorded in the peripheral versus the central part of the lung. Calculation of PI first necessitates accurate delineation of the lung margins, using radioactive gas inhalation images (it is assumed that gases reach the
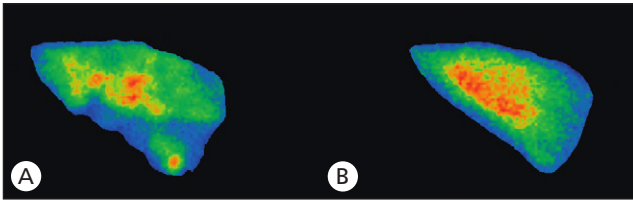
Fig. 13.1. Aerosol deposition images of a horse suffering from recurrent airway obstruction (lateral views of the right lung). Airway obstruction associated with recurrent airway obstruction impedes radioactive aerosol particle penetration into the peripheral lung, and favors patchy dispersal of the radiopharmaceutical (A). Following therapy with aerosolized drugs, lung function is improved and a more homogeneous distribution of the radiopharmaceutical is observed (B).

alveoli) or perfusion images. Second, within the lung margins, the central (including the major bronchi) and peripheral (containing mainly lung parenchyma) parts of the lung must be delineated.

Another quantitative index of the aerosol's ability to reach the peripheral lung is the "size" of the images as determined by an isocontour function. This function draws a line between pixels with a value equal to a defined percentage of the maximum pixel value in the image. The maximal pixel value in the image is influenced by the degree of aerosol penetration and is higher when more aerosol impacts within the airways. Consequently, the isocontour function, for the same defined percentage, tends to draw a smaller deposition image. The percentage of the isocontour function should be set high enough to exclude background radioactivity. The number of pixels within the isocontour line represents the size of the picture.

Assessment of Regional Lung Perfusion

Radiopharmaceuticals

In horses, perfusion scintigraphy alone has been performed mainly for research purposes, such as investigating alterations in regional perfusion induced by exercise or anesthesia. These studies employed either infusion of ^{81m}Kr gas or labeled entrapped particle methods. However, for clinical investigation of respiratory disorders, a combined ventilation–perfusion study is usually performed. The easiest way to study the ventilation–perfusion relationship is to use images of radiolabeled aerosol deposition and images of labeled entrapped particles. The latter involves administration of ^{99m}Tc-labeled microspheres or albumin aggregates into a jugular vein. These microspheres and albumin aggregates are supplied as lyophilized kits ready to label by adding sodium pertechnetate. A dose of 1.11 MBq/kg body weight gives satisfactory images of equine lung perfusion (Votion et al 1997). As the size of the particles in the suspension (at least 90% of the particles have a diameter above 10 μm and all are smaller than

90 μm) exceeds that of the pulmonary precapillary lumina, the particles temporarily embolize pulmonary capillaries and small arterioles according to the distribution of pulmonary blood flow (Beck 1987). This yields a picture of pulmonary blood flow distribution at the moment the particles were trapped. Removal of the entrapped particles occurs over several hours. Care must be taken during the intravenous injection: the solution must be gently homogenized before administration, blood must not be withdrawn into the syringe as it may form clots, and the tracer must be administered over several respiratory cycles to average the pulmonary blood flow distribution, which is influenced by changes in alveolar pressure associated with breathing (Jarvis et al 1992). In horses, the procedure appears to be safe because no adverse reaction has been reported.

Imaging of regional perfusion (to determine the ventilation to perfusion ratio)

When ventilation is imaged with ^{81m}Kr gas, ventilation and perfusion images may be obtained simultaneously, because the 140-keV emission of ^{99m}Tc and the 191-keV emission of ^{81m}Kr can be recorded simultaneously using two different acquisition channels. This reduces the time required for imaging and enables ventilation and perfusion images to be matched perfectly in the acquisition matrices. In contrast, when both ventilation and perfusion images are obtained using ^{99m}Tc radiopharmaceuticals, ventilation and perfusion images must be acquired in succession. Consequently, the radioactivity originating from the first study contaminates the subsequent study. To minimize this effect, the radioactivity within the lungs in the second study must exceed that of the first study. Labeled aerosol or Technegas imaging should be performed before perfusion imaging because it is easier to deliver a larger dose of ^{99m}Tc perfusion imaging agent than of ^{99m}Tc aerosol. With the recommended dosages, the background activity arising from the ventilation study is minimal when compared to that of the perfusion study.

Assessment of regional ventilation to perfusion ratio

In healthy horses, the radioactivity distribution patterns of ventilation (Fig. 13.2A) and perfusion (Fig. 13.2B) appear very similar. When respiratory disorders alter one or both of the functions, a direct visual comparison of the ventilation and perfusion images can be difficult to interpret. This problem can be overcome by the creation of computerized images of the ventilation to perfusion ratio. When labeled aerosols are used for lung ventilation imaging, the creation of ventilation to perfusion ratio images necessitates the exact matching of ventilation and perfusion images, using reference markers placed on the
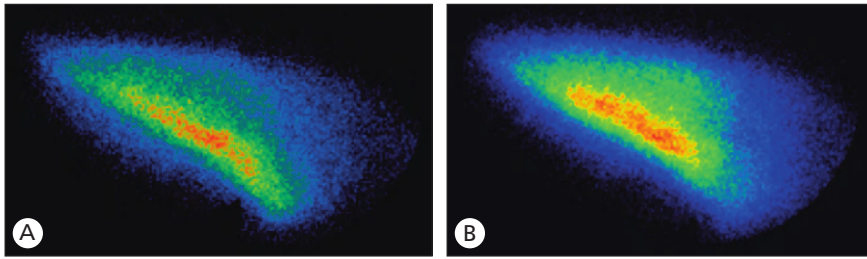
Fig. 13.2. (A,B) Ventilation and perfusion images (lateral views of the right lung). On visual inspection, ventilation (A) and perfusion (B) distribution patterns in healthy horses are comparable.

chest wall. Furthermore, radioactivity arising from the ventilation procedure that contaminates the perfusion scan must be subtracted from the perfusion image, taking into account the duration of time that has elapsed between the acquisition of the ventilation and perfusion images, because the radioactivity originating from the ventilation scan will have been attenuated by radioactive decay when compared to the ventilation image used for subtraction.

From "pure" ventilation (i.e. ^{81m}Kr or corrected ^{99m}Tc-labeled aerosol deposition images) and perfusion images, ventilation to perfusion ratio images may be created by computer. Considering that the same radioactive dose is present within the lungs after the ventilation and perfusion procedures, the mean pixel count of the ventilation images must be equalized to the mean of the perfusion scans using a corrective factor specific for the left and right lungs (see Votion et al 1997 for further details of this procedure). When the corrective factors have been applied, left and right ventilation to perfusion ratio images can be obtained by dividing the corresponding pixels in the ventilation image by those of the perfusion image. These ventilation to perfusion ratio images permit direct visualization of ventilation–perfusion mismatches. Additionally, a histogram of frequency ratio distribution can be produced. In healthy horses, the distribution is tightly centered on the theoretical ideal value of 1, i.e. ventilation closely matches perfusion. This matching ensures efficient gas exchange. Inequalities of the distribution of ventilation and perfusion ratio are the most common causes of inefficient gas exchange. Scintigraphy highlights these mismatches within the diseased lungs and enables assessment of the time course of a disease or its response to therapy (Votion et al 1999a).

Assessment of Alveolar Clearance

Radiopharmaceuticals

The alveolar–capillary membrane comprises the alveolar epithelial layer, the interstitial space, and the capillary endothelial layer. This barrier normally prevents plasma, cells, and proteins from flooding the air space, thereby maintaining normal gas exchange. Because intercellular junctions of alveolar cells are tighter than those of the endothelial cells, resistance to transmembrane diffusion

of hydrophilic molecules is primarily dependent on the alveolar epithelial component of this barrier. Therefore, soluble materials deposited in the alveoli passively diffuse into the pulmonary circulation and enter the bloodstream at a rate determined largely by the permeability of the alveolar epithelium. Elimination of ^{99m}Tc-DTPA from the lungs is considered to be an index of alveolar epithelial damage. Epithelial injury increases permeability of the alveolar epithelium, and hastens ^{99m}Tc-DTPA diffusion from lungs to blood. The ^{99m}Tc-DTPA is prepared as described earlier for ventilation studies.

Imaging of alveolar clearance

Numerous factors may influence the measurement of alveolar clearance rates, and thus a highly standardized method must be used (Votion et al 1998). Immediately after disconnecting the ^{99m}Tc-DTPA delivery system from the horse, the gamma camera is placed against the horse's left chest wall, and the caudal part of the lung is imaged at 30-second intervals for 20 min. The rate of alveolar clearance of hydrophilic solutes precludes the acquisition of additional satisfactory views of the lung. Thus, following imaging of alveolar clearance, a perfusion image should be obtained to further identify the lung boundary on the ^{99m}Tc-DTPA deposition images.

Measurement of alveolar clearance

The caudal half of the left lung may be outlined on the ^{99m}Tc-DTPA deposition scans using the perfusion image. The number of counts recorded by the gamma camera in each frame of the caudal lung is used to generate a (radio)activity versus time curve. The coefficient of time, estimated by fitting the monoexponential decay curve to the observed counts, measures the regional clearance rate (k). Results may also be expressed in half-time clearance from lung to blood ($t_{1/2} = \ln 2/k$). In healthy horses, the mean value for half-time ^{99m}Tc-DTPA alveolar clearance is approximately 40 min. One advantage of the alveolar clearance test is the detection of subclinical recurrent airway obstruction (RAO; Votion et al 1999b). The ^{99m}Tc-DTPA alveolar clearance rate is reduced in RAO-affected horses that have clinical signs of the disease but returns to baseline when horses are in full clinical

remission following their turn-out to pasture. RAO-affected horses that are maintained in a dust-controlled indoor environment have intermediate values for ^{99m}Tc-DTPA alveolar clearance rates, despite lacking abnormal clinical signs. In contrast, clinical examination and conventional pulmonary function tests are of insufficient sensitivity to differentiate those horses which were in clinical remission following turn-out to pasture from those of RAO horses which were maintained in a dust-controlled indoor environment. This demonstrates that measurement of ^{99m}Tc-DTPA alveolar clearance may allow detection of early functional changes at the alveolar level in horses with subclinical RAO. Detection of subclinical diseases is extremely useful for effective environmental and medical management, and for the prevention of irreversible lung remodeling. Measurement of ^{99m}Tc-DTPA alveolar clearance rates may also be useful in following both the time course and the severity of lung injury, as well as in monitoring the subsequent repair process.

Fig. 13.3. Exercise-induced redistribution of pulmonary perfusion (lateral view of the left lung). Computed exercise versus resting perfusion ratio image of lung demonstrating the redistribution of pulmonary perfusion to the caudodorsal region of the lung during exercise.

Research Applications of Equine Scintigraphy

Scintigraphic assessment of mucociliary clearance

Mucociliary clearance of inert insoluble particles may be imaged with scintigraphy, and the tracheal mucus transport rate can be determined. A dose of 370 MBq radioactive inert insoluble particles is deposited within the trachea using an endoscope or an intratracheal catheter (Nelson & Hampe 1983). Microspheres or albumin aggregates labeled with ^{99m}Tc may be used as radiopharmaceuticals. Sequential imaging of the trachea is used to follow the transport of the radioactive material up the trachea. The tracheal mucus transport rate is then calculated by dividing the distance moved by the radioactive tracer by the time taken. The method used must be highly standardized because of the numerous factors affecting the tracheal mucus rate, such as sedation, age, angulation of the trachea, and the presence of coughing. The study of tracheal mucociliary clearance is, however, of little value in routine clinical investigation, mainly because of large individual variation.

Study of pulmonary perfusion for detection of lung hemorrhage

A method to accurately detect and repeatedly quantify pulmonary hemorrhage in horses would greatly improve our knowledge of exercise-induced pulmonary hemorrhage (EIPH). In humans, scintigraphy employing labeled red blood cells (RBCs) is used to detect low-grade gastrointestinal bleeding, but unfortunately this method lacks sensitivity for detection of EIPH. Other techniques such as

indium-111 (^{111}In)-RBC/^{99m}Tc-RBC dual isotopes imaging (Votion & Lekeux 2003) or the radiolabeled peptide technique used to detect deep venous thrombosis (Taillefer 2001) should be evaluated for the detection of EIPH.

Scintigraphy, using labeled entrapped particles, showed that pulmonary blood flow is redistributed to the dorsocaudal regions of the lungs during exercise (Harmegnies et al 2002; Fig. 13.3). This may contribute to EIPH because bleeding arises from the dorsocaudal areas of both lungs.

Scintigraphic evaluation of inhalation therapy

The response to therapeutic aerosols is considered to be a function of the dose deposited at the appropriate site in the lung. This dose is dependent upon the system used to produce and deliver the aerosol, the physical characteristics of the inhaled aerosol, the mode of inhalation, and the geometry of the airways. Scintigraphy can facilitate the study of the efficiency of a delivery system (dose delivered, regional distribution), the techniques of drug administration (e.g. determination of the optimal time in the respiratory cycle to administer a bolus of drug to target a specific lung zone), the effect of respiratory disorders on aerosol distribution, and may also help determine the site of action of drugs. These studies may improve drug targeting.

Imaging of pulmonary infection and inflammation

In human medicine, lung scintigraphy with gallium-67 (^{67}Ga) is an established means to assess alveolar inflammation in a wide range of diffuse lung disorders. Immediately

following its intravenous injection, ^{67}Ga citrate binds to transferrin and the metal–protein complex diffuses passively through the vascular endothelium into the inflamed tissues. The circulating tracer is subsequently bound to iron-binding proteins produced by macrophages and bacteria within the inflamed tissue. Lung scintigraphy with ^{67}Ga could theoretically be used to monitor the extent and distribution of alveolar inflammation during the course of equine respiratory disorders and to evaluate the response to therapy. However, the major drawbacks of ^{67}Ga are its poor specificity, its long physical half-life (78 h) and its broad range of gamma-ray emission that produces poor-quality images.

Alternatively, pulmonary inflammatory foci may be imaged following the intravenous administration of radiolabeled leukocytes, which migrate rapidly into inflammatory sites. Radiolabeled leukocyte scintigraphy may aid the diagnosis and assessment of the severity of occult inflammatory or septic foci in the equine lung, such as those caused by *Rhodococcus equi* infection (Ramzan et al 2004).

Specific inflammatory cell involvement in equine lung diseases may also be studied using scintigraphy. Neutrophils, eosinophils, mononuclear cells, and platelets can be separated by density gradient centrifugation and subsequently selectively labeled *in vitro*, without alteration in their state of activation. These labeled cells may be re-injected into the donor horse to follow their distribution within the body, and to determine whether they are recruited to the lung. This technique was used to determine the time course of leukocyte recruitment to the lungs of RAO-affected horses following dust exposure, and to show that pulmonary leukocyte recruitment coincided temporally with the exacerbation of airway dysfunction (Fairbairn et al 1993). Scanning with labeled leukocytes is also particularly useful for investigating the effects of pro- or anti-inflammatory drugs on pulmonary leukocyte recruitment (Marr et al 1998).

A preliminary study suggests that a ^{99m}Tc-anti-granulocyte monoclonal antibody Fab′ fragment (LeukoScan®, Immunomedics Europe, Amsterdam, the Netherlands) could be valuable in investigating neutrophil recruitment to the equine lung (Votion et al 2000). Intravenously injected LeukoScan® particles bind to a granulocyte antigen and therefore can facilitate visualization of site(s) of granulocyte accumulation in the lung.

Human macrophages can be specifically labeled *in vivo* with a radiolabeled glycolipid, termed J001X, isolated from the membrane of *Klebsiella pneumoniae*. Administered as an aerosol, radioactive J001X binds selectively to macrophages, mainly in their activated state, by a protein-receptor-mediated process. In human medicine, scintigraphy with J001X facilitates the study of monocyte recruitment in lung diseases (Goupille et al 1995), but the technique remains to be evaluated in horses.

REFERENCES

Beck KC 1987 Regional trapping of microspheres in the lung correlates well with regional blood flow. Journal of Applied Physiology 63: 883–889

Burch WM, Sullivan PJ, McLaren CJ 1986 Technegas – a new ventilation agent for lung scanning. Nuclear Medicine Communications 7: 865–871

Coghe J, Votion D, Lekeux P 2000 Comparison between radioactive aerosol, Technegas and Krypton for ventilation imaging in healthy calves. Veterinary Journal 160: 25–32

Fairbairn SM, Page CP, Lees P et al 1993 Early neutrophil but not eosinophil or platelet recruitment to the lungs of allergic horses following antigen exposure. Clinical and Experimental Allergy 23: 821–828

Goupille P, Diot P, Valat JP et al 1995 Imaging of pulmonary disease in rheumatoid arthritis using J001X scintigraphy: preliminary results. European Journal of Nuclear Medicine 12: 1411–1415

Harmegnies NF, Duvivier DH, Vandenput SN et al 2002 Exercise-induced pulmonary perfusion redistribution in heaves. Equine Veterinary Journal 34(suppl): 478–484

Isawa, T, Lee BT, Hiraga K 1996 High-resolution electron microscopy of technegas and pertechnegas. Nuclear Medicine Communications 17: 147–152

Jarvis KA, Steffey EP, Tyler WS et al 1992 Pulmonary blood flow distribution in anesthetized ponies. Journal of Applied Physiology 72: 1173–1178

Marr KA, Lees P, Page CP et al 1998 Effect of the 5-lipoxygenase inhibitor, fenleuton, on antigen-induced neutrophil accumulation and lung function changes in horses with chronic obstructive pulmonary disease. Journal of Veterinary Pharmacology and Therapeutics 21: 241–246

Nelson R, Hampe DW 1983 Measurement of tracheal mucus transport rate in the horse. American Journal of Veterinary Research 44: 1165–1166

Ramzan PHL, Malton RJ, McGladdery AJ 2004 Use of ^{99m}technetium hexamethylpropyleneamine oxime labelled leucocyte scintigraphy in equine medical investigations: 8 cases. Equine Veterinary Education 16: 122–127

Taillefer R 2001 Radiolabelled peptides in the detection of deep venous thrombosis. Seminars in Nuclear Medicine 31: 102–123

Votion D, Vandenput S, Duvivier DH et al 1997 Analysis of equine scintigraphical lung images. Veterinary Journal 153: 49–61

Votion D, Vandenput S, Duvivier DH et al 1998 Scintigraphical evaluation of alveolar clearance in horses. Veterinary Journal 56: 51–58

Votion D, Ghafir Y, Vandenput S et al 1999a Analysis of scintigraphical lung images before and after treatment of horses suffering from chronic pulmonary disease. Veterinary Record 144: 232–236

Votion D, Vandenput S, Duvivier DH et al 1999b Alveolar clearance in horses with chronic obstructive pulmonary disease. American Journal of Veterinary Research 60: 495–500

Votion DM, Harmegnies NF, Lekeux PM 2000 Feasibility of monitoring neutrophils' migration into the lung with immunoscintigraphy: preliminary study. European Journal of Nuclear Medicine 27: 1089.

Votion DM, Lekeux P 2003 A dip into the world of veterinary nuclear medicine: equine lung scintigraphy. In: Birks E (editor) Clinical Techniques in Equine Practice. Elsevier, Oxford, pp.222–230

14 Blood Gas Analysis

R Eddie Clutton

Introduction

Technical advances have enabled modern blood gas analyzers to measure a range of hematological variables. Blood gas interpretation is also easier; advanced analyzer software has conferred some devices with primitive diagnostic abilities. Through text displays, some analyzers can prompt the minimally qualified to perform and report results – providing they can read the on-screen instructions. Reduced equipment size, increased robustness and the incorporation of fully rechargeable cells makes field use feasible, while the use of disposable cartridges has simplified calibration and improved reliability.

Applications

Arterial blood gas analysis is necessary for investigating pulmonary function because in measuring arterial tensions of O_2 (P_AO_2) and CO_2 (P_aCO_2) it quantifies the lung's ability to oxygenate and remove CO_2 from pulmonary arterial blood. Arterial pH (pH_a) and P_aCO_2 values also indicate the lung's influence on acid–base status. Blood gas determinations facilitate diagnosis and the management of respiratory diseases, such as maladaptive problems and pneumonias in foals and recurrent airway obstruction in adult horses. They are useful in all but the shortest general anesthesia, during which respiratory dysfunction is inevitable and occasionally severe. Serial arterial blood gas measurement is necessary for the proper management of positive pressure ventilation.

From pH and P_aCO_2 values, blood gas analyzers compute variables like plasma bicarbonate concentration [HCO_3^-] and base excess, which assist the diagnosis of metabolic disturbances, such as those occurring in gastrointestinal disease. Analysis of [HCO_3^-] can be used to estimate the duration of primary respiratory disturbances (see below). The analysis of both metabolic and respiratory function is important in exercise studies, including tolerance testing, and in the diagnosis and management of the mixed metabolic and respiratory disturbances that can occur in anesthetized horses with colic undergoing exploratory laparotomy.

Equipment

Technical notes

Improvements in blood gas analyzer performance have occurred because fluorescent optodes (optical electrodes) have replaced the traditional glass (pH), Clark (P_O_2) and Severinghaus (P_{CO_2}) electrochemical cells. Optodes measure the intensity of light emitted from fluorescent dyes in response to illumination from an excitatory light source. The quantity of light emitted is affected by the concentration of analyte (H^+ and O_2) to which the dye is exposed.

Equipment operation

Optode-based analyzers use single-use cassettes that contain the elements required for calibration, sample measurement, waste containment, and disposal. By physically isolating the blood sample from the analytical hardware, cassette-based systems avoid the single greatest limitation of older devices, namely equipment failure caused by aspiration of blood clots, which form in inadequately heparinized samples. Currently, a clotted sample only disables the cassette; and its prompt replacement delays analysis by no more than 2 or 3 min.

Depending on the analyzer, analysis often begins with the cassette package being "swiped" through a bar-code reader, which provides the software with cassette-specific calibration information. The cassette is then placed into the measurement chamber where it is warmed to $37°C$. Calibration verification is then performed on the P_{CO_2} and P_{O_2} sensors, by passing a precision calibration gas mixture across the optode sensors. The pH and electrolyte channels are calibrated with analytical grade buffer solution contained within the cassette. Once calibration is verified, the analyzer aspirates blood into the cassette and across the optode sensors. While measurement is in progress, the device prompts the supply of information such as body temperature, inspired O_2 concentration (F_IO_2), barometric pressure (P_B) and hemoglobin concentration [Hb], although some models measure P_B and [Hb] directly.

The analyzer measures pH and P_{CO_2} and its microprocessor derives dependent variables like [HCO_3^-], base

excess, and total CO_2. The oxygen optode measures and displays PO_2, which is used with supplied or co-measured data to calculate derivatives, such as the alveolar–arterial O_2 tension difference ($P_{(A–a)}O_2$) and percentage hemoglobin saturation. Once measurement is complete, the cassette and blood sample are removed from the analyzer and discarded. The detectors' output signal is displayed by liquid crystal as numerical data. The time taken for sample presentation, cassette calibration, sample analysis and result display varies between devices from 1 to 3 min, which far exceeds the needs for diagnosis, and suits rapid ventilator adjustment. The fixed temperature at which samples are analyzed (37°C) renders blood gas analyzers sensitive to environmental temperature extremes. Models operating under specific temperature ranges, such as 16–30°C, require insulating or cooling when operated in colder or warmer ambient temperatures (Silverman & Birks 2002).

Equipment availability

The purchase or leasing of modern "point-of-care" analyzers produced by reputable manufacturers is recommended for busy equine practices. Equipment that is regularly maintained and calibrated and protected from mechanical damage will perform reliably for years. The acquisition of second-hand laboratory desktop equipment is discouraged. Maintenance contracts, though strongly recommended because reliability is poor, are very expensive. Importantly, desktop devices cannot be used under field conditions and so blood samples must be returned, with altered values if examination is delayed, to the practice laboratory. Local hospitals with surgical/intensive care units will possess analysis equipment and may examine veterinary samples providing blood has been adequately heparinized. The inconvenience of transporting and submitting samples and waiting for results militates against this option for busy practitioners.

Sample Collection

Arterial blood collection is only marginally more difficult than venous blood collection. In conscious adult horses, samples may be taken from a number of sites (Table 14.1), with choice being based on personal experience and on the horse's size, temperament, and age. In foals, the dorsal metatarsal, brachial, and palmar arteries are easily punctured and the femoral artery may also be used.

Carotid artery

In adult horses, carotid arterial puncture is technically straightforward and well tolerated. The arteries run on the dorsolateral aspect of the trachea, medial to the external

jugular vein and adjacent to the recurrent laryngeal nerve and the vagosympathetic trunk. Sampling is performed in the distal third of the neck where the artery can often be palpated as a cord-like structure and fixed with the fingers of one hand while punctured with a 4.5 cm long 19- to 21-gauge needle held in the other. The needle is inserted at an angle to, and slightly above, the jugular vein to a depth of 1.25–2.5 cm. If the artery is impalpable, then a similarly sized needle is passed at a more cranial site through the midpoint of the jugular vein as it lies in the jugular furrow. The needle is directed in a dorsomedial direction to a depth of 3–4 cm. Accidental jugular puncture is readily distinguished from arterial penetration, because darker blood drips from the vein rather than flowing vigorously from the needle. The presence of pulsations before and after collection indicates the unlikelihood of inadvertent venous blood collection or contamination. Accidental damage to the recurrent laryngeal nerve or the vagosympathetic trunk is unlikely, but hematomas can occur especially if repeated sampling is performed at the same site (Willoughby & McDonell 1979). The carotid artery is "mobile" in foals and is consequently more difficult to puncture. The normally positioned carotid artery is unsuitable for cannulation.

Transverse facial artery

The transverse facial artery runs rostrally on the surface of the masseter muscle, about 1.5 cm ventral to the zygomatic arch and parallel to the axis of the palpebral fissure. Lying only a few millimeters below the skin, it is readily palpated and easy to puncture if the needle is advanced at a shallow angle over the artery and parallel to the axis of the rima palpebrarum. Unfortunately, the transverse facial vein runs in an adjacent parallel course and is equally easy to puncture. Hale & Chambers (1989) described severe bradycardia and hypotension in an anesthetized horse

Table 14.1. Arterial blood sampling sites in adult horses and foals
Adult horses
Common carotid artery
Transverse facial artery
Facial artery
Auricular artery
Dorsal metatarsal artery
Brachial or palmar (digital) arteries
Foals
Dorsal metatarsal artery
Brachial artery
Palmar artery
Femoral artery

that accompanied attempted catheterization of the transverse facial artery and which they attributed to accidental stimulation of the transverse facial nerve. Despite this, the artery is readily catheterized in adult horses, particularly after EMLA (eutectic mixture of local anesthetic) cream application (see below).

Facial artery

The facial artery is most suitable for blood collection in unconscious animals. It rounds the ventral border of the horizontal mandible (where it is readily palpated) and ascends along the rostral border of the masseter, before disappearing with the facial vein under the cutaneous faciei muscle. It is rostral to the vein and inadvertent puncture of the latter is uncommon. This artery is mobile in foals and more difficult to puncture, even in anesthetized subjects.

Auricular artery

The auricular artery is easy to puncture in small ponies, foals, and recumbent animals but access is more difficult in conscious tall animals or when the head cannot be immobilized. Once the convex surface of the pinna is clipped, the artery is identified as a straight vessel coursing approximately two-thirds the distance between the ear base and apex and closer to the medial than the lateral margin. It is readily distinguished from the more tortuous branches of the auricular vein, which are larger though less turgid and which do not pulsate. Applying a loose ligature to the ear base, which enlarges the veins while "damping" arterial pulsations, facilitates identification. This artery is easily catheterized in foals and adults under direct vision, especially after EMLA cream has been applied.

Dorsal metatarsal artery

The dorsal metatarsal artery descends the pelvic limb, becoming palpable in the oblique vascular groove on the dorsolateral aspect of the hock. It then descends superficially in the groove between the cannon and the lateral splint bone where it is readily palpated, immobilized, punctured, and catheterized if necessary. There are no adjacent veins that could be inadvertently sampled.

Brachial artery

The brachial artery, which courses distally on the medial surface of the brachium, is more superficial in foals than adults and has been recommended as a site for repeated sampling in foals. It is mobile in some foals, while others resent attempted puncture at this site vigorously unless local anesthetic is administered.

Palmar (digital) arteries

The palmar (digital) arteries are most easily palpated over the abaxial surfaces of the sesamoid bones. With the foreleg held up, the artery is punctured using a 2.5-cm long 23-gauge needle inserted parallel to the long axis of the leg (Rose & Rossdale 1981).

Puncture technique

The skin overlying the intended puncture site is clipped and surgically prepared. A 25- to 21-gauge needle and pre-heparinized (1 or 2 ml) syringe is advanced beneath that point where fingers of the other hand feel the maximum pulsation to be and along the vessel's axis. Mild negative pressure is applied to the syringe plunger, although larger needles, such as 19- to 21-gauge used with glass syringes, are filled by arterial pressure alone. Samples should be aspirated over three or four breaths when bradypnea is present, such as during anesthesia, because $P_{A}O_2$ values oscillate with breathing. If puncture is unsuccessful, the needle tip is withdrawn and redirected. Once sampling is completed, a sterile swab is applied with pressure over the puncture site and the needle is withdrawn. Firm pressure must be sustained for at least 5 min or until there is no evidence of hemorrhage.

Horses must not be stressed during arterial puncture otherwise hyperventilation will increase $P_{A}O_2$ and lower $P_{a}CO_2$ and confuse diagnosis. Calm, sympathetic handling is recommended. The application of local anesthetic ointment, such as EMLA cream, and then an occlusive polyethylene-lined dressing to the sampling site for 45–60 min desensitizes the skin and may prove beneficial. Alternatively, overlying skin may be infiltrated with non-irritant local anesthetic, such as mepivacaine. Sedatives may be necessary in restive animals. Acepromazine, a useful anxiolytic but an unpredictable sedative, is preferred over α_2-agonists because its effects on blood gas values are predictable and minor. In contrast, α_2-agonist drugs produce unpredictable blood gas changes (Table 14.2).

Arterial cannulation is recommended in animals requiring repeated samples, such as maladapted foals. Sedation is not necessary for this procedure in depressed youngsters. However, if struggling occurs, intravenous diazepam (10–20 mg) may be needed and O_2 can be provided by mask if clinical signs of dyspnea or hypoxemia appear. Animals should be restrained in sternal recumbency as repositioning in lateral recumbency may cause orthopnea and lower $P_{A}O_2$ (Kosch et al 1984). If struggling continues, a brief period of inhalation anesthesia with sevoflurane, halothane or isoflurane delivered by mask may be necessary, though this is not without risk. The dorsal metatarsal artery may be catheterized using a "cut-down" procedure, although the auricular artery is a more convenient site,

Table 14.2. The effects of commonly used sedatives on blood gas values in horses and ponies			
Drug	**Dose**	**Effect**	**Reference**
Azaperone	0.4–0.8 mg/kg IM	No effect on pH, P_aCO_2, P_AO_2	Lees & Serrano 1976
Acepromazine	0.65 mg/kg IV	Reduced response to severe hypoxemia and hypercapnia	Muir & Hamlin 1975
Diazepam	0.05–0.4 mg/kg IV	No significant changes in P_aCO_2 or P_AO_2	Muir et al 1982
Xylazine	0.5 mg/kg IV	Altered respiratory patterns	Lavoie et al 1992
	0.6 mg/kg IV	Significant reduction on f_R; insignificant decrease in P_AO_2	Reitemeyer et al 1986
	1.1 mg/kg IV	Altered respiratory patterns; apneic periods of 7–70 seconds; significant increases in P_aCO_2 and decreases in P_AO_2; reduced f_R, V_M	Lavoie et al 1992
	1.1 mg/kg IV	Reduced f_R; unchanged P_aCO_2; transiently and significantly reduced P_AO_2 [1.33–2.66 kPa (10–20 mmHg)]	Wagner et al 1991
Detomidine	10 µg/kg IV	Reduced f_R; unchanged P_aCO_2; transiently and significantly reduced P_AO_2 [1.33–2.66 kPa (10–20 mmHg)]	Wagner et al 1991
	20 µg/kg IV	Reduced f_R; unchanged P_aCO_2; transiently and significantly reduced P_AO_2 [1.33–2.66 kPa (10–20 mmHg)]	Wagner et al 1991
	Up to 40 µg/kg	Significant decrease in P_AO_2	
Medetomidine	5 µg/kg IV 10 µg/kg IV	Significant increases in P_aCO_2; insignificant decreases in P_AO_2 Significant increases in P_aCO_2; insignificant decreases in P_AO_2	Bryant et al 1996 Bryant et al 1996
Romifidine	80 µg/kg IV	Significant decreases in P_AO_2 5 min after injection Significant elevations in P_aCO_2 90 min after injection	Clarke et al 1991

f_R = respiratory rate, V_M = minute ventilation.

using 20- to 22-gauge catheters. Arterial catheters do not remain patent for much longer than 12–48 h unless continuous (rather than intermittent) flushing with heparinized saline is performed.

It is widely recommended that the rectal temperature be taken in animals in which it is likely to deviate from normal by more than 0.556°C (1°F), and appropriate corrections made to the analysis. These measures are deemed necessary because the analyzer, in maintaining blood temperature at 37°C during measurement, alters gas solubility and tension in samples drawn from animals at different temperatures. For example, body temperature reductions of 1°C decrease pH by 0.014 units, increase CO_2 solubility and lower P_aCO_2 (Hodgson 1987). The relationship between temperature and P_AO_2 is complex because temperature affects the oxyhemoglobin dissociation curve. If temperature corrections are not made then the high blood temperatures encountered during exercise (> 40°C) will yield spuriously low readings for P_AO_2 and P_aCO_2 while pH values will be excessive (Jones et al 1989). In contrast, blood from hypothermic foals will give high readings for P_AO_2 and P_aCO_2 and report low pH values (Hodgson 1987). However, there is controversy over the need for temperature correction in the medical intensive care setting (Shapiro 1995) with opponents pointing out the absence of a logical or scientific basis for the assumption that temperature-corrected values are better than values obtained at 37°C.

Sample Storage and Handling

Samples are drawn into 2-ml glass or plastic syringes whose hub is filled with 1000 IU/ml heparin. Excessive anticoagulant is undesirable because it lowers pH. Commercially available blood gas syringes containing freeze-dried heparin and supplied with a gas-tight cap for use when analysis is delayed are ideal, though expensive. Air bubbles must be eliminated from the sample and the syringe must be sealed from air using either a proprietary syringe cap or a needle whose tip is embedded in rubber. Otherwise air contamination will lower PCO_2 and alter PO_2 levels. When appreciable analysis delays (> 30 min) are anticipated, glass syringes should be used and placed on ice after sampling. Previous recommendations regarding equine blood storage conditions for blood gas analysis have erroneously relied on data from other species. Deane et al (2004) studied the effects of storage conditions on equine blood gases; their findings are summarized in Table 14.3.

	Storage temp.	pH	Po_2	Pco_2
Table 14.3. The effects of storage temperature, storage duration and syringe material on arterial blood gas variables in equine blood				
Glass	20°C*	Significant fall at 60 min	Significant fall by 60 min	Falls significantly by 120 min
	0°C†	Significant fall at 120 min	Significantly rise at 60 min, significant fall in 120 min	Falls significantly by 120 min
Plastic (Monoject)	20°C	Significant fall at 60 min	Unaffected at 10 min, falls significantly in 60 min	Falls significantly by 120 min
	0°C	Significant fall at 120 min	Unaffected at 10 min, significant rise at 60 min	Falls significantly by 120 min
Polypropylene (QS-50)	20°C	Significant fall at 60 min	Unaffected at 10 min, falls significantly in 60 min	Falls significantly by 120 min
	0°C	Significant fall at 120 min	Unaffected at 10 min, significant rise at 60 min	Falls significantly by 120 min

Samples were analyzed at 10, 60 and 120 min. From Deane et al 2004, with permission.
* Room temperature; † iced water.

Table 14.4. Some causes of blood pH changes in horses and foals			
			Examples
Acidosis	Metabolic	Loss of HCO_3^-	Chronic diarrhea; renal failure; saline overinfusion; secondary to chronic respiratory alkalosis
		Gain of H^+	Lactacidemia, e.g. anaerobic exercise, hypovolemic "shock"; renal failure; secondary to chronic respiratory alkalosis
	Respiratory	Elevated Pco_2	Hypoventilation: (1) neurological (drugs, hypothermia, maladaptive syndromes), (2) obstructive (laryngeal paralysis), (3) mechanical (chest wall injury, diaphragmatic hernia). Elevated metabolism
Alkalosis	Metabolic	Gain of HCO_3^-	Iatrogenic overadministration of HCO_3^-; secondary to chronic respiratory acidosis
		Loss of H^+	Gastrointestinal acid sequestration; secondary to chronic respiratory acidosis
	Respiratory	Reduced Pco_2	Psychogenic hyperventilation, e.g. anxiety, pain; secondary to hypoxemia, e.g. altitude, intra- and extra-cardiac "shunts"

Information Provided by Blood Gases and pH

The interpretation of blood gas values is not always an "exact science". If this were the case then modern analyzers would readily provide their own diagnosis. Consequently, attempts to interpret blood gas values should always be made with regard to the subject's medical history and the findings of physical examination, and not attempted *de novo*.

pH

The blood pH is the negative logarithm (base 10) of the hydrogen ion (proton) concentration [H^+] and is a convenient means of expressing [H^+] or acidity. Blood pH is of major physiological importance because [H^+] is a principal determinant of enzyme activity. One consequence of this logarithmic relationship is that small pH changes represent large changes in acidity. A "normal" pH of 7.4 corresponds to a [H^+] of 40 nmol/liter while a pH of 7.1 represents twice this concentration (80 nmol/liter). Minor pH deviations outwith the "accepted" normal range (7.35–7.45) represent a significant [H^+] change, which deserves enthusiastic management.

When describing acid–base changes, the suffix -emia refers to blood pH. Acidemia exists when pH values are <7.35; pH values >7.45 represent alkalemia. The suffix -osis describes respiratory or metabolic processes which act with a propensity to change blood pH but without necessarily prevailing. The principal causes of pH changes are described in Table 14.4.

"Safe" pH values for equids have not been established, but ranges of 6.9–7.8 are cited for other mammals. Arterial blood gas analysis measures extracellular pH, which may not reflect intracellular $[H^+]$. Thus, metabolic acidosis arising within cells may not immediately induce extracellular pH changes. In contrast, because CO_2 is rapidly diffusible, respiratory-induced pH changes rapidly become established in the intracellular environment.

Arterial carbon dioxide tension (P_aCO_2)

The arterial CO_2 tension (PCO_2) reflects the balance between metabolic CO_2 production ($\dot{V}CO_2$) and its elimination by the lungs. The latter depends on the minute alveolar ventilation ($\dot{V}_A$). When $\dot{V}CO_2$ is constant, P_aCO_2 reliably indicates the adequacy of minute alveolar ventilation (equation 1). Halving $\dot{V}_A$ doubles P_aCO_2.

Equation 1. P_aCO_2 (in kPa) $= \dot{V}CO_2/\dot{V}_A \times (P_B - 6.25) \times 0.83$

A second version (equation 2) is more suitable for use during anesthesia because by incorporating F_ICO_2 it recognizes rebreathing (the re-aspiration of expired CO_2) as a possible cause of hypercapnia.

Equation 2. P_aCO_2 is proportional to $F_ICO_2 + \dot{V}CO_2/\dot{V}_A$

Hypercapnia from rebreathing is possible when foals receive inadequate O_2 flows delivered with oversized facemasks. These equations indicate that hypercapnia $[P_aCO_2 > 5.85 \text{ kPa} (44 \text{ mmHg})]$ results from:

- decreased alveolar ventilation, such as results from profound sedation, anesthesia, severe neurological depression, hypothermia; severe respiratory disease
- increased CO_2 production, such as occurs with pyrexia
- both of above.

The mean normal P_aCO_2 in adult horses is 5.33 kPa (40 mmHg) but P_aCO_2 ranges from 4.79 to 6.13 kPa (36–46 mmHg). Values in foals are high at birth but then fall, approaching normal levels within a week or so (Table 14.5). Hypercapnia is usually a late feature in respiratory disease, its presence indicating the onset of respiratory failure. This is because CO_2 is (1) the normal determinant of ventilation, and so hypercapnia elevates $\dot{V}_A$ and resolves itself, and (2) readily diffusible across the alveolar–capillary membrane. However, hypercapnia does occur in chronic respiratory diseases including recurrent airway obstruction (Gillespie et al 1964), where elevated CO_2 levels represent a compromise between low plasma pH and a metabolically unaffordable work of breathing. P_aCO_2 values have been linked with survival prediction in induced, premature foals (Rose et al 1982).

Breathing 100% O_2 does not influence P_aCO_2. Indeed, when hypoxemic animals with chronic respiratory disease breathe high concentrations of O_2 it is not uncommon for ventilation to transiently cease, causing rapid and severe rises in P_aCO_2. This occurs because in chronic severe hypoxemia, P_AO_2, rather than P_aCO_2, becomes the principal determinant of $\dot{V}_A$, and when O_2 is provided, the surrogate respiratory stimulus is effectively removed.

Hypocapnia (respiratory alkalosis) is present when $P_aCO_2 < 4.78 \text{ kPa} (36 \text{ mmHg})$. It normally reflects hyperventilation in response to stress or pain, or overenthusiastic positive pressure ventilation. When severe cardiopulmonary disease causes hypoxemia, P_AO_2 may become the principal determinant of ventilation, in which case $\dot{V}_A$ may become excessive with respect to CO_2, causing hypocapnia. The same occurs when animals are moved from low to high altitudes without acclimatization; hypoxemia resulting from low P_IO_2 initiates hyperventilation and causes respiratory alkalosis (Greene et al 1999). Increased $\dot{V}_A$ and hypocapnia are the common initial responses to hypoxemia in foals. However, a failure of P_AO_2 to improve with time and O_2 therapy, coupled with rising P_aCO_2, heralds imminent respiratory failure and indicates the need for positive pressure ventilation or euthanasia. Hyperventilation is a normal rapid physiological response to primary metabolic acidosis, and serves to increase pH in the face of high plasma acid levels, such as occur after anaerobic exercise, or low plasma $[HCO_3^-]$ as occurs in severe diarrhea. In rare circumstances, hypocapnia can indicate reduced metabolic CO_2 production, as occurs in severely hypothermic, non-shivering foals.

Table 14.5. Arterial blood gas values from horses and foals (mean ± SEM)					
	P_AO_2			P_aCO_2	
Age	kPa	mmHg		kPa	mmHg
Birth	5.72 ± 0.52	43.0 ± 3.9		7.05 ± 0.24	53 ± 1.8	*
15 min	8.55 ± 0.52	64.3 ± 3.9		6.34 ± 0.17	47.7 ± 1.3	*
30 min	9.31 ± 0.57	70.0 ± 4.3		6.29 ± 0.08	47.3 ± 0.6	*
60 min	10.35 ± 0.69	77.8 ± 5.2		5.82 ± 0.16	43.8 ± 1.2	*
12 h	10.53 ± 0.53	79.2 ± 4.0		5.55 ± 0.13	41.7 ±1.0	*
24 h	11.33 ± 0.78	85.2 ± 5.9		5.69 ± 0.09	42.8 ± 0.7	*
1 week	10.64 ± 0.98	80 ± 7.4		5.61 ± 0.28	42.2 ± 2.1	*
Adult	10.64–14.89	80–112		4.79–6.12	36–46	†

*Rose et al 1982; †Brobst & Parry 1987.

Secondary acid–base effects of CO_2

Values of pH_a and $[HCO_3^-]$ elucidate the duration of respiratory changes and indicate whether they are primary or compensatory changes for primary metabolic disorders. The pH_a falls in hypercapnia because CO_2 forms carbonic acid (H_2CO_3) in plasma (equation 3).

Equation 3. $H_2O + CO_2 \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$
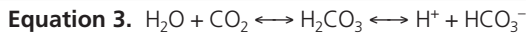
However, the magnitude of the pH fall depends on increases in plasma HCO_3^-. These are initially rapid (occurring within seconds) and result from H_2CO_3 dissociation. This corresponds with an initial rapid rise in $[HCO_3^-]$ of 1 mmol/liter per 1.33 kPa (10 mmHg) rise in P_aCO_2. Thereafter, $[HCO_3^-]$ continues to rise, but at a slower rate because renal tubular HCO_3^- reabsorption and H^+ secretion become active. In foals, renal compensation begins in approximately 6–12 h and reaches a maximum in 3–5 days. Renal compensation eventually increases plasma $[HCO_3^-]$ by 2 mmol/liter per 1.33 kPa (10 mmHg) rise in P_aCO_2. Consequently chronic respiratory acidosis is indicated by a rise in $[HCO_3^-]$ of 3 mmol/liter per 1.33 kPa (10 mmHg) rise in P_aCO_2.

In respiratory alkalosis, the immediate response of the bicarbonate buffer system is to increase plasma CO_2 levels. At the same time, the glycolytic pathway is activated and produces lactic acid. These processes acutely reduce plasma $[HCO_3^-]$ by about 2 mmol/liter per P_aCO_2 decrement of 1.33 kPa or 10 mmHg below normal. Eventually, the proximal convoluted tubules cease to reabsorb HCO_3^-. The net reduction in HCO_3^- resulting from these responses is approximately 5 mmol/liter per 1.33 kPa (10 mmHg) reduction in P_aCO_2 below 5.33 kPa (40 mmHg).

Arterial oxygen tension (P_AO_2)

P_AO_2 is the most sensitive measure of the lung's ability to oxygenate blood and consequently the best index of deteriorating pulmonary function. Most adult horses with recurrent airway obstruction have reduced P_AO_2 while pH_a or P_aCO_2 are rarely changed (Dixon et al 1995). Normal values in adults breathing air are 11.99–13.3 kPa (90–100 mmHg). Values in normal term foals vary considerably, and depend on the gestational and postnatal age (Rose et al 1982) and body position, with P_AO_2 values being higher in foals in sternal, rather than lateral, recumbency (Kosch et al 1984). A degree of hypoxemia and respiratory acidosis by adult standards is present after birth, but usually resolves by 12–24 h in full-term foals and slightly longer in preterm foals (Rose & Rossdale 1981). Impaired oxygenation is inevitable during general anesthesia in horses (Whitehair & Willits 1999) as $P_{(A–a)}O_2$ gradients are always increased (Stegman 1986, Thurmon 1990).

However, interpretation of P_AO_2 demands knowledge of the fractional inspired concentration of oxygen (F_IO_2) at sampling, the P_aCO_2, and to a lesser extent, the atmospheric pressure (P_B). This is because these factors are incorporated into the alveolar gas equation (equation 4), which predicts alveolar O_2 tension (P_AO_2), which in turn is the single most important determinant of P_AO_2 under non-pathological conditions.

Equation 4. $P_AO_2 = F_IO_2 (P_B − P_{H_2O}) − P_aCO_2/0.8$

The normal value for P_B at sea level is 101.3 kPa (760 mmHg). (Some blood gas analyzers incorporate electronic barometers.) P_{H_2O} is the saturated vapor pressure of water, which is 6.25 kPa (47 mmHg) at 37.5°C. The value for P_aCO_2 is taken from the blood gas analysis. The value 0.8 is the respiratory quotient (see Chapter 4 for details).

This complex approach is necessary because normal or elevated P_AO_2 values may be found when animals with severe respiratory disease breathe O_2-enriched gases (see below). During air breathing, $F_IO_2 = 0.21$. However, 100% O_2 delivered by tracheal or nasal insufflation, or from anesthetic breathing systems, does not always correspond with an F_IO_2 of 1.0, because O_2 may be diluted by atmospheric nitrogen, rebreathed CO_2 or methane. Oximeters measure and display the inspired and expired O_2 values on a breath-for-breath basis and circumvent this problem. However, whilst available and affordable, their use is not widespread.

Alveolar–arterial oxygen tension difference ($P_{(A–a)}O_2$)

In healthy horses breathing air, the normal $P_{(A–a)}O_2$ is approximately 0.66–1.33 kPa (5–10 mmHg) and represents normal arterial blood dilution by Thebesian and bronchial venous blood entering the left heart and pulmonary veins respectively. Abnormal elevations in $P_{(A–a)}O_2$ disclose and quantify imperfect gas exchange in animals which, through breathing high concentrations of O_2, may have normal or even elevated P_AO_2 levels.

The $P_{(A–a)}O_2$ is calculated from the alveolar gas equation (equation 4). For this, the P_B, F_IO_2, P_aCO_2, and the respiratory quotient should be known. The measured P_AO_2 is then subtracted from P_AO_2. For a horse with normal respiratory function [$P_aCO_2 = 5.3$ kPa (40 mmHg) and $P_AO_2 = 12.6$ kPa (95 mmHg)], breathing room air ($F_IO_2 = 0.21$) at sea-level [$P_B = 101$ kPa (760 mmHg)], P_AO_2 is given by:

Example 1. $P_AO_2 = 0.21 (101 − 6.25) − (5.3/0.8)$
$= 19.8975 − 6.625$
$= 13.3$ kPa (or 100 mmHg)
$P_{(A–a)}O_2 = 13.3 − 12.6$
$= 0.7$ kPa (5.3 mmHg)

which is normal. Increased $P_{(A–a)}O_2$ has three main causes in air-breathing animals. The most important, venous

admixture, arises from "shunt" and/or from blood draining alveolar units with low ventilation/perfusion ratios (see Chapter 4). The two are differentiated by providing an O_2-rich (>90%) mixture to breathe, which eliminates the hypoxemic effects of $\dot{V}_A/\dot{Q}$ disturbances and exposes "shunt" flow. Consequently, breathing 100% O_2 improves P_AO_2 in horses with recurrent airway obstruction but will not affect cyanosis in foals with congenital right-to-left cardiac anomalies, widespread pulmonary atelectasis, consolidation or a persistent fetal circulation. $P_{(A-a)}O_2$ values are also increased by reduced mixed venous O_2 tensions (see below) and diffusion limitation, though the latter is considered unimportant in humans and horses. The measured $P_{(A-a)}O_2$ value also increases as F_IO_2 rises for any given $\dot{V}/\dot{Q}$ situation, and it is imperative that the F_IO_2 be accounted for when comparisons are made. For example, when an animal breathes 100% oxygen (F_IO_2 = 1.0), P_AO_2 should rise to 88.1 kPa (663 mmHg), as shown in Example 2.

> *Example 2.* $P_AO_2 = 1.0 (101 - 6.25) - (5.3/0.8)$
> $= 94.75 - 6.625$
> $= 88.1$ kPa (or 663 mmHg)

If an arterial blood sample taken from this animal indicated P_AO_2 was 13.3 kPa (100 mmHg) the presence of satisfactory lung function might be assumed. However, calculating $P_{(A-a)}O_2$ reveals a value of 74.8 kPa (563 mmHg) indicating considerable venous admixture and severe dysfunction.

Tables are available for the conversion of P_AO_2 and $P_{(A-a)}O_2$ into estimated shunt flow. Alternatively, iso-shunt diagrams may be used (Benatar et al 1973). As a rule, when P_AO_2 exceeds 20 kPa (150 mmHg) and hemoglobin is fully saturated, the venous admixture is approximately 1% of cardiac output for every 2.66 kPa (20 mmHg) of $P_{(A-a)}O_2$.

Hypoxemia, that is inadequate O_2 tension in arterial blood, is defined as a P_AO_2 less than 7.98 kPa (60 mmHg). This value corresponds to the "shoulder" of the oxyhemoglobin dissociation curve, to the left of which small reductions in P_AO_2 cause large falls in hemoglobin saturation. "Hypoxia" describes subnormal O_2 tensions at a specified level, for example cerebral or tissue hypoxia. This can result from hypoxemia but can also occur with normal arterial O_2 tensions when tissue perfusion is inadequate, when inadequate levels of functional hemoglobin reduce the blood's O_2 content, or when tissue O_2 demand is abnormally high and exceeds delivery. The causes of hypoxia are described in Chapter 4.

Venous oxygen tension (P_vO_2)

In rare circumstances, venous blood samples can be used when arterial blood is unavailable. During anesthesia, cutaneous vasodilatation and hyperemia cause peripheral venous blood gas values to approach arterial levels,

increasing the confidence with which P_vCO_2 and pH_V values may be used as accurate estimates of ventilatory adequacy and acid–base status. Peripheral venous PO_2 does not accurately indicate P_AO_2, although if P_vO_2 values are greater than 7.98 kPa (60 mmHg) it can be assumed that arterial hypoxemia is absent.

Venous blood drains into, and is mixed in, the pulmonary artery, and its O_2 tension reflects whole body O_2 consumption ($\dot{V}O_2$) and cardiac output. The normal value of P_vO_2, 5.33 kPa (40 mmHg) is reduced by (1) arterial hypoxemia; (2) low cardiac output; and (3) if "whole body" O_2 consumption increases. Increased rectal temperature and other signs, such as cardiovascular hyperdynamism, indicate an increased metabolic rate. When these are absent, P_vO_2 is directly proportional to cardiac output. However, pulmonary arterial blood sampling is not feasible in general practice because cardiac catheterization is required.

Jugular venous O_2 tensions, which are easily obtained, may have value as premonitory indicators of cardiac arrest in anesthetized horses (McGoldrick et al 1998). It is known that while jugular venous O_2 tensions are always 0.66–1.33 kPa (5–10 mmHg) higher than mixed venous tensions, both correlate positively with cardiac output. In horses, mixed venous O_2 tension of 5.33–5.99 kPa (40–45 mmHg) corresponds with critically low values for cardiac output (Wetmore et al 1987). In humans, a P_vO_2 value of 3.99 kPa (30 mmHg) is held to indicate inadequate tissue oxygenation and is associated with a high risk of acute cardiac failure. In foals, a jugular venous PO_2 < 20 mmHg has been held to indicate the onset of hypoxemia or cardiac failure (Kosch et al 1984). Venous PCO_2 values greater than 7.99 kPa (60 mmHg) indicate the presence of hypercapnia.

When collecting venous samples for the investigation of metabolic or blood gas data, blood flow through the vein should not be restricted because this lowers values for P_vO_2 and pH while increasing P_vCO_2.

Arteriovenous oxygen content differences

Respiratory dysfunction resulting from left heart dysfunction is not uncommon in foals. The adequacy of cardiac output relative to whole body oxygen consumption ($\dot{V}O_2$) is estimated by calculating the arterial and mixed venous oxygen content ($C_{(a-v)}O_2$) difference. This requires taking arterial and mixed venous blood samples simultaneously. The value of $C_{(a-v)}O_2$ – traditional units are ml/dl – is calculated from:

$$C_{(a-v)}O_2 = \{([Hb] \times S_aO_2 \times 1.39) + (P_AO_2 \times 0.0225)\} - \{([Hb] \times S_vO_2 \times 1.39) + (P_vO_2 \times 0.0225)\}$$

where $C_{(x)}O_2$ is oxygen content of arterial (a) and venous (v) blood, [Hb] is hemoglobin concentration in g/dl (normal values are 12–15 g/dl), 1.34 indicates ml O_2 bound per g of hemoglobin, $S_{(x)}O_2$ percentage saturation of hemoglobin

in arterial (a) and venous (v) blood. Arterial P_{O_2} values of 13.3 kPa (100 mmHg) correspond to an $S_{a}O_2$ of 1.0. For venous blood, in which $P_{v}O_2$ is 5.3 kPa (40 mmHg), the value of $S_{v}O_2$ is 0.75. The coefficient 0.0225 indicates the solubility of oxygen dissolved in plasma; units are $ml \cdot dl^{-1} \cdot kPa^{-1}$. Normal $C_{(a-v)}O_2$ is 4–6 ml/dl. When whole body oxygen consumption is constant, decreased cardiac output, prolonged circulation time or sluggish perfusion increases the $C_{(a-v)}O_2$ value. Conversely, increased $C_{(a-v)}O_2$ indicates a hypermetabolic state when cardiac output is stable.

REFERENCES

Benatar SR, Hewlett AM, Nunn JF 1973 The use of iso-shunt lines for control of oxygen therapy. British Journal of Anaesthesia 45: 711–718

Brobst D & Parry BW 1987 Normal clinical pathology data. In: Robinson NE (editor) Current Therapy in Equine Medicine, volume 2. WB Saunders, Philadelphia, pp.725–729

Bryant CE, Clarke KW, Thompson J 1996 Cardiopulmonary effects of medetomidine in sheep and in ponies. Research in Veterinary Science 60: 267–271

Clarke KW, England GCW, Goossens L 1991 Sedative and cardiovascular effects of romifidine, alone and in combination with butorphanol, in the horse. Journal of Veterinary Anaesthesia 18: 25–29

Deane JC, Dagleish MP, Benamou AEM et al 2004 Effects of syringe material and temperature and duration of storage on the stability of equine arterial blood gas variables. Veterinary Anaesthesia and Analgesia 4: 250–257

Dixon PM, Railton DI, McGorum BC 1995 Equine pulmonary disease: a case–control study of 300 referred cases. Part 3: Ancillary diagnostic findings. Equine Veterinary Journal 27: 428–435

Gillespie JR, Tyler WS, Eberly VE 1964 Blood pH, O_2 and CO_2 tensions in exercised control and emphysematous horses. American Journal of Physiology 207: 1067–1072

Greene HM, Wickler SJ, Anderson TP et al 1999 High-altitude effects on respiratory gases, acid–base balance and pulmonary artery pressures in equids. Equine Veterinary Journal Supplement 30: 71–76

Hale GJ, Chambers JP 1989 Bradycardia and asystole following attempted arterial cannulation in a horse under general anaesthesia. Journal of the Association of Veterinary Anaesthetists 16: 10–11

Hodgson DR 1987 Blood gas and acid–base changes in the neonatal foal. Veterinary Clinics of North America; Equine Practice 3: 617–629

Jones JH, Taylor CR, Lindholm A et al 1989 Blood gas measurements during exercise: errors due to temperature correction. Journal of Applied Physiology 67: 879–884

Kosch PC, Koterba AM, Coons TJ 1984 Developments in management of the newborn foal in respiratory distress 1: Evaluation. Equine Veterinary Journal 16: 312–318

Lavoie JP, Pascoe JR, Kurpershoek CJ 1992 Effect of head and neck position on respiratory mechanics in horses sedated with xylazine. American Journal of Veterinary Research 53: 1653–1657

Lees P, Serrano L 1976 Effects of azaperone on cardiovascular and respiratory functions in the horse. British Journal of Pharmacology 56: 263–269

McGoldrick TME, Bowen IM, Clarke KW 1998 Sudden cardiac arrest in an anaesthetised horse associated with low venous oxygen tensions. Veterinary Record 142: 610–611

Muir WW, Hamlin RL 1975 Effects of acetylpromazine on ventilatory variables in the horse. American Journal of Veterinary Research 36: 1439–1442

Muir WW, Sams RA, Huffman RH, Noonan JA 1982 Pharmacodynamic and pharmacokinetic properties of diazepam in horses. American Journal of Veterinary Research 43: 1756–1762

Reitemeyer H, Klein HJ, Deegen E 1986 The effect of sedatives on lung function in horses. Acta Veterinaria Scandinavica 82: 111–120

Rose RJ, Rossdale PD 1981 Techniques and clinical application of arterial blood collection in the horse. Equine Veterinary Journal 3: 70–73

Rose RJ, Rossdale PD, Leadon DP 1982 Blood gas and acid–base status in spontaneously delivered term-induced and induced premature foals. Journal of Reproduction and Fertility Supplement 32: 521–528

Shapiro BA 1995 Temperature correction of blood gas values. Respiratory Care Clinics of North America 1: 69–76

Silverman SC, Birks EK 2002 Evaluation of the i-STAT hand-held chemical analyser during treadmill and endurance exercise. Equine Veterinary Journal Supplement 34: 551–554

Stegman GF 1986 Pulmonary function in the horse during anaesthesia. A review. Journal of the South African Veterinary Association 57: 49–53

Thurmon JC 1990 General clinical considerations for anesthesia in the horse. Veterinary Clinics of North America; Equine Practice 6: 485–494

Wagner AE, Muir WW, Hinchcliff KW 1991 Cardiovascular effects of xylazine and detomidine in horses. American Journal of Veterinary Research 52: 651–657

Wetmore LA, Derksen FJ, Blaze CA et al 1987 Mixed venous oxygen tension as an estimate of cardiac output in anesthetized horses. American Journal of Veterinary Research 48: 971–976

Whitehair KJ, Willits NH 1999 Predictors of arterial oxygen tension in anesthetized horses: 1,610 cases (1992–1994). Journal of the American Veterinary Medical Association 215: 978–981

Willoughby RA, McDonell WN 1979 Pulmonary function testing in horses. Veterinary Clinics of North America; Large Animal Practice 1: 171–196

15 Pulmonary Function Testing

David J Marlin and Christopher M Deaton

Objective assessment of pulmonary function can be useful in the diagnosis and prognosis of disease and in the monitoring of response to treatment, and is important in understanding normal lung function and the mechanisms of disease. In equine medicine, pulmonary function tests (PFT) have remained the domain of veterinary schools, research institutes, and referral practices. In human medicine the situation is very different. Measurements that accurately reflect the functional state of the lung, especially peak flow and the forced expiratory volume in one second (FEV_1), can be routinely measured with devices that are inexpensive, accurate, and widely available. Virtually no calibration or preparation is required. Measurement of these simple indices of pulmonary function is possible in people because they cooperate with the "forced" maneuvers. Because horses cannot cooperate, measuring pulmonary function in the horse is more aligned to the situation in infants than in adults.

Pulmonary function tests include measurements of lung volumes, tests of mechanical function, and tests of gas exchange. Each of these can be measured in the resting or exercising horse or when breathing is stimulated by use of drugs.

Indications for Measurement of Pulmonary Function

When managing a sick horse, the clinician needs to know the extent and severity of the functional impairment of the lung. This can be most easily assessed by evaluation of blood gases (see Chapter 14). In addition, understanding the type of functional abnormality can be a diagnostic clue. For example, in a horse with obvious respiratory distress of some duration, a finding of normal resistance to airflow would rule out a diagnosis of recurrent airway obstruction (RAO). If that observation was coupled with a low value for lung compliance, the horse is likely to have a restrictive lung disease such as pulmonary fibrosis. Unfortunately, the range of normal values for most PFTs in horses is quite large. For this reason, a single measurement of lung function may not be diagnostic unless the changes in the lung are severe. However, PFTs are useful to follow the course of disease because the day-to-day variation in lung function within an individual horse is not so great.

A progressive change in a variable lung function should be viewed as significant. To accurately follow changes in function within a horse, the factors that can affect measurements must be carefully controlled.

General Guidelines for Pulmonary Function Testing

Factors that may affect the values obtained from PFT include diurnal variation (Stadler & Deegen 1986), time of feeding (McDonell et al 1979), sedation, head position (Lavoie et al 1992), excitement, the type of measuring equipment, and the duration of the measurements. Equipment must not impose significant resistance nor significantly increase dead space. Sedation has many advantages for undertaking PFT in horses. It facilitates the placement of facemasks or esophageal catheters; reduces the mechanical noise arising from movement of the measuring devices; makes breathing more regular; reduces the reaction of the horse to equipment noise or to inhalation of bronchoconstrictor agents such as histamine; and allows standardization of the head and neck angle. The latter is an important variable in measurement of total pulmonary resistance because marked flexion of the neck increases resistance (Lavoie et al 1992). Unfortunately, upper airway resistance increases over time following sedation (Tomasic et al 1997), most likely as a result of congestion of the upper airways and relaxation of its dilator muscles. This is especially exaggerated if the head position is low.

Commonly used equine sedatives include the α_2-adrenoceptor agonists such as xylazine, detomidine, and romifidine. Xylazine reduces pulmonary resistance and increases pulmonary compliance in RAO-affected horses during clinical exacerbation (Broadstone et al 1992) by inhibiting acetylcholine release from cholinergic nerves in the airways (LeBlanc et al 1993). However, xylazine has no effect on RAO-affected horses in clinical remission or in healthy horses because smooth muscle is already relaxed. Because part of the effect of histamine is mediated via the parasympathetic nervous system, α_2-adrenoceptor agonists have the potential to inhibit the response to histamine. This effect may cause erroneous measurements of airway responsiveness.

Measurements to Evaluate the Mechanical Properties of Lungs and Airways

Pulmonary function tests can be used to assess the volume of air being breathed, the effort being used to ventilate the lung and its mechanical properties, gas exchange and its component parts such as diffusion and ventilation/perfusion matching, and the distribution of air and blood within the lung. As with most diagnostic tests, the simpler the measurement, the less information obtained. Because the lung has such a large functional reserve, simple measurements of lung function made in the resting horse may show little abnormality even when the horse has extensive disease.

When considering what to measure to assess the mechanical function of the lung, tidal volume immediately comes to mind. Because of their need to survive, animals tend to maintain their tidal volume even when lung disease is quite severe. However, they do this by generating more effort with their respiratory muscles and this effort is reflected in the magnitude of the pressure change in the pleural cavity. What is important to the horse is how much ventilation it is obtaining for a given amount of effort. For this reason, many measurements of the mechanical properties of the lung incorporate measurement of pleural or esophageal pressure, the latter being a good approximation of the former and far easier to measure.

The maximal change in pleural pressure during tidal breathing ($\Delta P pl_{max}$) is the simplest measurement of the mechanical properties of the lungs. When coupled with measurement of flow, this can be used to determine the total pulmonary resistance (R_L), which is an indicator of the degree of obstruction of the airways. Addition of a measure of tidal volume allows calculation of dynamic compliance (C_{dyn}). The latter reflects the elastic properties of the lungs and the magnitude of obstruction of the small peripheral airways.

In a continuing search for a more sensitive yet simple test of pulmonary function, veterinary scientists have adapted most tests used in humans for use in horses.

Forced oscillatory mechanics measurements offer the advantage that they can be superimposed on normal breathing and do not need an esophageal catheter. With more development, these measurements may be useful in studies conducted in the field. By contrast, measurements derived from forced expiration offer sensitivity but are not practical for large-scale use. Tidal-breathing flow–volume loops are derived during normal breathing and are therefore not very sensitive to small disturbances in lung function. It remains to be determined if inductance plethysmography offers any advantage over other tests. In humans, end-expiratory lung volume (EELV) is measured because it tends to increase with airway obstruction and decrease in cases of restrictive disease. The EELV has been measured in horses but not frequently enough to make it a useful diagnostic test.

Measurement of ventilation

The most basic test of pulmonary function is the measurement of respiratory airflow at rest, i.e. during quiet tidal breathing. From this, variables such as tidal volume (V_T), respiratory frequency (f_R), minute ventilation ($\dot{V}_E = V_T \times f_R$), peak inspired flow (PIF), peak expired flow (PEF) and inspiratory (T_i) and expiratory time (T_e) may be obtained. The airflow data may also be used to generate tidal breathing flow–volume loops (Petsche et al 1994, Guthrie et al 1995a). The current consensus of opinion is that measurements of ventilation in horses at rest are relatively insensitive for the detection of impaired pulmonary function. However, greater sensitivity may be obtained when measurements of flow are combined with simultaneous analysis of exhaled carbon dioxide concentration (see volumetric capnography).

Measurement of ventilation in the resting horse requires a facemask, a flow measuring device (pneumotachograph), a differential pressure transducer (except with ultrasonic devices), a means of calibrating either flow or volume, and a recording and analysis system (see Box 15.1).

Box 15.1. Flow measuring devices

The most common device used for measuring respiratory airflow in the horse is the capillary-type Fleisch pneumotachograph (Fig. 1.1) that determines flow from the pressure drop across the resistive element according to the Poiseuille equation:

$$P = 128\,\mu L \dot{V}'/\pi d^4$$

where P is the steady-state pressure drop, $\dot{V}'$ is flow, μ is the viscosity of the gas, L is length and d is diameter of the tube in which the gas is flowing.

For measurements in resting adult horses (approx 400–600 kg) a no.4 or no.5 Fleisch pneumotachograph is appropriate. A no.3 device is suitable for anesthetized animals, small ponies or foals. Many Fleisch-style pneumotachographs incorporate a heater that limits condensation by increasing the temperature of the screen by about 10°C above that of exhaled breath.

Fleisch pneumotachographs must be used with a differential pressure transducer. The Validyne-style transducer (Fig. 1.1A)

Continued
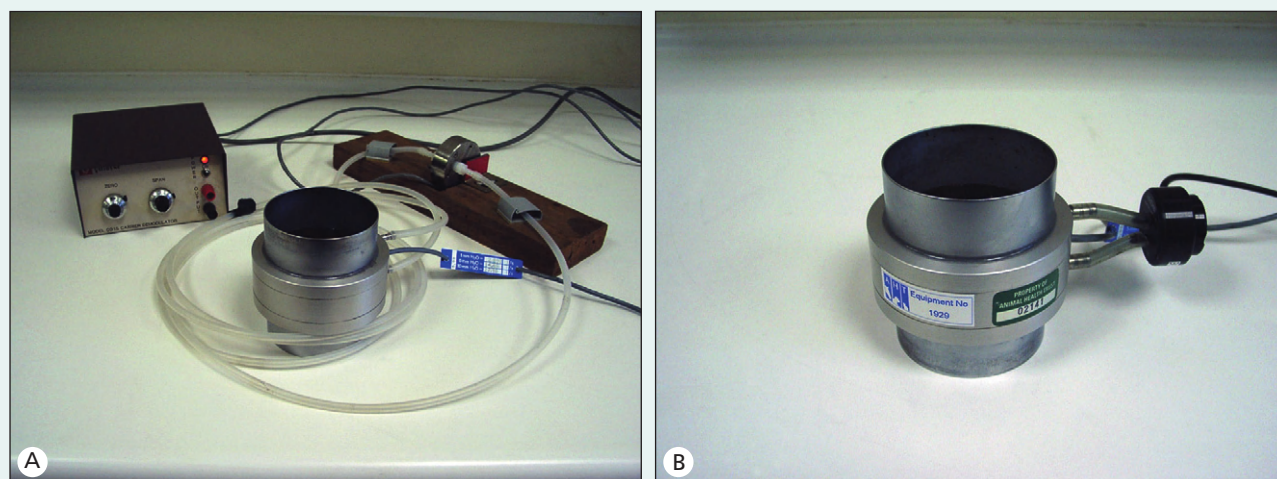
Fig. 1.1. (A) From right to left, Validyne pressure transducer, Fleisch No.5 heated pneumotachograph and Validyne carrier demodulator. (B) Piezoelectric differential pressure transducer mounted directly on a Fleisch No.5 heated pneumotachograph.

has excellent stability, sensitivity, and frequency response (the ability to follow rapid changes in pressure and flow). Disadvantages include expense, weight, and position sensitivity that make them unsuitable for mounting on the pneumotachograph. For the latter reason, they are usually mounted some distance from the horse and connected by two equal lengths of stiff tubing. An alternative is to use a piezoelectric-type transducer which is not orientation sensitive, is lighter in weight, and which can be mounted directly onto the pneumotachograph or mask resulting in less noise as a result of the movement of the horse's head (Fig. 1.1B).

Ultrasonic pneumotachographs are also available for use in horses (Flowmetrics, BRDL, Birmingham, UK; Exhalyzer V, Eco Medics, Switzerland). They are designed for use during exercise and generally lack the sensitivity required for resting measurements. Other possibilities for flow measurement include turbine pneumotachographs, pitot-based sensors and hot wire anemometers, although these are uncommonly used for resting measurements in horses.

Calibration of equipment

Calibration should be performed at the start of each day, immediately before making the measurements, and at the end of the series of measurements on each subject. Measurements should be accepted if the difference between pre- and post-calibrations is less than 10%. Calibration of the pneumotachograph requires either a volume or flow source that is calibrated and certified. Syringes are commonly used for volume calibration, while rotameters are used for flow calibration. The calibration should be performed at the same temperature and humidity as the measurements will be made. Flow rates displayed on the scale of rotameters are specific to particular conditions.

Calibrations must cover the expected range of measurements. In a 500-kg adult horse with a tidal volume of 1–7 liters, calibration solely with a 2-liter volume syringe is inappropriate. Furthermore, before undertaking measurements with new equipment or new configurations of existing equipment, it is advisable to establish linearity for both inspiratory and expiratory measurements. The stability of the baseline (zero flow) following calibration should also be determined. When calibrating equipment for measurements of gas concentrations, certified gas mixtures should be used.

Recording and analysis

Most flow measuring devices generate a voltage or digital signal proportional to the airflow rate. Some systems incorporate a display of the flow rate. However, in most cases it is desirable to record and analyze the signals. The output from pneumotachographs can be easily interfaced with computers running data acquisition and analysis hardware [in the form of analog to digital (A/D) converters] and software that has been specifically developed for analysis of respiratory signals (e.g. Biosystem XA, Buxco Research Systems, Wilmington, NC, USA; Po-Ne-Mah, Gould Instrument Systems Inc., OH, USA). Alternatively, some complete systems are available (e.g. Eco Medics).

Facemasks

Because many measurements of lung function require the use of facemasks, it is useful to review them here. Facemasks must be rigid, have low dead space, not impinge on the movement of the nares or compress the soft tissue overlying the nasomaxillary notch, be comfortable, and form a tight seal so that airflow does not escape from the mask. There are no commercially available masks for equine pulmonary function testing, with the exception of the Exhalyzer Eco Medics (Duernten, Switzerland) (Fig. 15.1) but it only accepts the Eco Medics ultrasonic flow meters. The Equine Aeromask (Trudell Medical
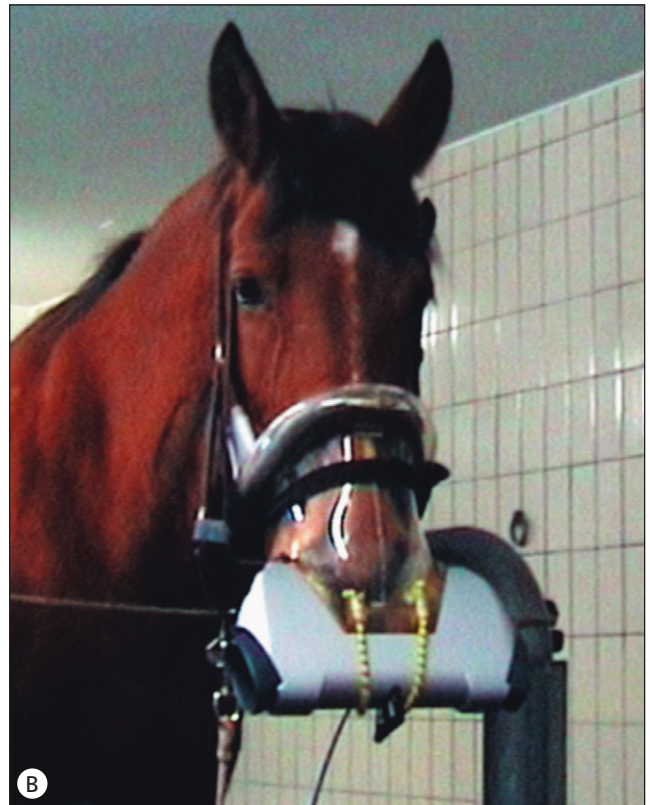
Fig. 15.1. The Exhalyzer ultrasonic pneumotachographs and mask from Eco Medics. (A) Original mask and ultrasonic flow transducer, (B) recently improved flow transducer. Reproduced with the permission of Eco Medics.

International, London, Ontario, Canada) can be modified for equine pulmonary function testing. Masks are available in three sizes and can be obtained before they have been drilled and fitted for medication delivery devices. They are made of clear, lightweight plastic and can be drilled and fitted with a holder that will accept a pneumotachograph (Fig. 15.2). Another alternative is to construct a mask using a head from a cadaver as a mold to build a cast (Fig. 15.3).

The seal to the horse's head should remain airtight, even when the horse moves. Rubber shrouds fashioned from tire inner tubes are ideal. The Eco Medics mask incorporates an inflatable rubber ring that ensures a firm and airtight fit to the face. Care should be taken to avoid seals that are excessively compliant in applications where the mask may become pressurized, such as for forced oscillatory mechanics or for provocation testing when using conventional mechanics measurements.

Pulmonary Resistance and Dynamic Compliance

With the addition of an esophageal pressure measurement (as an index of pleural pressure) to the flow measurement,

it is possible to calculate dynamic compliance (C_{dyn}) and total pulmonary resistance (R_L) (Box 15.2). These are the most common pulmonary function measurements found in the equine literature. An increase in R_L is indicative of airway obstruction that can be the result of narrowing of the upper and/or lower airway. As noted above, head flexion can obstruct the upper airway but if horses are allowed to stand with minimal restraint, this is not usually a problem. In the lower airway, obstruction can be caused by bronchospasm, mucus accumulation within the airway lumen, and by airway wall thickening as a result of inflammation-induced remodeling. Dynamic compliance decreases if the lungs become stiffer, for example as a result of fibrosis or pulmonary edema. Dynamic compliance also decreases when there is diffuse peripheral airway obstruction such as occurs in horses with RAO.

In recent years it has been acknowledged that measurements of compliance and resistance, especially using the more simplified analytical approaches, appear to be relatively insensitive to changes in pulmonary function in horses and there are many technical issues associated with their measurement. This has led a number of research groups to simply present data for maximal

Fig. 15.2. Horse in stocks wearing a modified Aeromask fitted with a No.5 heated Fleisch pneumotachograph. To the left of the mask the two thicker plastic tubes are those connected to the differential pressure transducer (out of picture). The thinner wire is the heater connection. To the right of the mask is the esophageal catheter, which passes through a rubber valve (not visible) into the mask.

change in pleural pressure (esophageal pressure) to describe the mechanical function of the lung, for example, in response to inhaled organic dust challenge of RAO-affected horses. However, measurements of conventional lung mechanics may still be required for regulatory procedures, such as in obtaining data to support registration of new pharmaceuticals.

Maximal Change in Pleural Pressure

Direct measurement of pleural pressure requires placement of a catheter in the pleural cavity. Fortunately, esophageal pressure provides an accurate estimate of pleural pressure and is a simple measurement to obtain. A relatively stiff catheter of approximately 2–3 m with an external diameter of around 3–4 mm is inserted down the esophagus so that

its tip lies within the mid-thorax. The final 10 cm or so of the catheter has holes placed in a spiral pattern. An unlubricated condom that is sealed to the catheter with fine suture material, cotton or silk, covers this perforated region. Once the catheter is in place, a small amount of air is introduced into the system (usually 2–3 ml). The volume introduced must not pressurize the system. The catheter is then connected to the pressure transducer and recording system. Technical details are provided in Box 15.3.

A potential problem with making measurements of esophageal pressure is swallowing, which causes transient but quite marked changes in esophageal tone and therefore pressure, often causing the recording to go out of range. Swallowing is easy to observe on a pressure trace and these pressure waves are excluded from the analysis. Many horses swallow frequently with an esophageal catheter in place and therefore it may not be possible to obtain reliable measurements in such individuals.

Measurement of the maximal change in pleural pressure (esophageal pressure) during tidal breathing ($\Delta P_{pl\,MAX}$) is the simplest PFT. The $\Delta P_{pl\,MAX}$ is directly proportional to R_L, tidal volume, and airflow rate, and indirectly proportional to C_{dyn}.

$$(\Delta P_{pl\,MAX}) = V_T/C_{dyn} + R_L \times flow$$

An increase in $\Delta P_{pl\,MAX}$ is not specific for any type of lung disease because it can increase if the lungs stiffen, the airways become obstructed, or if V_T and flow increase. If the diagnosis is known however, changes in $\Delta P_{pl\,MAX}$ can be used to track progress of the disease or response to treatment. In a horse with RAO for example, a reduction in $\Delta P_{pl\,MAX}$ after administration of atropine would indicate bronchodilation. Furthermore, if disease is affecting gas exchange, and there is a compensatory increase in ventilation (V_T and flow), this is also reflected in the measurement of $\Delta P_{pl\,MAX}$. For this reason it provides a good overall view of the lung's functional status and therefore Boehringer-Ingelheim used to market an intrapleural measurement system, the "Ventigraph", to measure and display esophageal pressure in horses.

Forced Oscillatory Mechanics

Forced oscillatory mechanics (FOM) is potentially a very attractive PFT because it is non-invasive and appears to be more sensitive to changes in lung function than are measurements of C_{dyn} and R_L. Flow and pressure are measured at the nares in response to impulses applied to the respiratory system (Figs 15.4 and 15.5). When C_{dyn} and R_L are measured, the horse generates the flow and pressure, whereas in the case of FOM, pressure and flow signals are superimposed on the normal breathing pattern either as multifrequency impulses or trains of single sine waves (Figs 15.4 and 15.5).
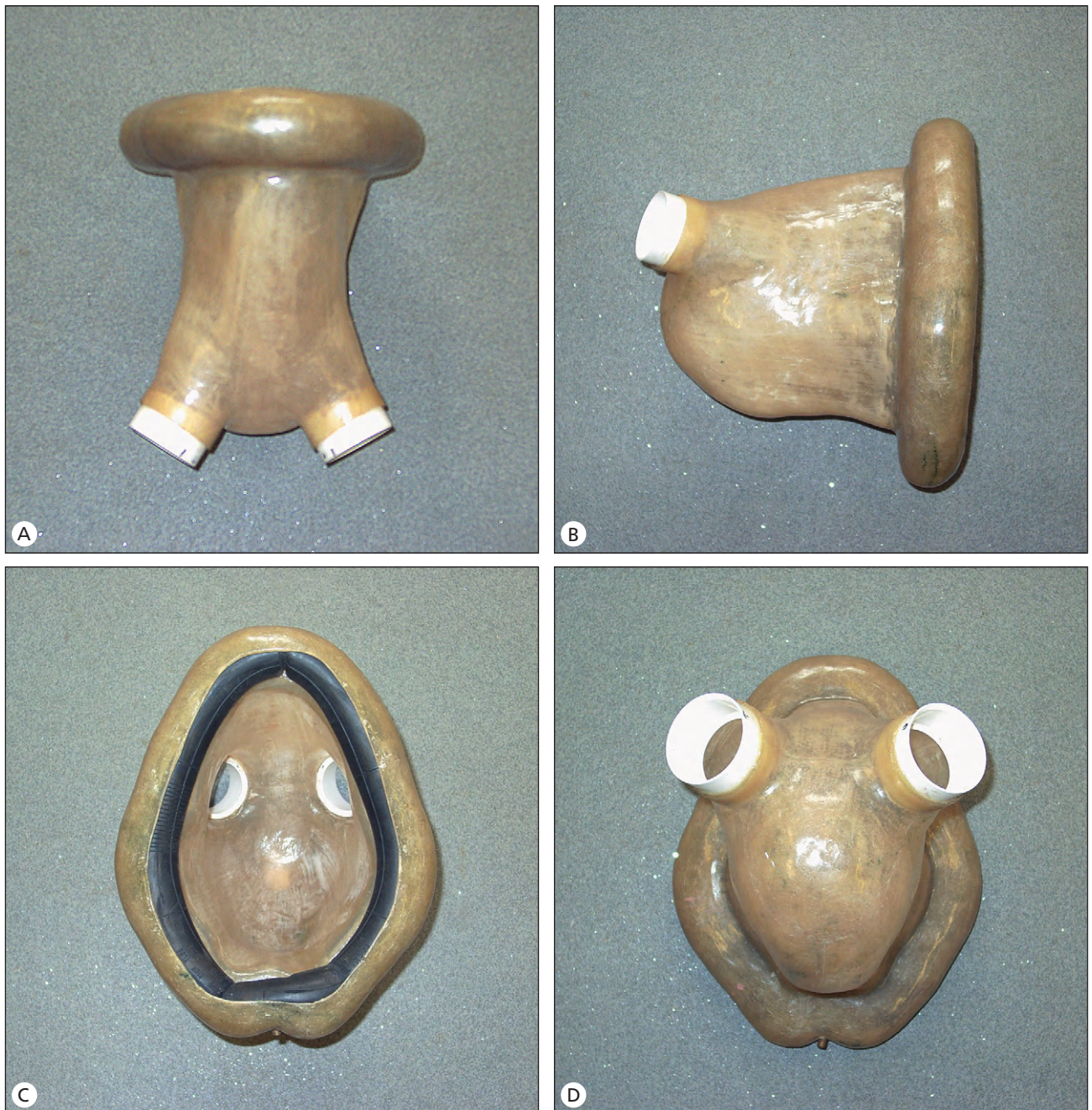
Fig. 15.3. Mask for pulmonary function testing constructed from fiberglass after a mold was made in plaster of Paris from the head of a cadaver. A head was then cast in plaster of Paris from the mold and the shape for the mask was built up using modeling material. This was then used to make a further mold and cast upon which the masks were made up by using layers of fiberglass sheets. An inflatable rubber seal is used to seal the mask around the horse's head. (A) Top view, (B) lateral view, (C) rear view, (D) front view.

Box 15.2. Calculation of pulmonary resistance and dynamic compliance

The simplest approach to calculating dynamic compliance (C_{dyn}) is to divide tidal volume by the change in pressure between the start and end of inspiration at points of zero flow (Fig. 2.1). Total pulmonary resistance (R_L) is determined by dividing the change in pressure between two points of equal volume (isovolume, the first during inspiration and the second during expiration) by the change in flow between the same two points (Fig. 2.2). The isovolume percentage is normally set to between 60 and 70%. Use of isovolume values in the range 50–90% makes minimal difference to the value of resistance in horses with and without methacholine-induced bronchoconstriction (Marlin, unpublished data).

The Mead–Whittenberger technique shown in Figs 2.1 and 2.2 was widely used prior to the introduction of computer-based signal acquisition and analysis, although it is still used, for example, by the Po-Ne-Mah data acquisition and analysis system (Gould Instrument Systems Inc., OH, USA). Whilst this is a simple approach, compliance and resistance are only calculated at discrete points within the respiratory cycle and

may not necessarily be representative of the whole cycle. An alternative approach is the least squares regression method (Fig. 2.3). Compliance is calculated from the slope of the line connecting the highest and lowest volume points (as in the Mead–Whittenberger approach) on a graphical representation of a pressure–volume loop, whilst resistance is determined by subtracting a calculated value for elastic recoil pressure (P_{el}) from transpulmonary pressure (P_{tp}) at each different volume (V) reading:

$$P_{res} = P_{tp} - P_{el}$$

$$P_{res} = \Delta P_{tp} - (\Delta V/C_{dyn})$$

P_{res} (the pressure required to overcome resistive forces) is then plotted against flow (V') at each volume reading and linear regression is used to obtain the slope of the line, which represents R_L.

Two more advanced techniques are the Mortola–Saetta method and the multiple linear regression (MLR) or covariance technique (Roy et al 1974).
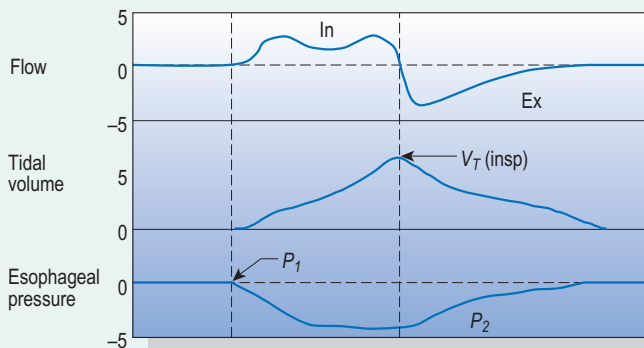
Fig. 2.1. Illustration of the calculation of dynamic compliance (C_{dyn}) from nasal flow and esophageal pressure recordings using the Mead–Whittenberger technique. In = inspiration; Ex = expiration; V_T (insp) = inspiratory tidal volume; P_1 and P_2 = pressures (dyn = $V_T / (P_1 - P_2)$).
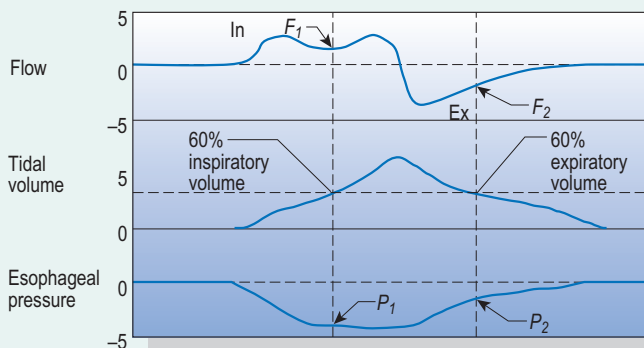
Fig. 2.2. Illustration of the calculation of total pulmonary resistance (R_L) from nasal flow and esophageal pressure recordings using the Mead–Whittenberger technique. $R_L = (P_2 - P_1) / (F_1 - F_2)$.

Continued

Box 15.2. Calculation of pulmonary resistance and dynamic compliance—cont'd
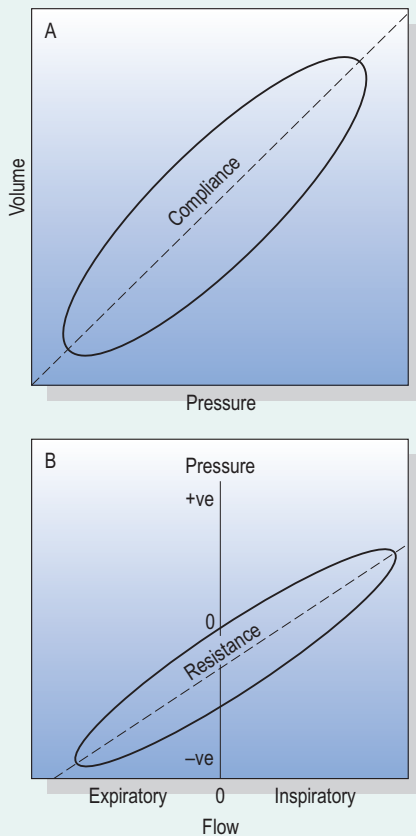
Fig. 2.3. (A) Representation of compliance as determined from a plot of volume as a function of pressure (common to both Mead–Whittenberger and least squares regression analysis/covariance techniques). (B) A plot of flow and pressure to obtain P_{res} and R_L, where $P_{res} = P_{tp} - (V_T/C_{dyn})$ as in the least squares regression method. P_{res} = the pressure required to overcome resistive forces; P_{tp} = transpulmonary pressure; R_L = total pulmonary resistance; V_T = tidal volume; C_{dyn} = dynamic compliance.

The MLR or covariance technique is based on solving a simplified general equation of motion for the lung for the constants E_L (elastance or $1/C_{dyn}$) and R_L:

$$P_{tp} = E_L \times V + R_L \times V' + k$$

where P_{tp} is transpulmonary pressure (represented by esophageal pressure), V is the volume above elastic equilibrium volume [this normally corresponds to functional residual capacity (FRC) under resting conditions in healthy individuals], V' is flow and k is a constant. As P_{tp}, or rather P_{es}, and flow are measured and volume can be obtained by integration, the equation can thus be solved to obtain E_L and R_L. With computer-based analysis the equation can be solved many times within each breath to analyze either discrete portions (e.g. inspiratory versus expiratory) or over the whole breath cycle. While the method is stable and less susceptible to waveform noise than other resistance and compliance methods, it is more difficult to validate. This is the analytical approach employed in the Biosystem XA software from Buxco (Buxco Research Systems, Wilmington, NC, USA).

The Mortola–Saetta method is a development of the least squares regression technique where the simplified equation of motion (see above) is transformed by dividing throughout by volume to obtain compliance and by flow to obtain resistance. These equations are solved for multiple points within the breath and the slope of the regression lines represents compliance and resistance. The whole breath cycle may be used or separated into inspiratory and expiratory components.

Whichever method is used should be made clear in publications, as this will affect the absolute value and also potentially the sensitivity to detect changes in response to disease, inhalation challenges, or other interventions.

Box 15.3. Measurement of esophageal pressure

The Validyne pressure transducer can be used with air-filled esophageal catheters but it must have the correct pressure range, typically ± 30 or ± 60 cmH$_2$O rather than the ± 3 cmH$_2$O used with a pneumotachograph. By using pressure transducers of the same make and model connected to the pneumotachograph and esophageal catheter by similar lengths and types of tubing, the risk of signals from flow and esophageal pressure being out of phase is minimized.

Whether two separate measuring systems are in phase or not is related to the frequency response of the complete measuring and recording system. A system with a good frequency response will accurately represent the true input signal

(Figs 3.1, 3.2 and 3.3). In the case of respiratory signals such as airflow and esophageal pressure, a frequency response up to 10–20 Hz is adequate for resting measurements. During exercise the rates of change of flow and pressure are greater and so equipment with a higher frequency response may be required.

Less common for the measurement of esophageal pressure are catheter-tip-mounted strain-gauge pressure transducers of the type commonly found on intravascular pressure transducers (e.g. Millar and Gaeltech). These have a much higher frequency response and may be desirable when the rate of change in pressure is rapid. Tip-mounted catheters are less subject to noise introduced by movement of the external part

of the catheter between the horse and the pressure transducer. However, the tip must be covered with a condom to prevent direct contact between the transducer and the esophageal wall. Without such a cover, the pressure is only measured locally where the catheter tip meets the esophageal wall.

To represent pressure changes within the pleural cavity, the esophageal balloon must rest between the base of the heart and the diaphragm. The catheter can be marked to the approximate length by aligning it from the external nares along the path of the esophagus. It can then be inserted and moved to and fro until the maximal change in pressure is recorded that is free of cardiac artifact. An alternative approach is to use radiography to position the balloon in a standard position. The catheter can then be marked at the nares so that it is easily replaced at the same location.

Depending on the rigidity of the catheter material, it may be possible to pass the esophageal balloon and catheter directly or it may be facilitated by use of a short stomach tube (about 60 cm long) placed into the rostral esophagus. The use of a twitch and/or application of a small amount of local anesthetic gel or spray on the mucosal surface inside the nares greatly facilitates catheter placement. The tricks used to pass a stomach tube can be used to pass the esophageal catheter.

An esophageal catheter can make it difficult to seal a facemask. A simple approach in our experience is to pass the catheter through a cable port valve inserted into the mask before inserting the catheter into the horse. When the catheter is in position the mask can be applied and the port valve tightened to lock the catheter in position.

The driving pressure for breathing is the difference between the pleural (esophageal) and external nares pressure. As the nares are inside the mask, it is appropriate to connect the second pressure port of the transducer to the inside of the mask rather than to leave it open to the atmosphere. However, when the horse is breathing quietly at rest, the difference between mask (nares) pressure and external (atmospheric) pressure is minimal and can essentially be ignored. However, when a horse has increased ventilation, the imposition of a small resistance by the pneumotachograph can create several cmH_2O difference in pressure between the inside of the mask and the atmosphere.
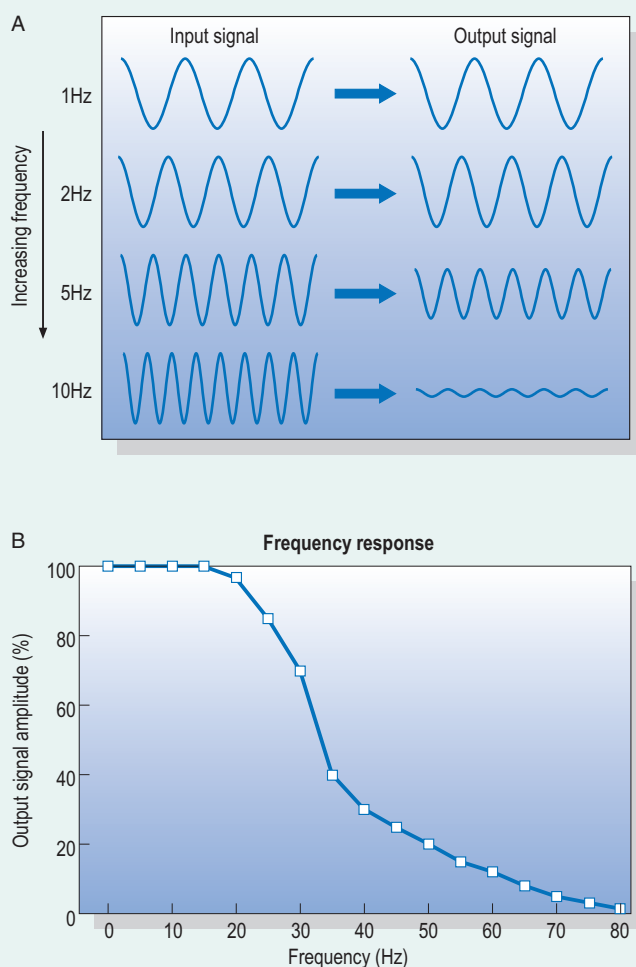
Fig. 3.1. (A) Illustration of the concept of frequency response. At 1 and 2 Hz input frequency the output frequency is faithfully reproduced. At 5 Hz there is some loss of signal amplitude, whilst at 10 Hz the output is almost completely lost. (B) The frequency response of a system is often expressed by showing the output amplitude as a function of the input frequency. In this example there is no loss of amplitude for frequencies between 0 and 15 Hz. This system would therefore be described as having a *flat frequency response* between 0 and 15 Hz. At 20 Hz there is a slight loss of amplitude, which becomes marked between 25 and 40 Hz. This is sometimes referred to as *roll-off*. This system would be suitable to measure resting esophageal pressure but unsuitable for measuring ventilation during exercise.

Box 15.3. Measurement of esophageal pressure—cont'd
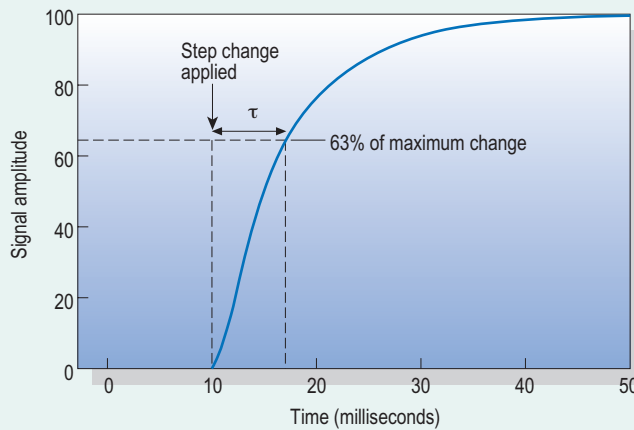
Fig. 3.2. The response of a measuring system can also be defined in terms of its response to an instantaneous change from one state to another, e.g. a rapid change from low to high or high to low pressure. The latter can be achieved for example by placing the measuring port of a pressure transducer inside a balloon and popping it. This is normally defined as the time taken for the amplitude of the transducer output to reach 63% (one time constant; tau) of its final steady-state value in response to a step change input.
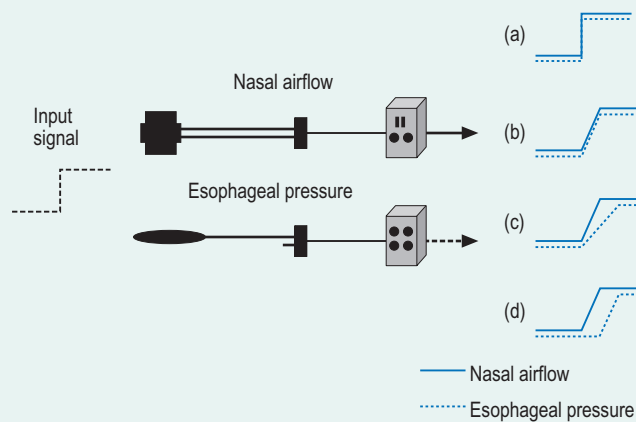
Fig. 3.3. Illustration of the frequency response of two different measurement systems. A common step change signal (dashed line, right of diagram) is applied to two transducer systems, one for measuring nasal airflow and one for measuring esophageal pressure. Four possible different outputs from these two systems are shown on the right of the diagram (labeled a to d): (a) both flow and pressure output signals faithfully reproduce the input signal; (b) both flow and pressure output signals are "slower" (lower frequency response) than the input signal but nevertheless are both in phase (they change together); (c) the flow signal responds faster than the pressure signal (i.e. the frequency response of the flow measuring system is faster or better than the pressure measuring system); (d) both flow and pressure signals are similar, except that the esophageal signal is delayed such that they are out of phase.

Although, estimation of respiratory system impedance (Z_{rs}) using forced oscillation was first described in 1956 (Du Bois et al 1956), FOM is relatively new to equine pulmonary function testing (Young & Hall 1989, Young & Tesarowski 1994, Young et al 1997, Mazan et al 1999, van Erck et al 2003, 2004a,b) and as yet is not in widespread use. At present there are two forced oscillation systems for use on horses, one based on impulse oscil-lometry (IOS; Masterscreen, Jaeger GmbH, Wurzburg, Germany; Fig. 15.6) and the other on forced oscillations (FOT) (EMMS, Bordon, Hants, UK; Fig. 15.7). Details of the two systems and technical aspects of interpretation of results are provided in Box 15.4.

Measurement of FOM produces values for resonant frequency of the respiratory system and for respira-tory impedance (Z_{rs}), which is composed of resistance (R_{rs})
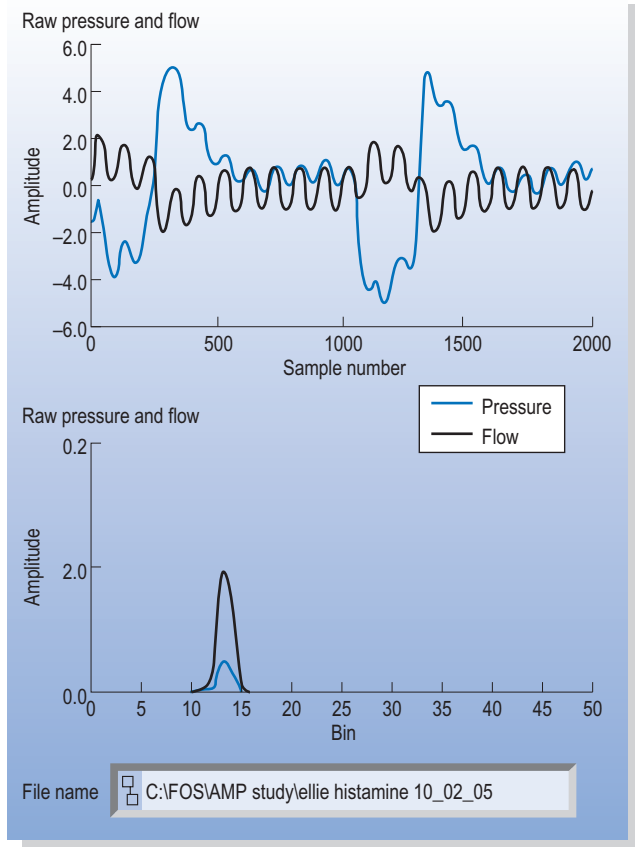
Fig. 15.4. Screen print from the EMMS FOM system to show the high frequency oscillations of flow and resulting pressure superimposed on the lower frequency flow and pressure signals generated as the horse takes two breaths (upper graph). The lower graph shows the amplitude of the flow and pressure signals after estimation by fast Fourier transformation (FFT) and removal of the breathing signal.

and reactance (X_{rs}) (see Box 15.4). Respiratory resistance includes all the components of the respiratory tract that contribute to friction, essentially the resistance to flow in the airways, which depends on airway caliber and the architecture of the airways (e.g. branching). In an animal with RAO for example, R_{rs} would be increased, the frequency dependence of resistance exaggerated, and reactance more negative, which also has the effect of increasing resonant frequency. Reactance reflects the stiffness of the lung.

Forced Expiration

In human medicine, the most commonly used lung function measurements are derived during a forced exhalation. To create a forced exhalation in horses, it is necessary to connect their airway to a large vacuum source. This was first performed in anesthetized horses within a plethysmograph using a vacuum reservoir
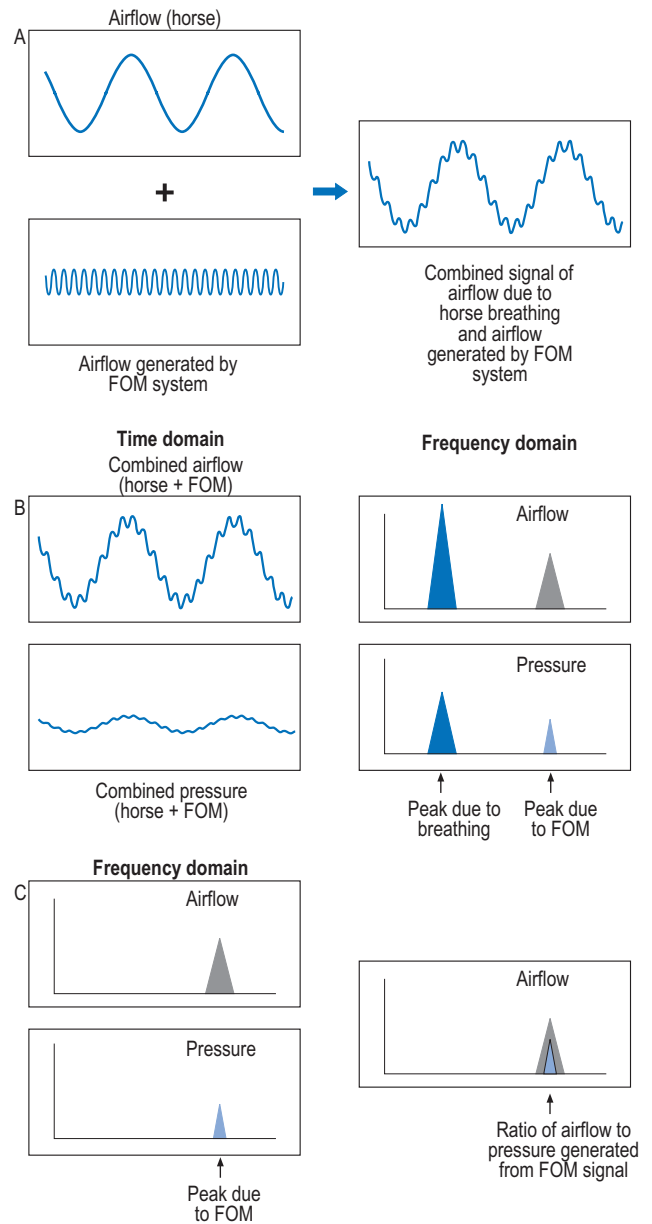
Fig. 15.5. (A) Low frequency signal representing horse's airflow, higher frequency oscillations generated by the FOM system and the two signals combined (right hand side). (B) Combined signals for airflow and pressure (left hand side) in the time domain and representation in the frequency domain on the right hand side. (C) Pressure and airflow peaks as a result of breathing of horse are ignored and the peaks generated from the forced oscillation are used to calculate the impedance variables.

connected by a tracheostomy tube (Gillespie 1974). Clearly this was not a technique with practical applications. More recently, forced expirations have been generated in sedated horses through a nasotracheal tube (Couetil et al 2000). Horses are mechanically ventilated and the lungs are inflated to total lung capacity. At this point,

Fig. 15.6. Impulse oscillometry system (IOS; reproduced with the permission of Dr Carmen Klein).
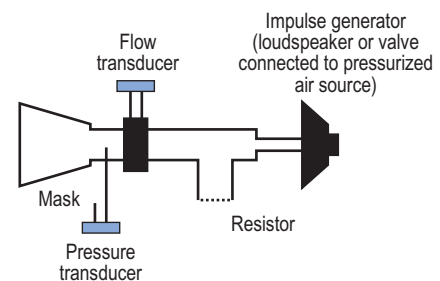
B

Fig. 15.7. (A) Measurement of pulmonary function in an unsedated adult horse using the technique of forced oscillatory mechanics (FOT; EMMS Ltd). Head and neck angle can be more difficult to standardize in unsedated horses, but if horses are to be sedated then in addition to standardization of the head and neck angle, the elevation of the head must be maintained to prevent excessive congestion of the upper airways, which will result in increased resistance. (B) A diagrammatic representation of the FOM equipment.

Box 15.4. Measurement of forced oscillatory mechanics

In the impulse oscillometry (IOS) system adapted for use in the horse, impulses are in the form of "clicks" generated by a loud-speaker and each click contains multiple frequencies in the range 0.1–50 Hz. In the case of the commercially available forced oscillometry (FOT) system for horses, the impulses are of single frequency (although sequential trains of different frequencies can be applied, usually in the range 1–5 Hz) and consist of pulses of air produced by a valve connected to a compressed air source. With IOS there is little sensation of pressure being applied whilst in the case of FOT, oscillations of pressure are more discernible.

The impedance of the respiratory system (Z_{rs}) is a function of the relationship between pressure and airflow and has two components: resistance (R_{rs}) and reactance (X_{rs}). R_{rs} is defined as the component of Z_{rs} where pressure and flow are in-phase (also referred to as the "real" part of Z_{rs}), whilst X_{rs} is the out-of-phase (or "imaginary") component. Real and imaginary in this context refer to the result of application of the Fast Fourier Transform to convert the signals from the time domain to the frequency domain. Both R_{rs} and X_{rs} vary with the frequency of oscillation being applied (Fig. 15.8).

The "resistance" of the respiratory system is determined from the relationship between pressure and flow at each frequency. As an example, if the entrance to the mask were completely blocked off then there would be zero flow and very high pressure, thus leading to high impedance. In practice with IOS or FOT there is a limit as to how high the impedance can be because the impulses are applied via a measurement head which has a leak resistor to avoid over-pressurization of the airways (Fig. 15.7). At the opposite extreme, if the measuring head were open to the air, there would be high flow and negligible pressure, so impedance would be close to zero.

In contrast, reactance (X_{rs}) is the ability of the lung to store energy and is defined as the degree to which oscillatory pressure leads flow, described as the phase angle. Reactance can be either positive or negative. When X_{rs} is negative,
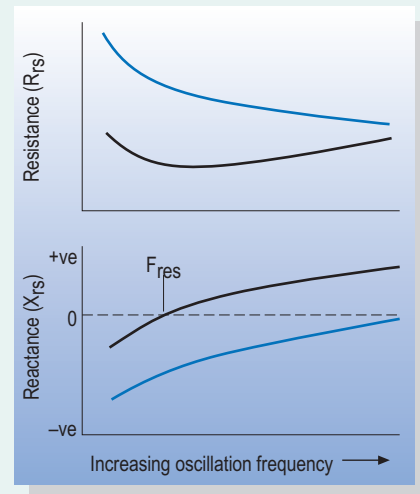
Fig. 4.1. Schematic representation of respiratory system resistance (R_{rs}), reactance (X_{rs}) and resonant frequency (F_{res}) as a function of increasing frequency (illustrating frequency dependence) in a healthy horse (black line) and in a subject with airway obstruction (blue line). Note that with airway obstruction the frequency dependence of resistance increases, reactance becomes more negative and resonant frequency increases.

pressure lags behind flow and when X_{rs} is positive, pressure leads flow. The negative part of X_{rs} reflects the capacitance (ability to store energy) of the pulmonary system. It is composed of the elastic components of both the lung and the chest wall, as well as the viscoelastic properties of the lung itself. The positive part of X_{rs} relates to the inertial properties of the air within the airways. In healthy subjects at low oscillation frequencies, elastic forces dominate over inertial forces and as a consequence X_{rs} is negative (Fig. 4.1), whilst at increasing frequencies inertial forces become increasingly dominant. If reactance is plotted as a function of increasing frequency, then a point is reached when reactance becomes zero. This frequency is termed the resonant frequency of the respiratory system.

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the airway is connected to a vacuum tank. The resulting forced expiration is used to generate a flow–volume curve (Fig. 15.8) from which is calculated forced vital capacity, FEV_1, forced expiratory flow at 75–95% exhaled vital capacity, and peak expiratory flow. Forced expiration is reported to be more sensitive than conventional measures of lung function in determining the early onset of pulmonary dysfunction in RAO-affected horses (Couetil et al 2001). The downside of this approach is that it is invasive and technically demanding and would not be considered practical in a routine clinical setting.

Tidal Breathing Flow–Volume Loop Indices

Tidal breathing flow–volume (TBFV) loops have the advantage that they can be recorded from conscious horses wearing a facemask and pneumotachograph to measure flow (see Box 15.1). Loops are formed by graphically plotting the volume against airflow for each breath (Fig. 15.9). A mean TBFV loop is usually generated from between 10 and 60 TBFV loops (Lumsden et al 1993, Connally & Derksen 1994, Petsche et al 1994, Herholz et al 2003a). TBFV loops have been produced from both exercising horses and horses at rest (Art & Lekeux 1988,
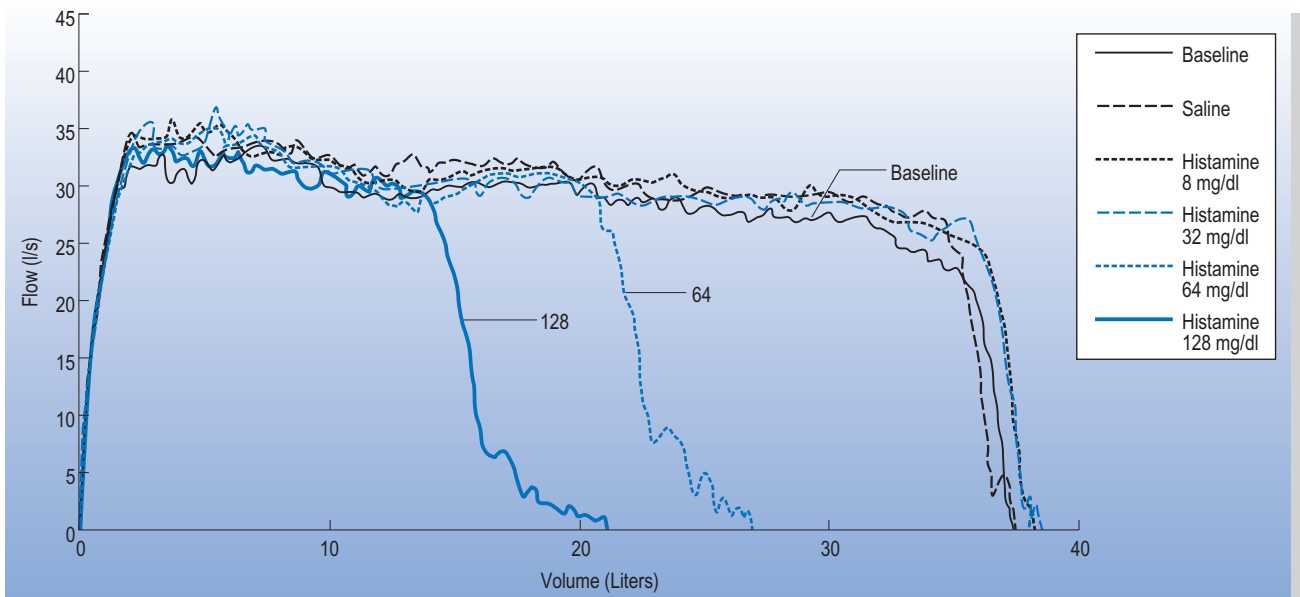
Fig. 15.8. Flow–volume curves obtained in a control horse during inhalation challenge with 0–128 mg/dl histamine. Exhalation begins at zero flow and volume. During the first part of exhalation, flow increases rapidly and reaches a plateau until late in exhalation when flow decreases rapidly. As the concentration of histamine increases to 128 mg/dl, airway obstruction causes the forced expiratory volume to decrease from 40 to 20 liters. In addition, the curve becomes more concave at the end of exhalation. Reproduced from Couetil et al 2000, with permission.
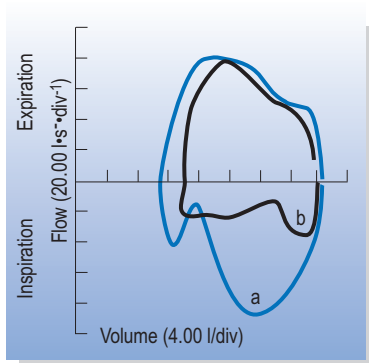
Fig. 15.9. Tidal breathing flow–volume loop of a control horse (a) and a horse with recurrent laryngeal neuropathy (b) exercising on a treadmill. Inspiration begins at the far right. As the horse inhales, its flow rate increases (downward deflection on the graph) to a peak, decreases and then increases to a second peak late in inhalation. At the end of inhalation, the horse has inhaled about 16–20 liters (four to five marks on the *x*-axis). Exhalation is uniphasic. Flow increases (upward deflection) and then decreases again. The horse with recurrent laryngeal neuropathy has no problem exhaling but the peak flow is dramatically reduced during inhalation.

Lumsden et al 1993, Connally & Derksen 1994, Petsche et al 1994, Guthrie et al 1995a,b, Herholz et al 2003a). A range of variables can be generated from a TBFV loop, including peak inspiratory flow, peak expiratory flow, and inspiratory and expiratory flows at 25% and 50% tidal volume. There is considerable intra- and inter-subject variation in TBFV loop indices (Guthrie et al 1995a,b). However, inspiratory flow at 50% tidal volume appears to be a useful indicator of disease severity in RAO (Petsche et al 1994, Herholz et al 2003a). The TBFV loop is very useful for identification of the severity of inspiratory flow limitation in horses with upper airway obstruction caused, for example, by recurrent laryngeal neuropathy.

Respiratory Inductance Plethysmography

Respiratory inductance plethysmography (RIP) provides a non-invasive measurement of lung volume using recording bands around the thorax and abdomen. Plethysmography bands are commercially available (Respibands, Ambulatory Monitoring Systems, Ardsley, NY, USA) or can be custom-made (Marlin et al 2002). The thoracic band can be placed at the seventh or eighth rib (Marlin et al 2002) or the 11th intercostal space (Miller et al 2000, Hoffman et al 2001) and the abdominal band at the level of the 16th, 17th (Marlin et al 2002) or 18th rib (Miller et al 2000, Hoffman et al 2001). The RIP has been used to study asynchrony between thoracic and abdominal movement in foals (Miller et al 2000). An index of airflow from the RIP bands has also been evaluated as a measure of airway obstruction (Hoffman et al 2001). RIP in conjunction with conventional techniques has been used to diagnose bilateral diaphragmatic paralysis in a pony (Amory et al 1994).
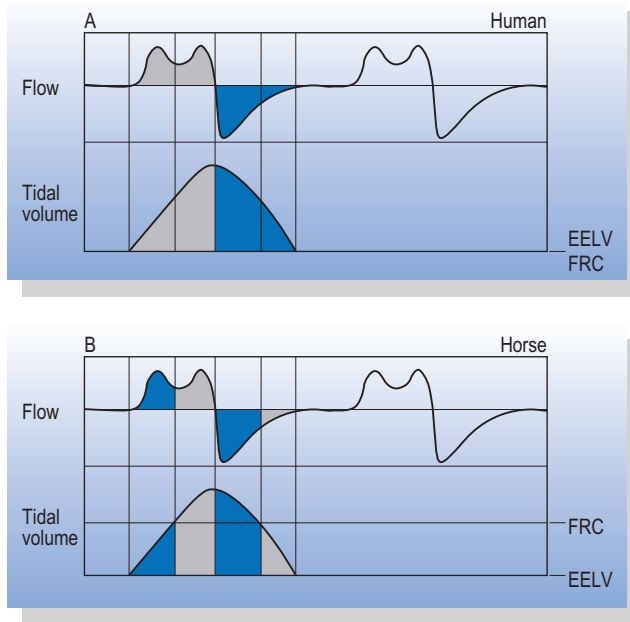
Fig. 15.10. (A,B) Representation of the breathing strategy and relative positions of functional residual capacity (FRC) and end-expiratory lung volume (EELV) in human (A) and horse (B). Gray areas represent active phases of breathing (i.e. involving muscular effort) whilst blue areas represent passive phases (i.e. relaxation and no muscular effort). Note that EELV and FRC are the same in human but EELV is always lower than FRC in horses because horses breathe around (above and below) their FRC whilst humans breathe from their FRC.

End-expiratory Lung Volume

At the end of a tidal exhalation, the lungs still contain a large volume of air that permits continuous gas exchange. In people, this end-expiratory lung volume (EELV) is identical to functional residual capacity (FRC) and represents the mechanical equilibrium of the respiratory system (Fig. 15.10). During the second phase of their exhalation, horses push their respiratory system below FRC (see Chapter 2) hence EELV is always smaller than the mechanically determined FRC (Fig. 15.10).

In humans, airway obstructions such as bronchiolitis, bronchopulmonary dysplasia, cystic fibrosis, emphysema, and chronic obstructive pulmonary disease tend to increase EELV. Restrictive conditions such as fibrosis or loss of surfactant in respiratory distress syndrome can decrease EELV. In addition, even in the absence of pathology, EELV can be elevated by processes such active laryngeal closure to slow exhalation (braking), inspiratory muscle activity during expiration, and a high respiratory rate.

There are few reports of measurement of EELV in horses and the role of changes in EELV in disease is unclear (Soma et al 1987). The two methods most applicable for determining EELV in horses are multiple breath nitrogen washout (Gallivan et al 1990) and helium dilution

(Aguilera-Tejero et al 1993, 1994, Deaton et al 2002). Whichever method is used to measure EELV, it should be remembered that gas trapped behind closed or obstructed airways, within cysts, or in regions of the lung with long time constants (i.e. poorly and slowly ventilated regions) is not measured by these dilution techniques. In the body plethysmograph method for determining EELV in humans and animals, however, trapped gas is included. Technical details of gas dilution techniques for measurement of EELV are provided in Box 15.5.

Measurement of Airway Responsiveness

Increased airway responsiveness, also referred to as bronchial or airway hyperresponsiveness (AHR), is an exaggerated narrowing of the airways in response to endogenous and exogenous stimuli. Human asthmatics typically have AHR; they develop airway narrowing in response to stimuli that do not affect non-asthmatics. These stimuli include antigens, cold air, and dusts. It is generally accepted that AHR is a consequence of inflammation. While allergic inflammation is a primary cause, viral infections such as influenza and exposure to environmental dusts can also temporarily induce AHR. The AHR may be present without other clinical evidence of airway inflammation such as cough or mucus hypersecretion. For this reason measuring AHR may provide a sensitive means to detect airway inflammation. Indeed, Klein and Deegen (1986) demonstrated that AHR to histamine is absent in horses without respiratory tract disease, present in around a quarter of horses with low-grade disease and present in all horses with severe airway inflammation.

Tests of AHR fall into two categories: those that use agents acting directly on airway smooth muscle to produce bronchoconstriction and those that induce bronchoconstriction indirectly through stimulation of release of endogenous mediators. Diagnosis of AHR in horses is most commonly undertaken using inhaled histamine or methacholine, which act directly on airway smooth muscle. Histamine binds to smooth muscle H_1 receptors but can also augment cholinergic bronchospasm (Olszewski et al 1999). Methacholine, an analog of acetylcholine, binds directly to airway smooth muscle muscarinic receptors. Exercise, dry air hyperventilation, distilled water, cold air, mannitol, hypertonic saline, and adenosine monophosphate (AMP) have been used in humans and animals. Measurement of AHR is described in Box 15.6.

Tests of Gas Exchange Properties of the Lung

Measurement of arterial blood gas tensions (P_AO_2 and P_aCO_2) provides the best overall evaluation of pulmonary gas exchange (Chapter 14). If P_AO_2 is depressed by an amount approximately equal to the elevation in P_aCO_2, the

Box 15.5. Measurement of end-expiratory lung volume

Multiple breath nitrogen washout

The horse is fitted with a facemask, a heated pneumotacho-graph, and a non-rebreathing valve. A reservoir bag containing 100% oxygen is connected to the inspiratory port of the non-rebreathing valve. The exhaled nitrogen concentration is recorded with a rapid-response analyzer. The washout is halted when the exhaled nitrogen concentration is below 1%. EELV is calculated from the following equation (Rollin et al 1996):

$$EELV = \{V_E \times F_{N_2}(mixed) / [F_{N_2}(initial) - F_{N_2}(end)]\} - V_D$$

where V_E is the total volume of gas exhaled, $F_{N_2}(mixed)$ is the fraction of nitrogen in the total volume of gas exhaled, $F_{N_2}(initial)$ is the fraction of nitrogen in the lung prior to washout, $F_{N_2}(end)$ is the fraction of nitrogen in the final breath of the washout, and V_D is the instrumental dead space. The nitrogen fraction in the total volume of exhaled gas can be determined by collecting the exhaled gas into a reservoir bag or by integrating the exhaled nitrogen fractions during the breath-by-breath washout.

Measurements of EELV in horses using the multiple-breath nitrogen washout technique have reasonable within-day reproducibility but differ significantly between days (Gallivan et al 1990). The measurement is critically affected by the presence of leaks in the equipment, most likely around the facemask. Leaks are more likely to occur when measuring EELV by the multiple-breath nitrogen washout technique compared to

the helium dilution method as it can take approximately 10 min to wash out the nitrogen from a horse's lungs. In contrast it only takes around 90 seconds to measure EELV by helium rebreathing.

Helium rebreathing

The horse breathes from a bag of air containing a known volume and concentration of helium in air in a closed system. The helium equilibrates with the air within the horse's respiratory tract over approximately six to twelve breaths. Provided there are no leaks in the system, the volume (i.e. respiratory tract and bag) is constant and the volume of helium in the system is the same at the beginning and end of the equilibration. However, as a result of dilution by air within the respiratory tract, the helium concentration is reduced at the end of equilibration (Fig. 5.1).

The technique requires a tight-fitting facemask, a giant three-way valve, a 20–50 liter reservoir bag (Douglas bag), a cylinder of medical grade 10% helium in air, a dry-gas meter or a volume syringe to measure volume, a thermometer and a helium analyzer. A means of displaying flow and helium concentration in real-time is helpful for timing valve operation and detecting leaks, but is not essential.

The reservoir bag is flushed several times with the rebreathing gas containing helium and is evacuated using a vacuum source. The bag is then filled to the required volume (60 ml
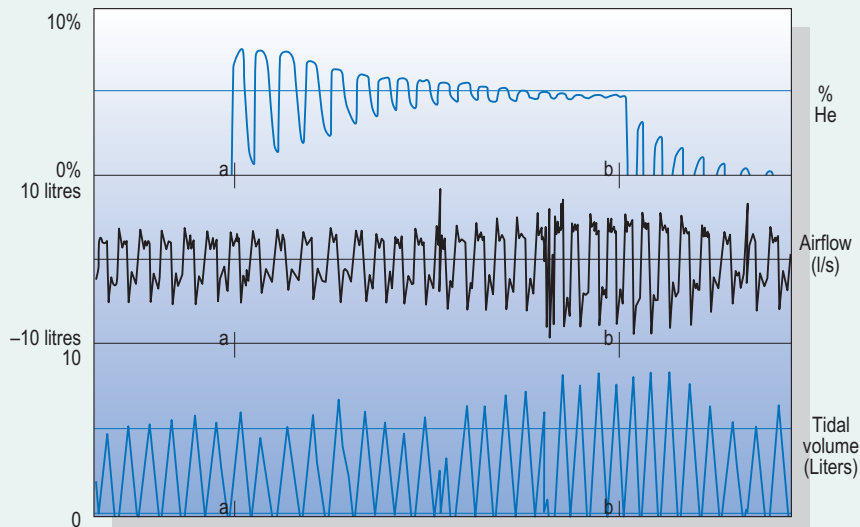
Fig. 5.1. Changes in respired helium concentration during measurement of EELV in a horse using the rebreathing (dilution) method. Before point "a" the horse is breathing room air. At point "a" (end expiration) the valve is closed to air and opened to the bag containing ~10% helium. Over approximately 15 breaths this equilibrates with the air in the horse's lung and at point "b", again corresponding to end expiration, the valve is closed to the bag and the horse breathes room air again (point "b" to end of trace). Respired airflow rate and tidal volume are also shown in the middle and lower panels, respectively.

gas/kg body weight) with the resulting gas. This is most easily accomplished by filling through a dry-gas meter. The bag is agitated to ensure thorough mixing and connected to the helium analyzer. The aspiration rate of the analyzer must be known or measured and the volume of the bag adjusted for the volume of gas removed. For example, if the aspiration rate is 500 ml/min and the analyzer requires 60 seconds for a stable reading, then for a desired bag volume of 15 liters, the bag should be filled to 15.5 liters.

The facemask that is connected to the pneumotachograph and helium analyzer is fitted to the horse and the bag containing the helium and air is attached to the large valve. The horse is allowed to breathe room air through the valve for several minutes or until a regular breathing pattern has been achieved. By following the flow signal or by observing the horse, the valve is switched at the end of a breath, i.e. end expiration, so that the horse now breathes from the reservoir bag. The helium concentration is monitored until equilibration occurs. Changes in breathing pattern that occur after the horse was switched to the bag may affect the rate of equilibration but not the volume for EELV measured. Once equilibration has occurred, the valve is switched back to room air and the bag is sealed. The timing of the closing of the valve has no bearing on the measurement of EELV. Leaks in the system have a dramatic impact on the value for EELV. If the helium concentration is monitored continuously, leaks are obvious and the measure-

ment must be repeated. Measurements should always be made in duplicate and the mean of two measurements that are no more than 10% different should be expressed at BTPS.

To calculate the EELV, the equilibrated helium concentration in the bag is measured. The dead space of the connectors and mask must be subtracted from the calculated EELV. It should also be noted that the volume for EELV includes the volume of air in the extrathoracic airways (i.e. trachea and upper airway). EELV is calculated as follows:

$$EELV = (V_B \times He_i/He_e) - (V_B + V_D)$$

where V_B is the initial bag volume, He_i and He_e are the initial and equilibrated helium concentrations, respectively, in the bag, and V_D is the instrumental dead space, which includes the dead space of the mask. If the bag is filled through a dry-gas meter from a cylinder, then the initial bag volume (V_B) will be at conditions close to 0% relative humidity and ambient barometric pressure. As EELV measurements should be presented at BTPS, it is necessary therefore to also record the temperature of the gas entering the dry-gas meter.

Neon has also been used as a tracer gas to determine EELV by rebreathing in ponies (Gutting et al 1991). Sodium cyanide was injected into the jugular vein at the start of rebreathing to stimulate ventilation and speed equilibration of neon between the rebreathing bag and the horse. EELV was calculated as described for helium.

hypoxemia is the result of alveolar hypoventilation and no further investigation is necessary. If, however, $P_{A}O_2$ is depressed more than $P_{a}CO_2$ is elevated, the animal has additional causes of hypoxemia such as loss of diffusing area or, more likely, ventilation/perfusion mismatching. Both diffusing capacity and ventilation/perfusion matching have been investigated in horses but the methods are highly technical and useful only as research tools. More recently, volumetric capnography has been introduced as a method to examine ventilation/perfusion inequalities. The usefulness of this technique awaits broader confirmation.

Carbon monoxide diffusing capacity

The carbon monoxide (CO) diffusing capacity test (DL_{CO}, also referred to as CO transfer factor) assesses the efficiency of oxygen transport across the alveolar–capillary membrane and into hemoglobin in red blood cells (Comroe 1975). Diffusing capacity has been measured in horses using the single breath (Gillespie & Tyler 1968), steady state (Mauderley 1974) and rebreathing (Aguilera-Tejero et al 1993) methods.

Ventilation and perfusion matching/mismatching

Because disease rarely occurs uniformly throughout the lung, there are regions of normal lung and regions in which there is airway obstruction and altered compliance. These regional changes cause abnormal distribution of airflow within the lung. In addition, disease can also cause abnormal distribution of blood flow. These changes lead to the mismatching of ventilation ($\dot{V}$) and blood flow ($\dot{Q}$), which is the most common cause of hypoxemia in lung disease. The degree of matching or mismatching between ventilation and perfusion within the lung can be assessed using either the multiple inert gas elimination technique (MIGET) or by imaging of ventilation and perfusion (Votion et al 1997, 1999a) (Chapter 13).

Multiple inert gas elimination technique

MIGET requires the infusion of six inert gases (sulfur hexafluoride, ethane, cyclopropane, enflurane, diethyl ether and acetone) dissolved in isotonic sodium chloride solution

Box 15.6. Measurement of airway responsiveness

The standard approach is to administer increasing doses of the challenge agent by means of a jet, ultrasonic or Piezo nebulizer (Fig. 6.1). It is important to know the particle size distribution of the output and the output rate (i.e. ml solution nebulized/min). In general terms, particles of less than 5 µm diameter are required to penetrate to the small airways. The nebulizer output is normally quoted as the median mass aerodynamic diameter (MMAD). For example, for the Ultra-Neb 2000 (DeVilbiss) ultrasonic cup nebulizer, the quoted MMAD is <4 µm at a rate of 6 ml/min. The latter can be adjusted using a dial on the unit. In the case of jet nebulizers, the output rate and the particle size distribution vary with the pressure used to generate the aerosol. For example, the Sidestream jet nebulizer (Profile Respiratory Systems Ltd, Bognor Regis, Sussex, UK) has an output of 0.37 and 0.46 g/min at the recommend 80–124 kPa. During a challenge, the concentration of the agent, the output rate of the nebulizer, and the duration of nebulization determine the dose delivered. Whilst many authors often

Fig. 6.1. (A) Histamine being nebulized to a horse using a Devilbiss Ultraneb 2000 ultrasonic nebulizer. The aerosol is being nebulized directly into the pneumotachograph, which avoids having to remove the device and disturb the horse. Note the masks being worn by technicians. (B) Jet nebulizer chamber (Sidestream durable nebulizer, Profile Respiratory Systems Ltd) and compressor; and (C) close-up of nebulizer chamber.

display provocation tests in terms of response (e.g. resistance) to a nebulized concentration (e.g. 1, 2, 4, 8 mg/ml), it is more appropriate to express the response as a function of challenge dose (i.e. concentration × time × output rate).

If the nebulizer does not have a calibrated dial to adjust flow rate (output), output can be determined by weighing the nebulizer chamber before and after a measured period of nebulization. Because the rate of nebulization may vary with the nature of the solution to be nebulized and also with the volume within the nebulizer, the calibration should use a similar duration, volume, and type of solution to that being nebulized during challenges. Viscous solutions such as hay dust suspension and some preparations of corticosteroids nebulize poorly in ultrasonic nebulizers. For these solutions, jet nebulizers may be preferred. Either jet or ultrasonic nebulizers are suitable for methacholine and histamine.

Nebulizing the aerosol into an open mask is simple but a lot of aerosol enters the room. A closed delivery system can be scavenged of excess aerosol and also allows more precise control of delivery. Whichever system is used, personnel should wear suitable fitted facemasks to prevent unnecessary exposure to aerosol. The closed delivery system shown in Fig. 6.2 not only limits exposure of personnel in the room, but also generates a bolus of aerosol for inhalation by back-filling the inspiratory arm during expiration or breath pause.

Solutions for provocation testing should be made fresh daily. Histamine and methacholine powders should be stored below 0°C and allowed to reach room temperature in a desiccator before weighing. Solutions for nebulization should be allowed to reach room temperature. Cold solutions (i.e. solutions stored on ice or direct from the fridge) should not be used as they can induce bronchoconstriction.

It is good practice to first challenge with the vehicle solution (usually 0.9% saline or 0.9% phosphate-buffered saline) to ensure that the animal does not react to the vehicle itself. This is especially important when an animal reacts to the lowest dose of a provocative agent that has been administered. Without using a vehicle inhalation challenge, the proportion of the reaction that is as a result of the agent cannot be ascertained.

Sample protocol for histamine provocation testing
Requirements
- Equipment for measurement of lung function
- Nebulizer and delivery system
- Scavenging system or masks for personnel
- Balance to weigh histamine
- Timer (minutes and seconds).

Protocol
- Bronchoprovocation testing can be undertaken in unsedated or sedated horses but the results under the two conditions should not be compared within or between horses. Testing should not be performed less than 4 h after feeding.
- Weigh histamine and make up solutions.
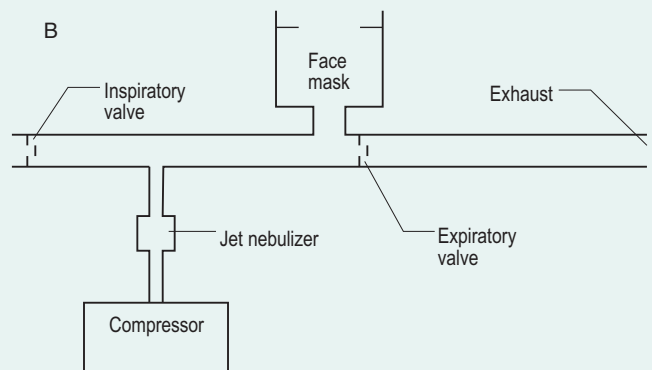
Fig. 6.2. (A,B) Closed system for bronchoprovocation testing using a Jet nebulizer.

- Fit equipment for pulmonary function measurement and establish baseline. A good baseline measurement is essential against which to judge responses. If in doubt about the quality of the measurement, make several.
- Nebulize saline for 2 min.
- Measure pulmonary function immediately after nebulization.
- Nebulize the lowest concentration of histamine (e.g. 1 mg/ml) for 2 min.
- Measure pulmonary function immediately after nebulization.
- Repeat with increasing doses of histamine 2, 4, 8, 16, and 32 mg/ml until the required response is obtained.

Box 15.6. Measurement of airway responsiveness—cont'd

Determination and expression of response

The dose at which an animal first responds provides the level of sensitivity whilst the slope of the dose–response curve is the reactivity. The reactivity is commonly expressed as the dose of bronchoprovocation agent resulting in a specific reduction in compliance or increase in resistance. For example, PC_{20} is used to indicate the dose of an agent resulting in a 20% decrease in pulmonary compliance, whilst PD_{200} would be used to indicate the dose of an agent resulting in a doubling of resistance. The precise values obtained depend on the health of the animals being studied, the type and efficiency of the nebulizer, the efficacy of the delivery system, the duration of nebulization, the range of doses used, the timings of the challenge protocol, and the sensitivity of the pulmonary function testing equipment.

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into the jugular vein. For studies performed in the resting horse, an infusion rate of 30 ml/min for 60 min has been employed (Hedenstierna et al 1987). At the end of the infusion period, mixed expired gas is collected into a heated mixing chamber and arterial and mixed venous blood samples are collected. The retention and excretion of each gas are determined by the ratios of arterial to mixed venous gas concentrations and mixed expired to mixed venous gas concentrations, respectively (Wagner et al 1974). The solubility of each gas in the blood of each horse is measured using a two-step procedure as described by Wagner et al (1974). The retention, excretion and solubility data are entered into a 50-compartment lung model with each compartment having a specific ventilation–perfusion ratio (Nyman & Hedenstierna 1989, Nyman et al 1991). Data for ventilation and blood flow are plotted against the logarithm of the ventilation–perfusion ratios. Ventilation–perfusion ratios have been determined during exercise as well as at rest using MIGET (Seaman et al 1995, Funkquist et al 1999, Nyman et al 1999). This research technique has been used to understand the matching of ventilation and blood flow in the resting horse and during exercise. It also demonstrated that horses with RAO have a large volume of lung that receives little blood flow and that they have a large volume of alveolar dead space (Nyman et al 1999).

Volumetric capnography

Volumetric capnography, which is also referred to as the single breath test for carbon dioxide (SBT-CO_2), has been used as an index of ventilation/perfusion matching in the horse (Herholz et al 2003b). The volumetric capnogram is a plot of the fraction of carbon dioxide in exhaled gas against exhaled breath volume (Fletcher et al 1981). Volumetric capnography has been performed using an ultrasonic pneumotachograph and an infra-red carbon dioxide analyzer in both unsedated and sedated horses

(Herholz et al 2001a,b,c, 2002a,b). Measurements of tidal volume and CO_2 fraction are recorded and the volumetric capnogram is generated off-line. Specific software is available (e.g. SBD-CO_2calc, Oberli-Engineering, Berne, Switzerland) to calculate physiological dead space volume, alveolar dead space volume and an index of CO_2 elimination. Volumetric capnography indices are of sufficient sensitivity to differentiate between healthy horses and asymptomatic RAO-affected horses (Herholz et al 2003b). This technique is attractive as it is non-invasive, can be performed in unsedated animals, and requires limited equipment. However, there is relatively little information regarding the sensitivity of this technique in comparison to other more commonly used pulmonary function tests in the horse, and this test has only become established for use by the group at University of Berne.

Scintigraphy

Imaging of pulmonary ventilation and perfusion can be performed by nuclear scintigraphy using radioactive tracers; this is fully described in Chapter 13.

Other Measurements of Lung Function

Alveolar clearance

The ability of inhaled technetium-99m–diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) to diffuse across the alveolar epithelium, which is a drawback when measuring ventilation, has been used to assess alveolar clearance (Votion et al 1998, 1999b). The permeability of the pulmonary vascular endothelium to ^{99m}Tc-DTPA is far greater than that of the alveolar epithelium such that the rate of clearance of ^{99m}Tc-DTPA reflects alveolar epithelial integrity. The alveolar clearance rate appears to be a sensitive indicator of alveolar epithelial damage in RAO (Votion et al 1999b).

Mucociliary clearance

The tracheal mucociliary clearance rate has been determined by following the transport of radiographic or radioactive markers up the trachea (Nelson & Hampe 1983, Dixon 1992). Colored markers, for example Indian ink in syrup, can also be placed in the distal trachea and their transport monitored with a bronchoscope (Turgut & Sasse 1989).

Pulmonary function measurement during exercise

Ventilation is the most common indicator of pulmonary function measured during exercise. Measurements of ventilation in horses during exercise are most commonly made during treadmill exercise but can be undertaken in the field using the Eco Medics Exhalyzer 5 system or a system produced by Cosmed (Rome, Italy). The latter system still needs careful validation for use in horses. Peak flow, tidal volume, respiratory frequency, minute ventilation, flow–volume loops and observations on respiratory–locomotory coupling may all provide information on pulmonary function. Measurements of gas exchange (oxygen uptake, carbon dioxide production and respiratory exchange ratio) and indicators of blood gas and acid–base status (arterial and mixed venous blood gases and blood lactate concentration) may also be informative. Measurements made during exercise may be helpful to identify mild disease or dysfunction (Connally & Derksen et al 1994, Couetil & Denicola 1999, Courouce-Malblanc et al 2002). For measurement of ventilation during exercise the Eco Medics Exhalyzer 5 and the BRDL flowmetrics ultrasonic pneumotachograph systems are highly suitable because of their very low resistance and dead space (Butler et al 1993). However, to estimate oxygen uptake and carbon dioxide production these systems need to be combined with a respiratory mass spectrometer. Alternatively, oxygen uptake and carbon dioxide production can be measured with open-flow systems, although these do not provide any information on ventilation.

Conclusions

Many of the pulmonary function tests that have been described either lack sensitivity for routine application in equine practice or require expensive and sophisticated equipment and are time consuming and technically challenging, which limits their use to referral centers or in research. However, with the development of new techniques such as FOM and IOS, it is conceivable that sensitive, specific and simple pulmonary function testing may become more accessible to clinicians in equine practice.

REFERENCES

Aguilera-Tejero E, Pascoe JR, Amis T C et al 1993 Measurement of pulmonary diffusing capacity for carbon monoxide and functional residual capacity during rebreathing in conscious thoroughbreds. American Journal of Veterinary Research 54: 1752–1757

Aguilera-Tejero E, Pascoe JR, Smith BL et al 1994 Evaluation of a technique for detection of pulmonary hemorrhage in horses, using carbon monoxide uptake. American Journal of Veterinary Research 55: 1032–1036

Amory H, Lomba F, Lekeux PM et al 1994 Bilateral diaphragmatic paralysis in a pony. Journal of the American Veterinary Medical Association 205: 587–591

Art T, Lekeux P 1988 Respiratory airflow patterns in ponies at rest and during exercise. Canadian Journal of Veterinary Research 52: 299–303

Broadstone RV, Gray PR, Robinson NE et al 1992 Effects of xylazine on airway function in ponies with recurrent airway obstruction. American Journal of Veterinary Research 53: 1813–1817

Butler PJ, Woakes AJ, Smale K et al 1993 Respiratory and cardiovascular adjustments during exercise of increasing intensity and during recovery in thoroughbred racehorses. Journal of Experimental Biology 179: 159–180

Comroe JH 1975 Pulmonary diffusing capacity for carbon monoxide (DLCO). American Review of Respiratory Diseases 111: 225–228

Connally BA, Derksen FJ 1994 Tidal breathing flow-volume loop analysis as a test of pulmonary function in exercising horses. American Journal of Veterinary Research 55: 589–594

Couetil LL, Denicola DB 1999 Blood gas, plasma lactate and bronchoalveolar lavage cytology analyses in racehorses with respiratory disease. Equine Veterinary Journal Supplement 30: 77–82

Couetil LL, Rosenthal FS, Simpson CM 2000 Forced expiration: a test for airflow obstruction in horses. Journal of Applied Physiology 88: 1870–1879

Couetil LL, Rosenthal FS, DeNicola DB et al 2001 Clinical signs, evaluation of bronchoalveolar lavage fluid, and assessment of pulmonary function in horses with inflammatory respiratory disease. American Journal of Veterinary Research 62: 538–546

Courouce-Malblanc A, Pronost S, Fortier G et al 2002 Physiological measurements and upper and lower respiratory tract evaluation in French Standardbred Trotters during a standardised exercise test on the treadmill. Equine Veterinary Journal Supplement 34: 402–407

Deaton CM, Marlin DJ, Schroter RC 2002 An improved technique for measuring functional residual capacity (FRC) in horses. Proceedings of the 20th Veterinary and Comparative Respiratory Society Symposium, p.50

Dixon PM 1992 Respiratory mucociliary clearance in the horse in health and disease, and its pharmaceutical modification. Veterinary Record 131: 229–235

Du Bois AB, Brody AW, Lewis DH et al 1956 Oscillation mechanics of lungs and chest in man. Journal of Applied Physiology 19: 653–658

Fletcher R, Jonson B, Cumming G et al 1981 The concept of deadspace with special reference to the single breath test for carbon dioxide. British Journal of Anaesthesia 53: 77–88

Funkquist P, Wagner PD, Hedenstierna G et al 1999 Ventilation-perfusion relationships during exercise in standardbred trotters with red cell hypervolaemia. Equine Veterinary Journal Supplement 30: 107–113

Gallivan GJ, Viel L, McDonell WN 1990 An evaluation of the multiple-breath nitrogen washout as a pulmonary function test in horses. Canadian Journal of Veterinary Research 54: 99–105

Gillespie J 1974 The role of the respiratory system during exertion. Journal of the South African Veterinary Association 45: 305–309

Gillespie JR, Tyler WS 1968 Chronic alveolar emphysema in the horse. Advanced Veterinary Science and Comparative Medicine 13: 59–99

Guthrie AJ, Beadle RE, Bateman RD et al 1995a Characterization of normal tidal breathing flow-volume loops for thoroughbred horses. Veterinary Research Communications 19: 331–342

Guthrie AJ, Beadle RE, Bateman RD et al 1995b The effects of three models of airway disease on tidal breathing flow-volume loops of thoroughbred horses. Veterinary Research Communications 19: 517–527

Gutting SM, Forster HV, Lowry TF et al 1991 Respiratory muscle recruitment in awake ponies during exercise and CO_2 inhalation. Respiratory Physiology 86: 315–332

Hedenstierna G, Nyman G, Kvart C et al 1987 Ventilation-perfusion relationships in the standing horse: an inert gas elimination study. Equine Veterinary Journal 19: 514–519

Herholz C, Straub R, Busato A 2001a Ultrasound-spirometry and capnography in horses: analysis of measurement reliability. Veterinary Research Communications 25: 137–147

Herholz C, Straub R, Busato A 2001b The variability and repeatability of indices derived from the single-breath diagram for CO_2 in horses with chronic obstructive pulmonary disease and the effect of lobelin hydrochloride on these indices. Veterinary Research Communications 25: 401–412

Herholz C, Straub R, Moens Y et al 2001c Statistical shape analysis of volumetric capnograms: evaluation of a new approach for the assessment of pulmonary function in horses with chronic obstructive pulmonary disease. Journal of Veterinary Medicine Series A – Physiology Pathology Clinical Medicine 48: 75–84

Herholz C, Straub R, Luthi S et al 2002a Differences in pulmonary functional indices derived from the single-breath diagram for CO_2 (SBD-CO_2) in horses related to age, sex and usage. Veterinary Research Communications 26: 467–478

Herholz C, Straub R, Luthi S et al 2002b Validity of pulmonary function indices derived from the volumetric capnogram in horses with recurrent airway obstruction (RAO). Research in Veterinary Science 72: 141–146

Herholz C, Straub R, Braendlin C et al 2003a Measurement of tidal breathing flow-volume loop indices in horses used for different sporting purposes with and without recurrent airway obstruction. Veterinary Record 152: 288–292

Herholz CP, Gerber V, Tschudi P et al 2003b Use of volumetric capnography to identify pulmonary dysfunction in horses with and without clinically apparent recurrent airway obstruction. American Journal of Veterinary Research 64: 338–345

Hoffman A, Kuehn H, Riedelberger K et al 2001 Flowmetric comparison of respiratory inductance plethysmography and pneumotachography in horses. Journal of Applied Physiology 91: 2767–2775

Klein HJ, Deegen E 1986 Histamine inhalation provocation test: method to identify nonspecific airway reactivity in equids. American Journal of Veterinary Research 47: 1796–1800

Lavoie JP, Pascoe JR, Kurpershoek CJ 1992 Effect of head and neck position on respiratory mechanics in horses sedated with xylazine. American Journal of Veterinary Research 53: 1652–1657

LeBlanc PH, Eberhart SW, Robinson NE 1993 In vitro effects of alpha 2-adrenergic receptor stimulation on cholinergic contractions of equine distal airways. American Journal of Veterinary Research 54: 788–792

Lumsden JM, Derksen FJ, Stick JA et al 1993 Use of flow-volume loops to evaluate upper airway obstruction in exercising standardbreds. American Journal of Veterinary Research 54: 766–775

Marlin DJ, Schrotert RC, Cashman PM et al 2002 Movements of thoracic and abdominal compartments during ventilation at rest and during exercise. Equine Veterinary Journal Supplement 34: 384–390

Mauderley JL 1974 Evaluation of the grade pony as a pulmonary function model. American Journal of Veterinary Research 35: 1024–1029

Mazan MR, Hoffman AM, Manjerovic N 1999 Comparison of forced oscillation with the conventional method for histamine bronchoprovocation testing in horses. American Journal of Veterinary Research 60: 174–180

McDonell WN, Hall LW, Jeffcott LB 1979 Radiographic evidence of impaired pulmonary function in laterally recumbent anaesthetised horses. Equine Veterinary Journal 11: 24–32

Miller C, Hoffman AM, Hunter J 2000 Thoracoabdominal asynchrony failed to grade airway obstructions in foals. Journal of Applied Physiology 88: 2081–2087

Nelson R, Hampe DW 1983 Measurement of tracheal mucous transport rate in the horse. American Journal of Veterinary Research 44: 1165–1166

Nyman G, Hedenstierna G 1989 Ventilation-perfusion relationships in the anaesthetised horse. Equine Veterinary Journal 21: 274–281

Nyman G, Lindberg R, Weckner D et al 1991 Pulmonary gas exchange correlated to clinical signs and lung pathology in horses with chronic bronchiolitis. Equine Veterinary Journal 23: 253–260

Nyman G, Bjork M, Funkquist P 1999 Gas exchange during exercise in standardbred trotters with mild bronchiolitis. Equine Veterinary Journal Supplement 30: 96–101

Olszewski MA, Zhang XY, Robinson NE 1999 Pre- and post-junctional effects of inflammatory mediators in horse airways. American Journal of Physiology 277: L327–L333

Petsche VM, Derksen FJ, Robinson NE 1994 Tidal breathing flow-volume loops in horses with recurrent airway obstruction (heaves). American Journal of Veterinary Research 55: 885–891

Rollin F, Desmecht D, Verbanck S et al 1996 Multiple-breath washout and washin experiments in steers. Journal of Applied Physiology 81: 957–963

Roy R, Powers SR Jr, Kimball WR 1974 Estimation of respiratory parameters by the method of covariance ratios. Computers in Biomedical Research 7: 21–39

Seaman J, Erickson BK, Kubo K et al 1995 Exercise induced ventilation/perfusion inequality in the horse. Equine Veterinary Journal 27: 104–109

Soma LR, Beech J, Gerber NH 1987 Effects of cromolyn in horses with chronic obstructive pulmonary disease. Veterinary Research Communications 11: 339–351

Stadler P, Deegen E 1986 Diurnal variation of dynamic compliance, resistance and viscous work of breathing in normal horses and horses with lung disorders. Equine Veterinary Journal 18: 171–178

Tomasic M, Mann LS, Soma LR 1997 Effects of sedation, anesthesia, and endotracheal intubation on respiratory mechanics in adult horses. American Journal of Veterinary Research 58: 641–646

Turgut K, Sasse HH 1989 Influence of clenbuterol on mucociliary transport in healthy horses and horses with chronic obstructive pulmonary disease. Veterinary Record 125: 526–530

van Erck E, Votion D M, Kirschvink N et al 2003 Use of the impulse oscillometry system for testing pulmonary function during methacholine bronchoprovocation in horses. American Journal of Veterinary Research 64: 1414–1420

van Erck E, Votion D, Art T et al 2004a Measurement of respiratory function by impulse oscillometry in horses. Equine Veterinary Journal 36: 21–28

van Erck E, Votion D, Kirschvink N et al 2004b Influence of breathing pattern and lung inflation on impulse oscillometry measurements in horses. Veterinary Journal 168: 259–269

Votion D, Vandenput S, Duvivier H et al 1997 Analysis of equine scintigraphical lung images. Veterinary Journal 153: 49–61

Votion D, Vandenput S, Duvivier D H et al 1998 Scintigraphical evaluation of alveolar clearance in horses. Veterinary Journal 156: 51–58

Votion D, Ghafir Y, Vandenput S et al 1999a Analysis of scintigraphical lung images before and after treatment of horses suffering from chronic pulmonary disease. Veterinary Record 144: 232–236

Votion D, Vandenput S N, Duvivier D H et al 1999b Alveolar clearance in horses with chronic obstructive pulmonary disease. American Journal of Veterinary Research 60: 495–500

Wagner PD, Naumann PF, Laravuso RB 1974 Simultaneous measurement of eight foreign gases in blood by gas chromatography. Journal of Applied Physiology 36: 600–605

Young SS, Hall LW 1989 A rapid, non-invasive method for measuring total respiratory impedance in the horse. Equine Veterinary Journal 21: 99–105

Young SS, Tesarowski D 1994 Respiratory mechanics of horses measured by conventional and forced oscillation techniques. Journal of Applied Physiology 76: 2467–2472

Young SS, Tesarowski D, Viel L 1997 Frequency dependence of forced oscillatory respiratory mechanics in horses with heaves. Journal of Applied Physiology 82: 983–987

16 Treadmill Endoscopy

Safia Barakzai

High-speed treadmill examination of the equine athlete has become an indispensable tool for assessment of the upper respiratory tract (URT), for both clinical and research purposes. The use of high-speed treadmill endoscopy (HSTE) has provided clinicians with a gold standard for accurately identifying dynamic causes of upper airway obstruction which are not present at rest, such as intermittent dorsal displacement of the soft palate, vocal fold or arytenoid cartilage collapse, nasopharyngeal collapse, intermittent epiglottal entrapment, axial deviation of the aryepiglottic folds, and epiglottic retroversion. These conditions could previously only be speculated upon in horses that made abnormal respiratory noises during exercise. Endoscopic examination during nasal occlusion or after swallowing at rest, or immediately post exercise may alert the clinician's suspicions to the possibility of various disorders occurring at exercise, but they do not accurately replicate the appearance and function of the URT at exercise. However, even treadmill testing does not reproduce racing conditions, so that when horses are taken to the point of fatigue on a treadmill, the level of exertion may not replicate that of a race. Factors such as the weight of the jockey, the excitement or stress of race day and varying underfoot conditions cannot be reproduced in an exercise laboratory, and this may explain why an endoscopic diagnosis is not achieved during HSTE for a proportion of horses with a history of abnormal respiratory noise and poor performance during racing (Lane 1999).

"Dynamic" disorders of the URT only become apparent during exercise because of the dramatically increased volume of air moving through the tract at this time – from 4 liters/second in a resting horse to approximately 75 liters/second in a galloping horse (Parente 1997). To transport such sizeable volumes of air in and out of the lungs and meet the horse's increased oxygen requirement, large subatmospheric pressures must be generated within the respiratory tract during inspiration. These subatmospheric pressures tend to collapse the non-rigid parts of the upper airway such as the nasopharynx and larynx, and active muscular effort is required to resist such collapse and maintain an adequate, functional airway.

HSTE is also useful in horses that have previously undergone surgery of the URT, for both clinical cases and research work (Stick & Derksen 1989, Kannegieter & Dore 1995, Tetens et al 1996, Ducharme et al 2003). In fact repeated pre- and post-treatment HSTE represents the gold standard for assessment of the efficacy of any medical or surgical treatment for naturally occurring dynamic disorders of the URT. However, as a result of practical and economic considerations, this is unfortunately rarely possible in clinical practice (Franklin et al 2002, Barakzai et al 2003).

HSTE Equipment, Patient Preparation, and Training

Conducting a high-speed treadmill examination is a labor-intensive procedure that requires experienced personnel and expensive equipment. A minimum of three handlers (plus the endoscopist) are necessary – one to operate the treadmill, one to steady the horse's head and one to stand at the side of the horse and encourage it to move forward. It is strongly recommended that handlers positioned in the immediate vicinity of the exercising horse should wear safety helmets.

The treadmill itself should be housed in a room large enough to safely maneuver both the animal and the equipment. For racehorses, the treadmill must be capable of speeds of up to 14 m/second and an incline of up to 10° uphill, to replicate racing conditions as accurately as possible (Fig. 16.1). The use of an incline allows the animal to perform increased work effort at submaximal speeds. Rails should be present on either side of the treadmill belt to maintain the horse's position and protect handlers. Cooling fans (either fixed overhead or moveable units which can be positioned in front of the horse) must be used in warm weather to increase sweat evaporation in the exercising horse and prevent hyperthermia.

Before any horse is exercised on a treadmill, a full clinical examination should be performed, including examination for lameness, to rule out conditions that may render the horse unsuitable for high-speed exercise. A resting endoscopic examination may reveal obvious structural or functional abnormalities in some horses, which will then not require an HSTE examination to make a definitive diagnosis. Ideally, the horse should be fit at the time of examination, as this maximizes the chance of

Fig. 16.1. Horse galloping on the treadmill while undergoing HSTE. Note the uphill incline used to maximize work effort.

diagnosing a suspected problem, and minimizes the risk of injury or of postexercise myopathy. If the horse is not fit, this should be taken into account during the exercise sessions. Shoes should be left on, and loose shoes should be re-fitted because if the horse "throws" a shoe whilst exercising on the treadmill, the fast-moving flying metal object can cause serious injury to personnel. Neoprene exercise boots are worn on all four lower limbs, and rubber over-reach boots on the front feet, to prevent injuries caused by interference. If a heart rate monitor is to be used, this is positioned under the safety harness, which is placed around the horse's girth. This harness can be attached to the overhead safety "cut-out" line which will stop the treadmill belt if for some reason excessive tension is placed upon it, for example if the horse falls or does not keep up with the treadmill speed, causing it to be carried backwards on the belt. A nylon halter or a bridle may be used on the horse's head, depending on its temperament and thus the amount of control required. Hobbles and harness are recommended for pacing standardbreds and other horses which usually run in such equipment, such as Norwegian coldblooded trotters.

Horses should be acclimatized to the treadmill before HSTE is performed. This usually requires admission of the horse to the hospital so that between one and three training sessions can be scheduled, although in some clinics, treadmill testing is performed on an outpatient basis. The length of time required to acclimatize to the treadmill varies widely between individual horses, and

horses should be comfortable working at a variety of different gaits (including galloping at close to maximal speed) with smooth transitions between gaits before HSTE is attempted.

Exercise test protocol

There are many variations in testing protocol between the clinics that perform HSTE (Morris 1991, Parente 1996, Ducharme et al 1998). Protocols involving evaluating horses when exercising at various percentages of maximum heart rate (HR_{max}) require a separate exercise test to be performed initially to establish what this value is, and are therefore not as useful as alternative methods for evaluation of clinical cases. Use of a heart rate monitor is, however, recommended to determine the horse's heart rate relative to its speed. A heart rate of approximately 220 beats/min (bpm) guarantees that the horse is at maximal exertion (Parente 1996), and heart rates in excess of 240 bpm suggest that the test should be terminated (Ducharme et al 1998).

A rapid incremental test is usually considered to be the most useful for clinical HSTE evaluations, particularly for National Hunt-type racehorses, which race over long distances. A typical protocol requires a heart rate monitor and is given in Table 16.1.

For racehorses that sprint over short distances, a thorough warm-up period followed by a very rapid acceleration to close to maximal speed (usually 12–14 m/second)

Table 16.1. A typical protocol for rapid incremental high speed treadmill testing

Phase 1: Warm up – 2 m/s for 4 min, 4.5 m/s for 1 min, 7 m/s for 2 min

Phase 2: Walk until heart rate < 70 bpm

Phase 3: Gradually accelerate to 9 m/s, incline treadmill to 3° (for thoroughbreds).
Then accelerate to 11 m/s for 600 m, 12 m/s for 600 m, 14 m/s for 1,600 m before decelerating to 12 m/s for 600 m.

Most horses will not be capable of completing all these steps as described, but they should be exercised as close to the desired speed as possible for the predetermined distances.

Reproduced from Parente 1996, with permission.

Table 16.2. Submaximal exercise test, suitable for unfit racehorses or yearlings

- The horse is worked at 4 m/s for the first 4 min, of which 3 min is performed on the flat, and the final minute is performed up a 3°–5° incline.
- The treadmill is then accelerated to 6 m/s for 800 m, 8 m/s for 800 m and 8.5 m/s for 1,600 m or until the animal fatigues.

Reproduced from Ducharme et al 1998, with permission.

Fig. 16.2. Attachment of endoscope to the halter using Velcro.

up an incline, which is maintained until the horse begins to fatigue, may be more useful to mimic racing conditions and reduce the risk of musculoskeletal injury.

Unfit racehorses (especially yearlings) or show horses can be worked using a submaximal test (Table 16.2) (Ducharme et al 1998).

High-speed treadmill video-endoscopy

Video-endoscopic equipment with either digital or video recording is essential if HSTE is to be performed because very rapid movements of the nasopharynx and larynx are often seen more clearly during a slow-motion playback. After the horse has undergone a warm-up period, the treadmill is stopped, a twitch is applied if necessary, and a flexible video-endoscope is passed up the right ventral meatus. The end of the endoscope should be positioned just rostral to the epiglottis to allow clear visualization of the caudal nasopharynx and larynx. Many methods have been used to secure the endoscope in the nasopharynx by attaching it to the head collar, including Velcro straps and use of a latex Penrose drain (Fig. 16.2). Some movement of the end of the endoscope within the nasopharynx is inevitable, and high-quality video-endoscopic equipment is necessary to minimize the detail lost by movement blur.

Adjunctive diagnostic tests

There are many additional techniques that can be performed on horses undergoing HSTE which include recording of respiratory noises made at exercise, intrapharyngeal and intratracheal pressure measurements, respiratory gas analysis, collection of arterial and venous blood samples, exercise electrocardiograms, and high-speed gait analysis (Morris & Seeherman 1991, Parente 1996, 1997, Ducharme et al 1998, Franklin et al 2003).

Normal Endoscopic Appearance of the URT During HSTE

During exercise, the arytenoid cartilages should attain and maintain full abduction (most of the corniculate process lies horizontally at 90° to the midline of the rima glottidis) bilaterally and no movement of the arytenoid cartilages (apart from during swallowing) should occur until ventilation returns to resting levels (Fig. 16.3). The roof of the nasopharynx is normally displaced ventrally at the end of expiration (Morris & Seeherman 1988) and this finding should only be considered abnormal if it obstructs more
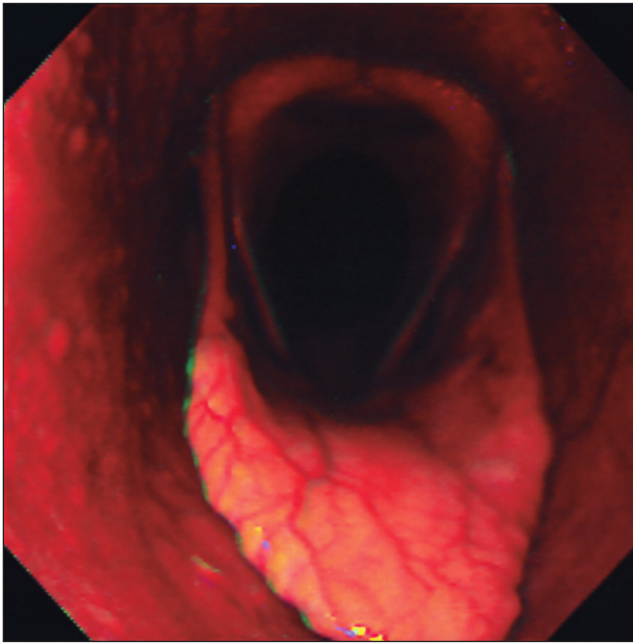
Fig. 16.3. Normal larynx at exercise. Note the bilaterally symmetrically fully abducted arytenoids.

than one-third of the rima glottidis (Ducharme et al 1998). The horse remains able to swallow during strenuous exercise, during which full adduction of both arytenoids should occur transiently, followed by a rapid return to the fully abducted position. During swallowing, the constrictor action of the circular muscles of the pharyngeal walls may be evident very transiently. This can be differentiated from cases of circumferential nasopharyngeal collapse by the timing and transient duration of the constriction during swallowing. Normal horses may swallow several times during submaximal exercise, but repeated swallowing usually indicates an irritating stimulus (Morris 1991).

Dynamic Obstructions of the URT

Intermittent dorsal displacement of the soft palate (DDSP)

Intermittent DDSP is the most commonly diagnosed cause of URT obstruction in horses undergoing HSTE examination for investigation of poor performance (Morris & Seeherman 1991, Lane 1999). Parente and Martin (1995) found that many horses which dorsally displaced their soft palates during nasal occlusion at rest (Fig. 16.4) did not experience DDSP at exercise, and conversely, some horses that did not displace their soft palates during nasal occlusion at rest did develop DDSP during HSTE. Therefore, the overall correlation between resting and HSTE findings with respect to DDSP is poor.

The incidence of ulceration of the caudal border of the soft palate in horses that experience DDSP at exercise is historically reported to be high (up to 90%; Hogan et al 2002, Rodgerson 2004). In contrast, studies that have definitively diagnosed DDSP using HSTE have found a much lower incidence of soft palate ulceration in DDSP-affected horses (10%; Kannegieter & Dore 1995, Parente et al 2002). It is possible that these differences may reflect breed differences in the study population of these authors. Epiglottic hypoplasia and flaccidity have also been reported to be associated with intermittent DDSP (Tulleners et al 1997, Robertson 1998), but many such studies have not confirmed using HSTE that DDSP occurs. There are clinical and experimental data showing that the epiglottis is not essential to maintain the palate in a normal sub-epiglottic position (Holcombe et al 1997, Parente et al 1998), and other studies that have confirmed all DDSP cases using HSTE have found that epiglottic length and/or appearance in the vast majority of these horses is normal (Kannegieter & Dore 1995, Rehder et al 1995, Parente et al 2002). In addition, research has shown that contraction of the hyoepiglotticus muscles (whose activity increases
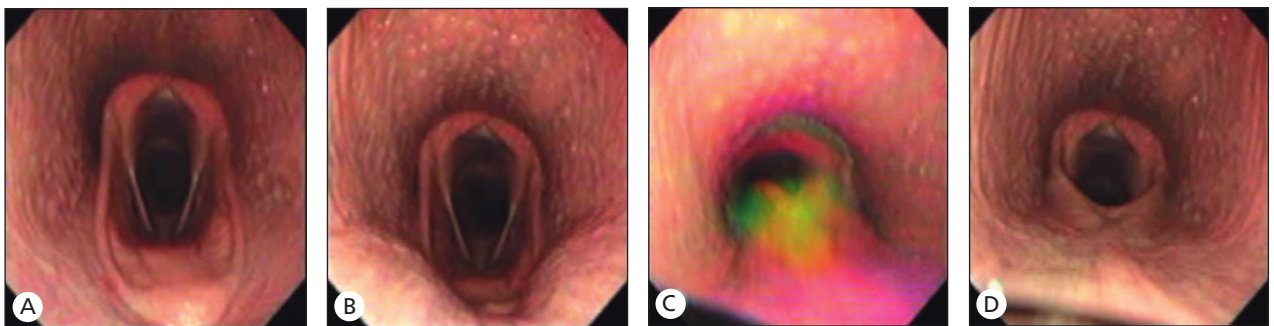
Fig. 16.4. DDSP induced by nasal occlusion at rest. (A) The epiglottis appears to be pulled ventrally by the action of the hyoepiglotticus muscles. (B) The soft palate begins to billow dorsally during inspiration. (C) The palate flips up over the epiglottis. (D) The caudal border of the palate has displaced dorsal to the epiglottis, and remains displaced despite numerous attempts at swallowing.
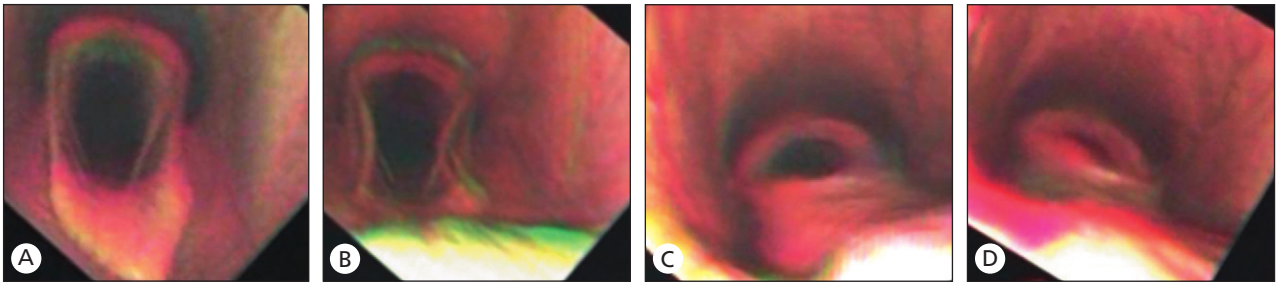
Fig. 16.5. Sequence of events related to DDSP. (A) Larynx appears normal at start of exercise. (B) Palatal instability of the soft palate. Note the moderate bilateral axial displacement of the aryepiglottic folds as the epiglottic tip is lifted dorsally by the billowing soft palate. (C) and (D) DDSP has now occurred – during inspiration, the displaced palate is "sucked" progressively dorsally, almost completely obstructing the rima glottidis. Reproduced from Barakzai 2006, with permission.

with increased intensity of breathing) causes conformational changes of the epiglottis in some horses, where the epiglottis assumes a similar shape to that described in dynamic epiglottic hypoplasia or epiglottic flaccidity (Dixon 1995, Holcombe et al 2002). It has been hypothesized that dynamic changes in the appearance of the epiglottis during nasal occlusion or exercise may simply be a result of the normal physiologic action of the hyoepiglotticus muscle and not a conformational abnormality of the epiglottis (Holcombe et al 2002).

Palatal instability (Fig. 16.5) (vertical "billowing" of the soft palate without displacement of its caudal border) is a common endoscopic observation before the occurrence of DDSP (Kannegieter & Dore 1995, Lane 1999, PM Dixon personal communication). This wave-like billowing is thought to begin at the junction of the hard and soft palates, and progresses caudally (Lane 1999). As the soft palate "billows" dorsally, it may lift the epiglottis and in turn cause a degree of axial deviation of the aryepiglottic folds prior to DDSP occurring (Fig. 16.5; Dart et al 2001, Rodgerson 2004, PM Dixon personal communication). Some horses may displace the base of the epiglottis for a time before the soft palate fully displaces (Fig. 16.6) (Parente 1997).

After the soft palate becomes displaced dorsal to the epiglottis, it causes a marked obstruction of the rima glottidis (Fig. 16.5) and affected horses usually make a loud expiratory, and possibly an inspiratory, "gurgle" and are often unable to continue galloping at high speed. Interestingly, in one study, 38% of horses (and particularly standardbreds) that experienced DDSP at exercise on the treadmill did not have a history of abnormal respiratory noise at exercise (Parente et al 2002). Clinically normal horses may transiently displace their soft palates at exercise but will typically swallow and replace the palate to a normal subepiglottic position quickly.

It should be remembered that a large number of other disorders such as epiglottal entrapment, subepiglottic cysts, epiglottitis, congenital and iatrogenic soft palate defects and
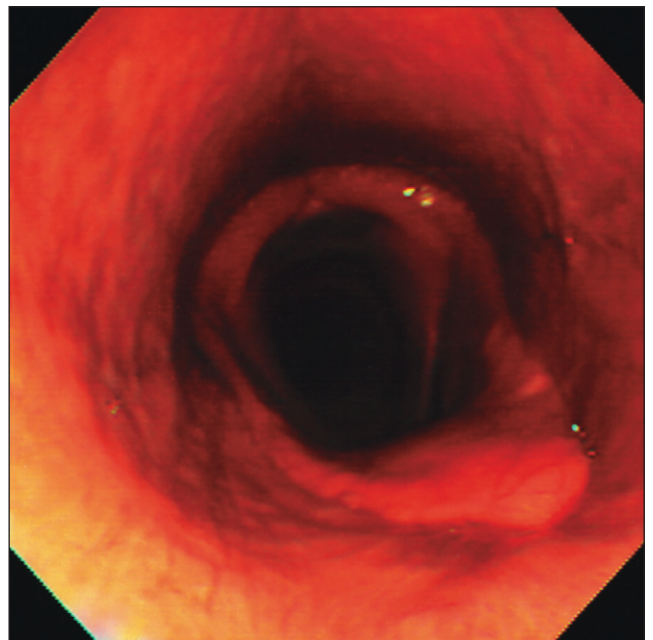
Fig. 16.6. The base of the epiglottis has "sunk" below the caudal border of the soft palate prior to DDSP occurring.

palatal cysts may predispose horses to intermittent DDSP, and therefore a thorough examination of the entire region should be completed before electing to perform surgery on the soft palate or adjacent structures.

Recurrent laryngeal neuropathy (RLN)

RLN is the second most commonly diagnosed cause of URT obstruction in horses undergoing HSTE examination for investigation of poor performance. Endoscopic grading of laryngeal function at rest is described in Chapter 34. Endoscopic grading of laryngeal function at exercise can be categorized as grade A (Fig. 16.3), B (Fig. 16.7) or C
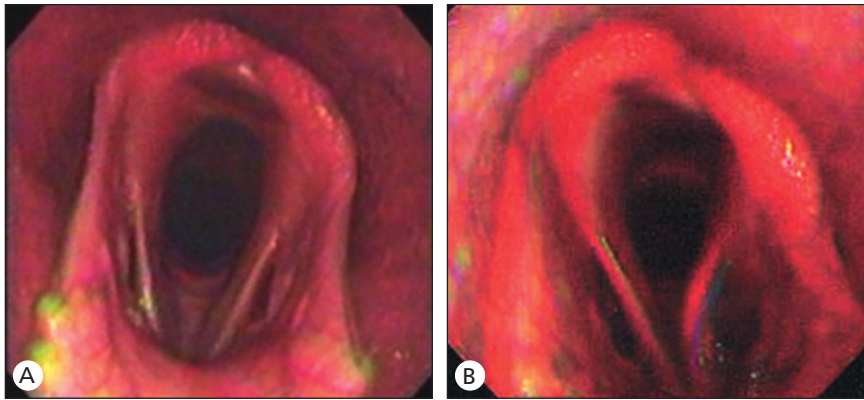
Fig. 16.7. Two horses during HSTE with grade B laryngeal function – partial abduction of the left arytenoid is maintained at maximal exertion in both. (A) No vocal fold collapse has occurred. (B) The left vocal fold is collapsing axially to cause respiratory obstruction and abnormal noise. Reproduced from Barakzai 2006, with permission.

(Figs 16.8 and 16.15), and definitions of these grades are shown in Table 16.3.

The endoscopic grade of laryngeal function at rest (I–IV) (see Chapter 34) will usually give an indication of laryngeal function at exercise, with the majority of resting grade I and II horses (i.e. those which are able to attain and maintain full bilateral arytenoid abduction) being classified as grade A at exercise (Parente & Martin 1995, Lane 2003), and all those with grade IV RLN at rest being classified as grade C at exercise (Morris & Seeherman 1990, Hackett et al 1998, Lane 2003). Occasionally, horses with grade I or II RLN at rest may experience some degree of collapse of the arytenoid cartilage or vocal fold during HSTE (Kannegieter & Dore 1995, Lane 2003). Therefore the presence of exercise-related dynamic RLN cannot be completely ruled out on the basis of resting endoscopic findings alone, and if abnormal inspiratory sounds are present during exercise, clinicians who do not have facilities for treadmill testing should not immediately discount RLN as a cause of URT obstruction in horses with grade I and II RLN at rest.

HSTE is particularly useful for evaluation of horses with resting grade III RLN. One study which evaluated 26 racehorses with resting grade III RLN found that at maximal exercise (12 m/second for standardbreds and 14 m/second for thoroughbreds), 4% were grade A, 19% were grade B, and 77% were grade C (Hammer et al 1998). Conversely, another study (Rakestraw et al 1991) found that five out of six horses with resting grade III RLN were able to maintain full abduction at exercise, but these horses
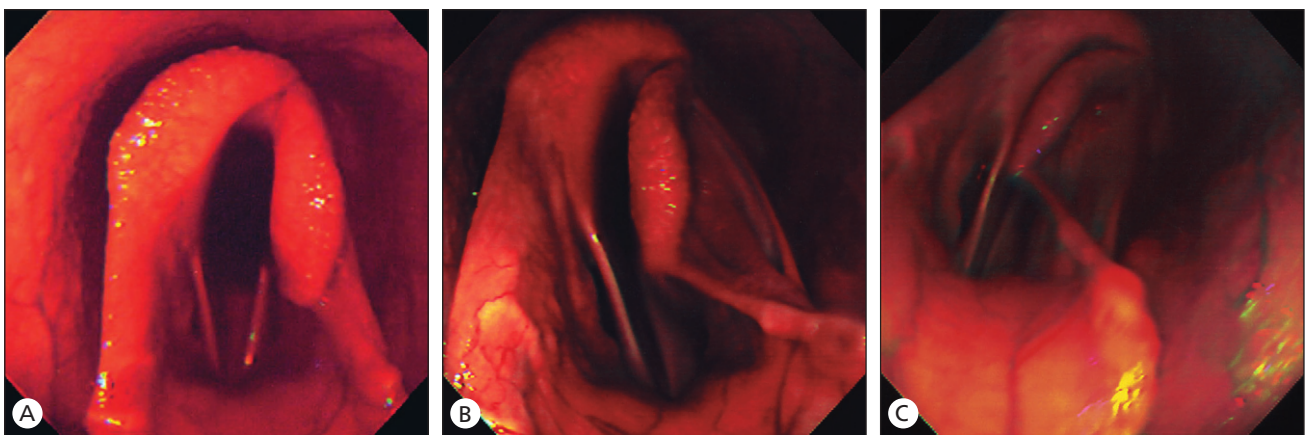
Fig. 16.8. (A–C) Horse with grade C laryngeal function shown during HSTE. There is progressive complete collapse of the left arytenoid across the midline until it completely obstructs the contralateral side of the rima glottidis during inspiration.

Table 16.3. Grading system of laryngeal function*
as assessed in the horse during exercise†

Laryngeal grade	Definition
A	Full abduction of the arytenoid cartilages during inspiration.
B	Partial abduction of the affected arytenoid cartilages (between full abduction and the resting position).
C	Abduction less than resting position including collapse into the contralateral half of the rima glottidis during inspiration.

*Description generally refers to the left arytenoid cartilage in reference to the right. However, this grading system can apply to the right side (i.e. right grade III.1-B).
†Updated from Rakestraw et al 1991, with permission.

only ran at a maximum speed of 8.5 m/second. Horses with grade B RLN during exercise often have the same or a better degree of arytenoid abduction than that obtained after laryngoplasty (Dixon et al 2003). It has also been reported that some horses may maintain adequate abduction of the arytenoid cartilage during exercise but that the vocal fold and laryngeal ventricle of the affected side can collapse axially on inspiration and partially obstruct the airway (Fig. 16.7B) (Kannegieter & Dore 1995, Dart et al 2001). In these cases, vocalcordectomy or ventriculocordectomy should be an effective surgical treatment and is associated with fewer complications than prosthetic laryngoplasty (Lane 1993, Ducharme et al 1998, Barakzai & Dixon 2003). Therefore, for horses with resting grade III RLN, HSTE allows differentiation between horses that do and do not require surgery, and if surgery is required, will guide the surgeon as to which surgical procedure may be most suitable (Stick & Derksen 1989, Ducharme et al 1998, Barakzai & Dixon 2003).

Intermittent epiglottal entrapment

Entrapment of the epiglottic cartilage in the glossoepiglottic and aryepiglottic folds is commonly diagnosed in the resting horse but occasionally occurs intermittently as a dynamic cause of respiratory obstruction at exercise (Morris & Seeherman 1990, 1991, Kannegieter & Dore 1995) (Fig. 16.9). Horses with epiglottal entrapment may be asymptomatic or demonstrate a range of clinical signs. Respiratory noise varies from a vibrant expiratory noise to both inspiratory and expiratory noises or no abnormal noise at all during fast work (Morris 1991). Similarly, some cases of epiglottal entrapment do not appear to impair ventilation, even at maximal exercise and this is thought to be related to individual variation in the tightness of the entrapping fold of mucosa (Morris 1991). During inspiration, the subatmospheric pressures within the airway are greater dorsal to the entrapping fold than ventral to it, and this pressure differential will force the entrapping fold to be drawn ventrally, towards the epiglottis (Morris & Seeherman 1990). During expiration, the entrapping membranes fill with air, ballooning outwards and significantly obstructing airflow in many cases. Epiglottal entrapment, whether intermittent or persistent, is frequently associated with DDSP during exercise (Kannegieter & Dore 1995).
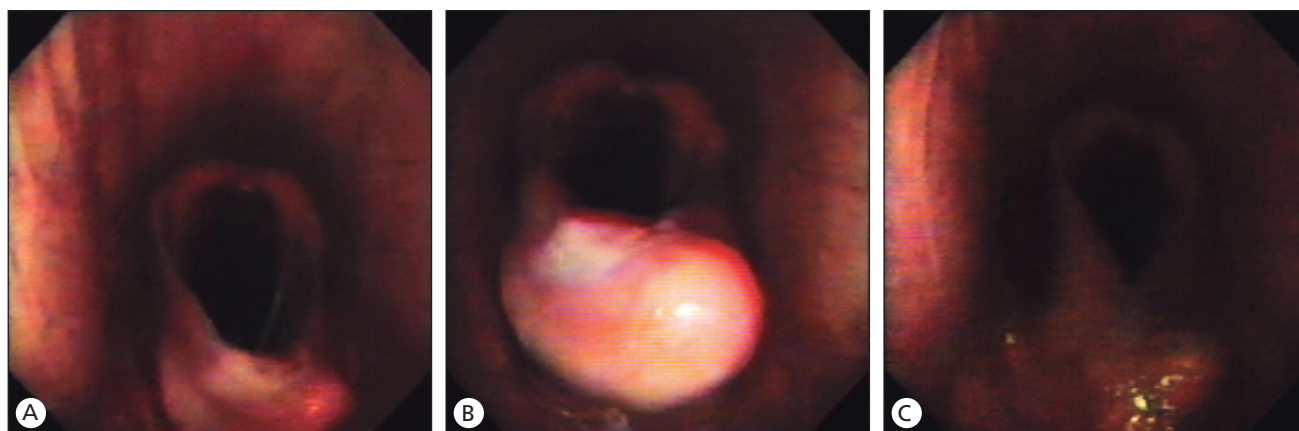
Fig. 16.9. Horse with epiglottal entrapment during HSTE: (A) during inspiration, normal epiglottic outline is not visible because of the entrapping mucosal fold; (B) during expiration, the entrapping mucosa fills with air and causes significant airway obstruction; (C) epiglottal entrapment predisposes to DDSP which has occurred in this photograph. The tip of the entrapped epiglottis can be seen bulging dorsally against the ventral aspect of the soft palate.
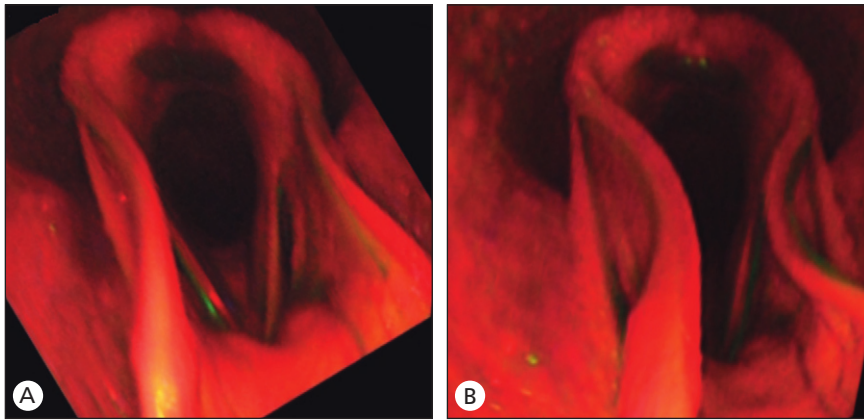
Fig. 16.10. ADAF: (A) bilateral ADAF – the aryepiglottic fold does not pass the line of the vocal fold; (B) severe bilateral right-sided ADAF – the aryepiglottic fold has moved halfway between the vocal cord and midline.

Axial deviation of the aryepiglottic folds (ADAF)

In ADAF, the membranous portion of the aryepiglottic fold, extending between the lateral corniculate process of the arytenoid cartilage and the lateral edge of the epiglottis, deviates axially during inspiration at strenuous exercise (Kannegieter & Dore 1995, King et al 2001). The aryepiglottic folds are usually tensed between the arytenoids and the epiglottis, and slight loss of abduction of the former or elevation of the latter will remove tension from these folds and so predispose to ADAF. This disorder is observed in many exercising horses to a mild, clinically insignificant degree (Fig. 16.10A) but occasionally can be characterized as moderate or severe (Fig. 16.10B) and cause significant airway obstruction (Table 16.4). The degree of deviation in some horses can progress from mild through to severe, depending on the degree of fatigue (King et al 2001). In horses with ADAF during HSTE, concurrent dynamic upper airway disorders have been reported in 36.5% of cases (King et al 2001). These include axial collapse of one or both vocal cords, left laryngeal hemiparesis, intermittent DDSP, right laryngeal dysfunction and dorsal pharyngeal collapse, although the relationship between ADAF and these concurrent dynamic URT disorders is unknown.

Nasopharyngeal collapse

The caudal two-thirds of the nasopharynx lack rigid support and rely on neuromuscular function to resist collapse during inspiration (Strand & Staempfli 1993). Dynamic nasopharyngeal collapse is, therefore, thought to be related to neuromuscular dysfunction of the nasopharynx. It can be classified as lateral, circumferential

Table 16.4. Definition of mild, moderate and severe ADAF (King et al 2001)		
Grade of ADAF	**Definition of ADAF grade**	**% obstruction of glottis**
Mild	Axial collapse of one or both aryepiglottic folds, with folds remaining abaxial to the vocal cords	<20%
Moderate	Axial deviation of one or both aryepiglottic folds less than halfway between the vocal cord and midline	21–40%
Severe	Collapse of one or both aryepiglottic folds more than halfway between the vocal cord and midline	41–63%

Reproduced from King et al 2001, with permission.

(Fig. 16.11) or dorsoventral (Fig. 16.12) (Parente 1997). Collapse of the roof of the nasopharynx is considered abnormal if it obstructs more than one-third of the rima glottidis (Ducharme et al 1998). Any lateral collapse of the nasopharynx that encroaches over the rima glottidis is also considered abnormal (Ducharme et al 1998). In more severely affected horses, nasopharyngeal collapse is usually associated with abnormal respiratory noises, which may vary from a low-intensity, low-pitched "grunt" to a vibrant inspiratory and/or expiratory "snore" or "gurgle". Nasopharyngeal collapse may occasionally be associated with flexion of the head and neck, and affected horses may appear normal when the head and neck are extended during HSTE (Dixon & Barakzai, personal observations).
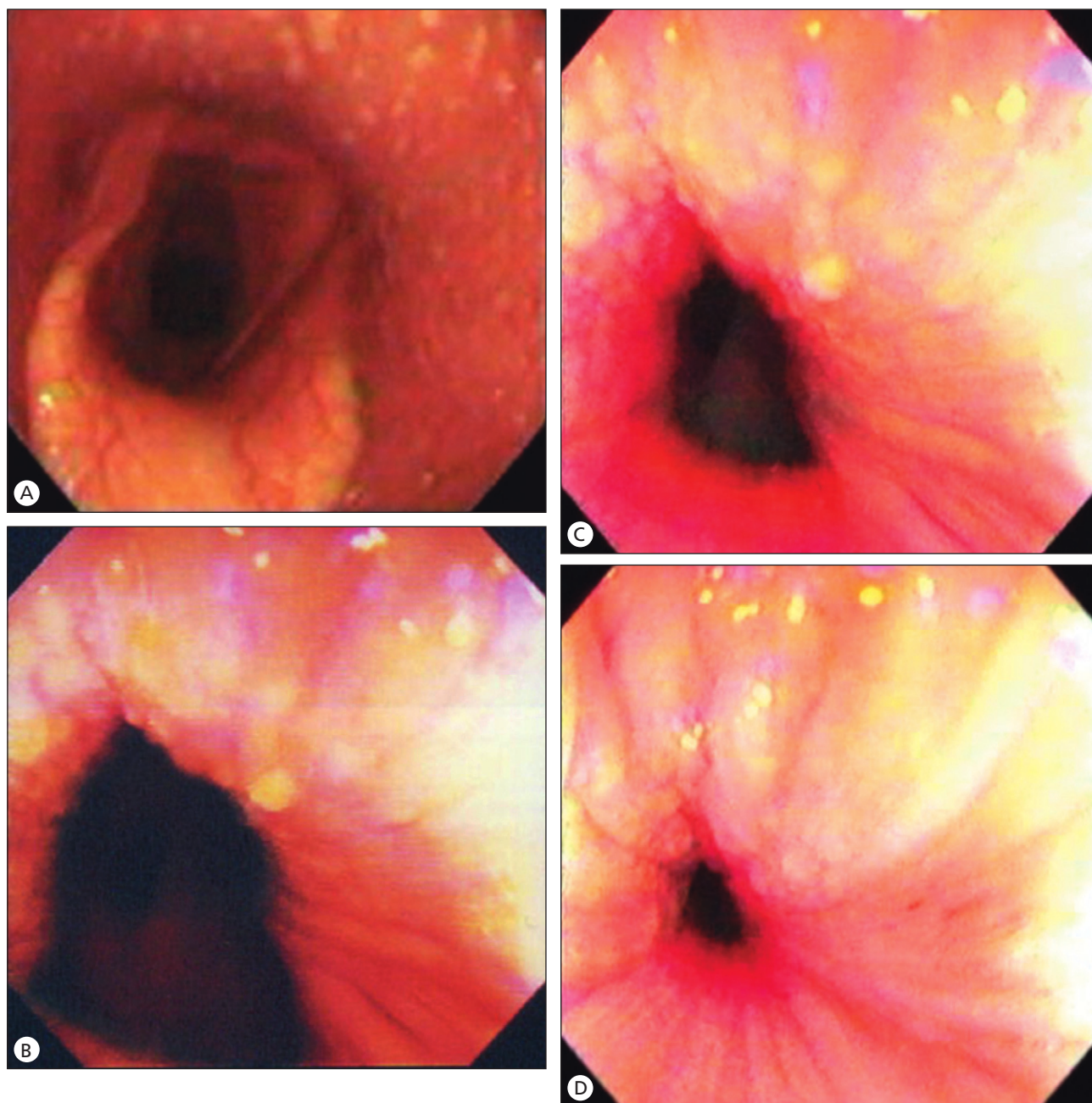
Fig. 16.11. Circumferential nasopharyngeal collapse: (A) horse at the start of HSTE, (B) nasopharyngeal collapse begins, (C) and (D) progressive collapse of the nasopharynx, leaving a very limited airway during every inspiration. Note that this type of collapse can be differentiated from a normal "swallow" by its timing (instigated during inspiration) and duration (lasts longer than a swallow). Reproduced from Barakzai 2006, with permission.

Nasopharyngeal collapse can be induced experimentally by topical local anesthesia of the laryngeal mucosa, and it has therefore been hypothesized that dysfunction of the mucosal mechanoreceptors and the branches of the superior laryngeal nerve may be involved in this disorder (Holcombe et al 2001). Dysfunction of the stylopharyngeus muscles may have a role in dorsal naso-pharyngeal collapse (Tessier et al 2004, Tessier et al 2005).

Fourth branchial arch defects (4-BAD, cricopharyngeal–laryngeal dysplasia)

The vast majority of horses with 4-BAD have abnormalities which may be detected during resting endoscopy, but a small percentage of affected horses will be normal at rest, with a variety of abnormalities evident on HSTE (Lane 2001). These include dynamic rostral displacement of the palato-pharyngeal arch (Fig. 16.13), dynamic right-sided
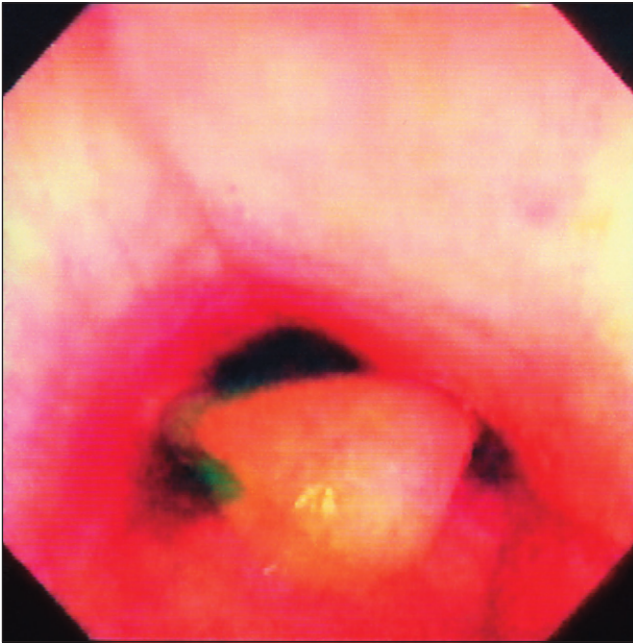
Fig. 16.12. Dorsal nasopharyngeal collapse. Reproduced from Barakzai 2006, with permission.
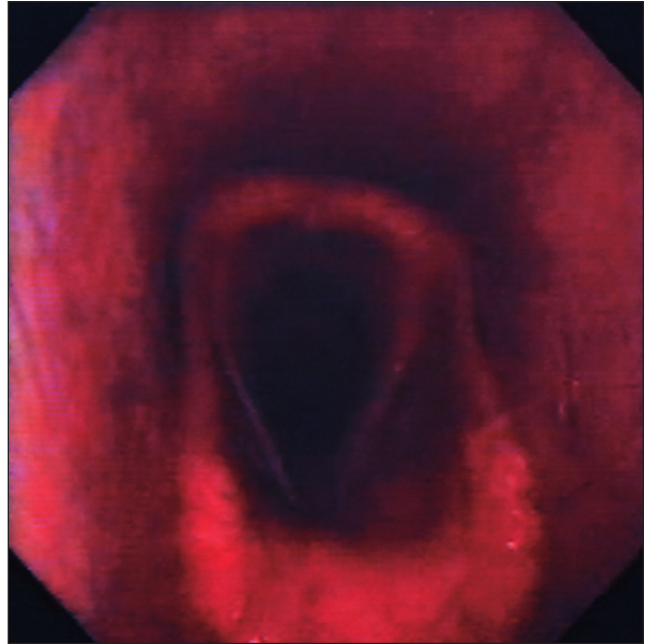
Fig. 16.13. Dynamic unilateral rostral displacement of the palato-pharyngeal arch. This horse had 4-BAD syndrome but was normal during resting endoscopy. During HSTE, the right palato-pharyngeal arch became rostrally positioned over the right arytenoid cartilage. Some ADAF of the right aryepiglottic fold has also occurred.

arytenoid cartilage and vocal fold collapse, bilateral vocal fold collapse (right > left), bilateral ADAF with DDSP, and unilateral right-sided ADAF (Lane 2001).

Epiglottic retroversion and axial collapse of the lateral margins of the epiglottis

Epiglottic retroversion is a rare disorder observed during HSTE where the epiglottis is retroverted dorsally and caudally into the rima glottidis on each inspiration (Dixon 1995, Parente et al 1998). In one horse, the epiglottis appeared to roll up in a tube-like fashion before lifting dorsally (Parente et al 1998). The few documented cases have occurred in horses with a history of severe upper respiratory infection and possible disruption of the normal hyoid musculature (Parente et al 1998). Although the epiglottis may become fully retroverted, the soft palate remains stable in its normal position at all times during the breathing cycle. This condition can be reproduced by local anesthesia of the hypoglossal nerve, which innervates the hyoepiglotticus and geniohyoid muscles that control epiglottic position (Holcombe et al 1997).

Axial collapse of the lateral margins of the epiglottis is diagnosed when the left or right sides of the epiglottis are observed to vibrate or displace axially during inspiration, often at the same time as production of an abnormal respiratory noise. It can occur as a lone abnormality, but has also been reported to occur with ADAF and bilateral arytenoid cartilage and vocal fold collapse (Fig. 16.14) (Kannegieter & Dore 1995, Dart et al 2001, Strand et al
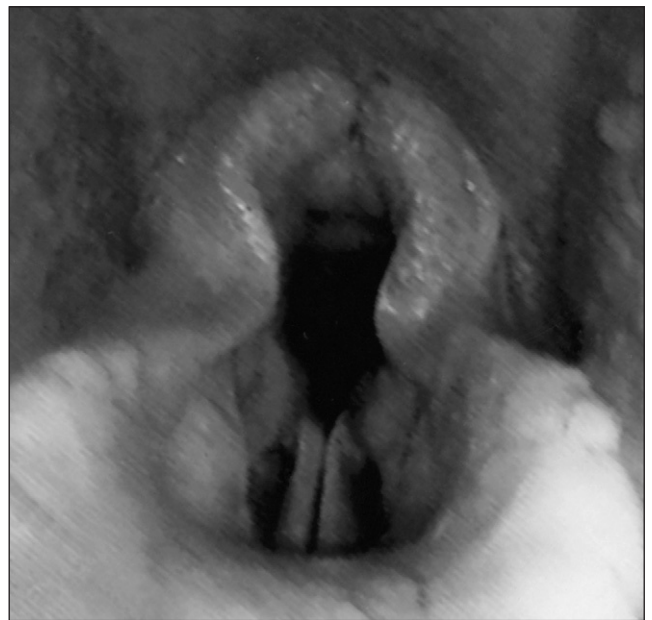
Fig. 16.14. HSTE photograph of a Norwegian coldblooded trotter during exercise with induced neck flexion. There is bilateral loss of abduction of the arytenoid cartilages and bilateral axial collapse of the vocal folds and the aryepiglottic folds. The left lateral margin of the epiglottis is also mildly collapsed in an axial direction. Photograph courtesy of E. Strand, Norwegian School of Veterinary Medicine and reproduced from Barakzai 2006, with permission.
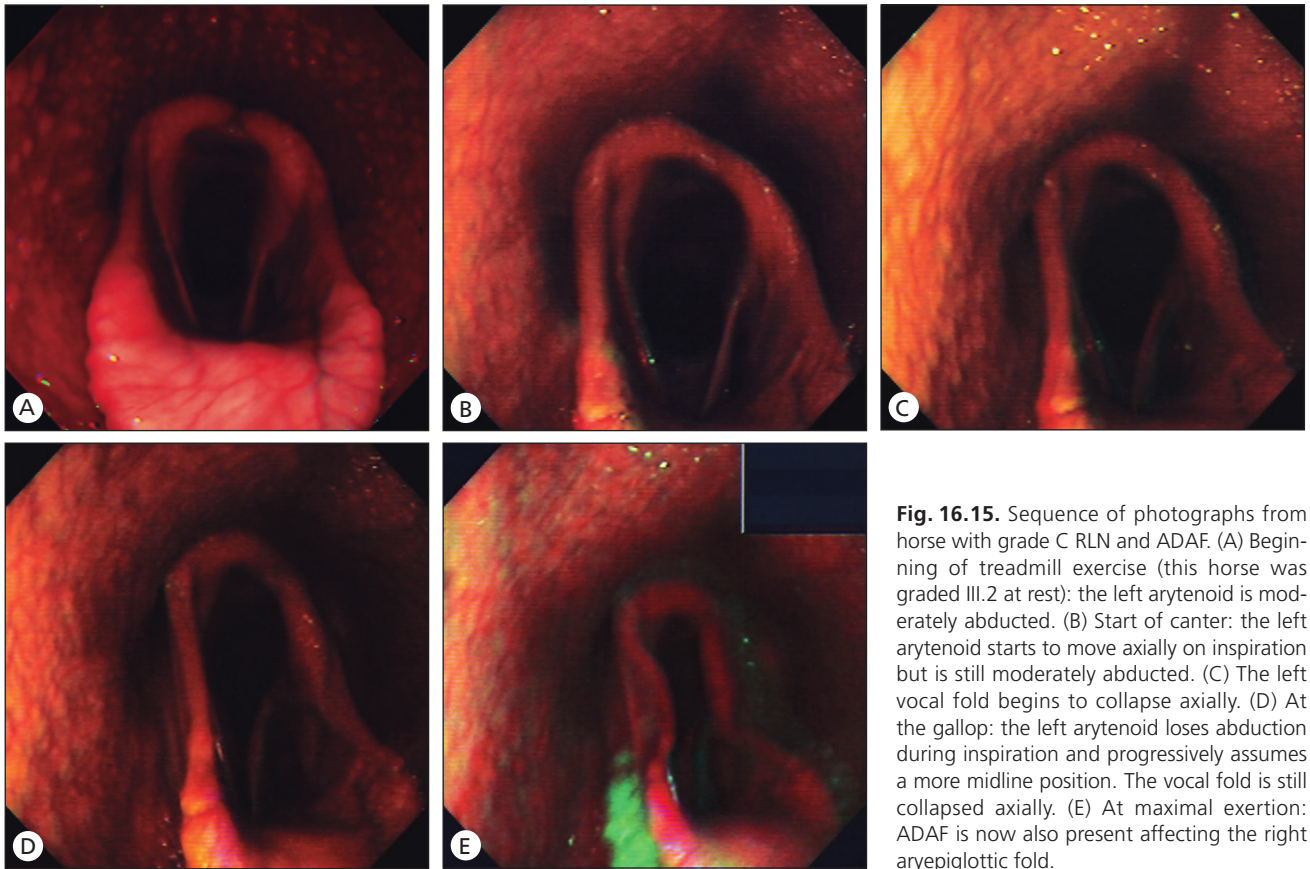
Fig. 16.15. Sequence of photographs from horse with grade C RLN and ADAF. (A) Beginning of treadmill exercise (this horse was graded III.2 at rest): the left arytenoid is moderately abducted. (B) Start of canter: the left arytenoid starts to move axially on inspiration but is still moderately abducted. (C) The left vocal fold begins to collapse axially. (D) At the gallop: the left arytenoid loses abduction during inspiration and progressively assumes a more midline position. The vocal fold is still collapsed axially. (E) At maximal exertion: ADAF is now also present affecting the right aryepiglottic fold.

2004). It is sometimes associated with epiglottic hypoplasia or flaccidity, which is observed during resting endoscopy, but also occurs in horses with a normal epiglottic appearance at rest.

Bilateral arytenoid cartilage and vocal fold collapse

This disorder has been reported in association with head flexion in Norwegian coldblooded trotter racehorses (Strand et al 2004), but could possibly occur in other breeds which are exercised with a similar head-carriage. Affected horses present with a history of abnormal respiratory noise and poor performance, and initial resting endoscopy and HSTE (performed without tension applied to the reins) do not reveal URT abnormalities. When the horses undergo HSTE wearing full harness race-tack, including a "head-check", and tension is applied to the bit via long reins, bilateral dynamic collapse of both vocal folds and reduced abduction of both arytenoid cartilages occurs (Fig. 16.14). Concurrent moderate ADAF associated with mild to moderate dorsoaxial collapse of the lateral edges of the epiglottis occurred in two of the five reported cases (Strand et al 2004). Given that arytenoid function is bilaterally normal in affected horses when they are run with their head and neck extended, it is unlikely that this condition is a manifestation of bilateral laryngeal neuropathy. It is hypothesized that this abnormality is the result of a failure of the arytenoid cartilages' ability to abduct, secondary to conformational changes in the throat region associated with head flexion and the use of particular tack, in predisposed horses (Strand et al 2004).

Multiple abnormalities (Figs 16.5 and 16.15)

Multiple abnormalities of the URT have been reported during HSTE in between 7 and 38% of horses presenting with abnormal respiratory noise or poor performance at exercise (Kannegieter & Dore 1995, Dart et al 2001, Durando et al 2002, Parente et al 2002). Multiple abnormalities have been found to be more commonly associated with abnormal exercising blood gases than single URT disorders (Durando et al 2002). Therefore, treadmill testing has been recommended for horses when an abnormal URT is observed at rest, to make a complete diagnosis (Dart et al 2001).

REFERENCES

Barakzai SZ 2006 Handbook of Equine Respiratory Endoscopy. WB Saunders, Philadelphia.

Barakzai SZ, Dixon PM 2003 Ventriculocordectomy for treatment of recurrent laryngeal neuropathy: 75 cases in a mixed population of horses. Proceedings of the Havemeyer Foundation Workshop on Idiopathic Laryngeal Hemiplegia, R&W Publications, Newmarket, UK pp.71–73

Barakzai SZ, Johnson VS, Baird DH et al 2003 The efficacy of composite surgery for treatment of dorsal displacement of the soft palate in 53 racing Thoroughbreds. Equine Veterinary Journal 36: 175–179

Dart AJ, Dowling BA, Hodgson DR et al 2001 Evaluation of high-speed treadmill videoendoscopy for diagnosis of upper respiratory tract dysfunction in horses. Australian Veterinary Journal 79: 109–111

Dixon PM 1995 A review of the role of the epiglottis in equine upper airway obstruction. Equine Veterinary Education 7: 131–139

Dixon RM, McGorum BC, Railton DI et al 2003 Long-term survey of laryngoplasty and ventriculocordectomy in an older, mixed-breed population of 200 horses. Part 1: Maintenance of surgical arytenoid abduction and complications of surgery. Equine Veterinary Journal 35: 389–396

Ducharme NG, Hackett RP, Stick JA 1998 Treadmill diagnostics for upper airway abnormalities. In: White NA, Moore JN (editors) Current Techniques in Equine Surgery and Lameness. WB Saunders, Philadelphia, pp.119–121

Ducharme NG, Hackett RP, Woodie JB 2003 Investigations into the role of the thyrohyoid muscles in the pathogenesis of dorsal displacement of the soft palate in horses. Equine Veterinary Journal 35: 258–263

Durando MM, Martin BB, Hammer EJ et al 2002 Dynamic upper airway changes and arterial blood gas parameters during treadmill exercise. Equine Veterinary Journal Supplement 34: 408–412

Franklin SH, Naylor RRJ, Lane JG 2002 The effect of a tongue-tie in horses with dorsal displacement of the soft palate. Equine Veterinary Journal Supplement 34: 430–433

Franklin SH, Usmar SG, Lane JG et al 2003 Spectral analysis of respiratory noise in horses with upper airway disorders. Equine Veterinary Journal 35: 264–268

Hackett RP, Ducharme NG, Stick JA 1998 Assessment of laryngeal and pharyngeal function. In: White NA, Moore JN (editors) Current Techniques in Equine Surgery and Lameness. WB Saunders, Philadelphia, pp.117–118

Hammer EJ, Tulleners EP, Parente EJ et al 1998 Video-endoscopic assessment of dynamic laryngeal function during exercise in horses with grade III left laryngeal hemiparesis at rest: 26 cases (1992–1995). Journal of the American Veterinary Medical Association 211: 399–403

Hogan PM, Paler SE, Congelosi M 2002 Transendoscopic laser cauterization of the soft palate as an adjunctive treatment for dorsal displacement in the racehorse. Proceedings of the American Association of Equine Practitioners 48: 228–230

Holcombe SJ, Derksen FJ, Stick JA et al 1997 Effects of bilateral hypoglossal and glossopharyngeal nerve blocks on epiglottic and soft palate position in exercising hoses. American Journal of Veterinary Research 58: 1022–1026

Holcombe SJ, Derksen FJ, Berney C et al 2001 Effect of topical anesthesia of the laryngeal mucosa on upper airway mechanics in exercising horses. American Journal of Veterinary Research 62: 1706–1710

Holcombe SJ, Cornelisse CJ, Berney C et al 2002 Electromyographic activity of the hyoepiglotticus muscle and control of epiglottis position in horses. American Journal of Veterinary Research 63: 1617–1621

Kannegieter NJ, Dore ML 1995 Endoscopy of the upper respiratory tract during treadmill exercise: a clinical study of 100 horses. Australian Veterinary Journal 72: 101–107

King DS, Tulleners EP, Martin BB et al 2001 Clinical experiences with axial deviation of the ary-epiglottic folds in 52 racehorses. Veterinary Surgery 30: 151–160

Lane JG 1993 Recurrent laryngeal neuropathy. Proceedings of the 15th Bain Fallon Lectures, Australian Equine Veterinary Association. Australian Veterinary Association, Artamon, pp.173–192

Lane JG 1996 Larynx. In: Traub-Dargatz JL, Brown CM (editors) Equine Endoscopy, 2nd edn. Mosby, St Louis, pp.74–96

Lane JG 1999 Endoscopy during high speed treadmill exercise: what are the advantages of this technique over endoscopy during quiet breathing? In: Proceedings of the BEVA Specialist Meeting on Equine Sports Science, Bristol, UK

Lane JG 2001 Fourth branchial arch defects in thoroughbred horses: a review of 60 cases. Proceedings of the World Equine Airways Symposium and Veterinary and Comparative Respiratory Society Annual Conference, Edinburgh, UK

Lane JG 2003 Differences between resting and treadmill endoscopic findings in regard to RLN. Proceedings of the Havemeyer Foundation Workshop on Idiopathic Laryngeal Hemiplegia, R&W Publications, Newmarket, UK, p.23

Morris EA 1991 Dynamic evaluation of the equine upper respiratory tract. Veterinary Clinics of North America; Equine Practice 7: 403–416

Morris EA, Seeherman HJ 1988 The dynamic evaluation of upper respiratory function in exercising horses. Proceedings of the American Association of Equine Practitioners 34: 159–160

Morris EA, Seeherman HJ 1990 Evaluation of upper respiratory tract function during strenuous exercise in racehorses. Journal of the American Veterinary Medical Association 196: 431–438

Morris EA, Seeherman HJ 1991 Clinical evaluation of poor performance in the racehorse: the results of 275 evaluations. Equine Veterinary Journal 23: 169–174

Parente EJ 1996 Testing methods for exercise intolerance in horses. Veterinary Clinics of North America; Equine Practice 12: 421–433

Parente EJ 1997 Treadmill endoscopy. In: Traub-Dargatz JL, Brown CM (editors) Equine Endoscopy, 2nd edn. Mosby, St Louis, pp.106–116

Parente EJ, Martin BB 1995 Correlation between standing endoscopic examinations and those made during high-speed exercise in horses: 150 cases. American Journal of Veterinary Research 41: 170–175

Parente, EJ, Martin BB, Tulleners EP 1998 Epiglottic retroversion as a cause of upper airway obstruction in two horses. Equine Veterinary Journal 30: 270–272

Parente EJ, Martin BB, Tulleners EP et al 2002 Dorsal displacement of the soft palate in 92 horses during high-speed treadmill examination (1993–1998). Veterinary Surgery 31: 507–512

Rakestraw PC, Hackett RP, Ducharme NG et al 1991 Arytenoid cartilage movement in resting and exercising horses. Veterinary Surgery 20: 122–127

Rehder BS, Ducharme NG, Hackett RP et al 1995 Measurement of upper airway pressures in exercising horses with dorsal displacement of the soft palate. American Journal of Veterinary Research 56: 269–274

Robertson JT 1998 Dorsal displacement of the soft palate. In: White NA, Moore JN (editors) Current Techniques in Equine Surgery and Lameness. WB Saunders, Philadelphia, pp.131–135

Rodgerson D 2004 Treadmill pharyngoscopy and laryngoscopy. In: Slovis NM (editor) Atlas of Equine Endoscopy. Mosby, St Louis, pp.225–238

Stick JA, Derksen FJ 1989 Use of videoendoscopy during exercise for determination of appropriate surgical treatment of laryngeal hemiplegia in a colt. Journal of the American Veterinary Medical Association 195: 619–622

Strand E, Staempfli HR 1993 Dynamic collapse of the roof of the nasopharynx as a cause of poor performance in a standardbred colt. Equine Veterinary Journal 25: 252–254

Strand E, Hanche-Olsen S, Gronvold A et al 2004 Dynamic bilateral arytenoid and vocal fold collapse associated with head flexion in 5 Norwegian Coldblooded Trotter Racehorses. Equine Veterinary Education 16: 242–254

Tessier C, Holcombe SJ, Derksen FJ et al 2004 Effects of stylopharyngeus muscle dysfunction on the nasopharynx in exercising horses. Equine Veterinary Journal 36: 318–323

Tessier C, Holcombe SJ, Derksen FJ et al 2005 Electromyographic activity of the stylopharyngeus muscle in exercising horses. Equine Veterinary Journal 37: 232–235

Tetens J, Derksen FJ, Stick JA et al 1996 Efficacy of prosthetic laryngoplasty with and without ventriculocordectomy as treatments for laryngeal hemiplegia in horses. American Journal of Veterinary Research 57: 1668–1673

Tulleners E, Stick JA, Leitch M et al 1997 Epiglottic augmentation for treatment of dorsal displacement of the soft palate in racehorses: 59 cases (1985–1994) Journal of the American Veterinary Medical Association 211: 1022–1028

17

Evaluation of Upper Respiratory Tract Sounds

Frederik J Derksen

Introduction

Veterinarians have long recognized that when horses make abnormal respiratory sounds (hereafter referred to as "respiratory noise") during exercise they often perform poorly (Williams 1874). Furthermore, specific respiratory noises heard during exercise may indicate the presence of distinct respiratory conditions, each with their own pathogenesis and implications for exercise tolerance. For these reasons, veterinarians of the past listened carefully for these sounds, and noises such as blowing, snuffling, soft and dry whistling, snoring, roaring, and rattling were described in detail (Williams 1874). Readers of these accounts, however, have difficulty understanding exactly what sounds are being described, and how these sounds relate to specific upper airway conditions cannot be reliably determined.

Respiratory sounds do not always occur throughout the exercise period. In some instances the sounds are more obvious at maximum exercise, while in other cases the sounds are intermittent, or occur as the horse is pulling up. The listener on the ground therefore is often not in a position to hear these sounds, and the jockey, rider, or driver is distracted by concurrent exercise-related noises such as those caused by footfall and wind. For these reasons, in horses with suspected upper airway problems, the history regarding noise production during exercise is often unreliable and non-specific, and subjective sound evaluation has limited usefulness in reaching a specific diagnosis.

In recent years, there has been a growing interest in respiratory acoustic measurements in people as well as horses, and advances in computer technology have made sound analysis practical and potentially useful in the diagnosis of airway disorders (Pasterkamp et al 1997). This chapter will describe how upper airway sound in exercising horses can be recorded, analyzed, and used as a diagnostic tool.

Upper airway disorders are common in horses, and are often difficult to diagnose in the standing animal. For example, dorsal displacement of the soft palate is rarely diagnosed at rest, but it is one of the most common causes of airway obstruction in exercising horses (Raphel 1982, Morris & Seeherman 1990, Martin et al 2000). Upper airway obstructions are often more apparent during exercise, because the large increase in airflow and the large intraluminal pressure swings that occur during exercise encourage dynamic airway collapse. Thus, examination of the upper airway in the standing horse is often insufficient for making a diagnosis, and video-endoscopy is often required. Video-endoscopy during exercise requires a high-speed treadmill, available only at referral institutions. Furthermore, examination on the treadmill takes the horse out of the environment in which it normally exercises, and examinations on the treadmill often fail to reveal the cause of the problem identified by the owner. Thus, a simple and inexpensive diagnostic technique that can be used in the field would aid in the diagnosis of upper airway disease in horses. Upper airway sound evaluation is such a technique.

Evaluation of respiratory sound during exercise is also useful in assessing the effectiveness of surgical procedures used in the treatment of upper airway conditions (Derksen et al 2001, Brown et al 2003, Brown et al 2004). Upper airway noise is sometimes the only reported clinical sign of upper airway obstruction, or it can be accompanied by exercise tolerance. In show horses, upper airway noise is often the most important presenting complaint, as it interferes with the aesthetics of riding, and may result in reduced grading or even disqualification of the affected animal from competition. Thus, in many horses with upper airway conditions, the objective of surgery is to reduce the respiratory noise. In these cases, evaluation of sound production before and after surgery is a good way to determine surgical success.

Fundamentals of Sound

A sound wave is the vibration of air. When a sound source sets the molecules of the air into vibration, a varying pressure with the same frequency as the source is produced. Human hearing can detect these varying pressures if the frequency is between 0.02 and 20 kHz and vibrations in this frequency range are called sound. In exercising horses, most of the upper airway sound energy is in the 0.02–8 kHz range, putting it well within the range of human hearing. However, the average human ear is most sensitive between 2 and 4 kHz. Thus, while human hearing can detect almost the entire frequency range of vibrations made by the upper airway, upper airway sounds in the

2–4 kHz range are most acutely appreciated (Strong & Plintnik 1992).

What is the difference between sound and noise? Noise can be defined as any unwanted or annoying sound, much as a weed is an unwanted plant. Thus, normal respiratory sounds made by exercising horses are usually not referred to as noise, while abnormal sounds associated with upper airway diseases can be considered noises.

Sound has time, frequency, and amplitude characteristics, and analyses of these characteristics can yield important information. Time is an important component of upper airway sound analysis in horses. From a clinical perspective it is important to know if a particular sound is made during inhalation or exhalation, because sounds associated with different upper airway conditions occur during specific portions of the respiratory cycle. For example, sounds associated with recurrent laryngeal neuropathy (RLN) occur throughout inhalation, whereas sounds associated with dorsal displacement of the soft palate are predominant during exhalation (Derksen et al 2001).

The frequency components of upper airway sounds also contain important information. Upper airway sounds made by exercising horses, like most other sounds, are caused by a complex wave. A complex wave is made up of many sinusoidal components. The frequency characteristics of these sinusoidal components can be analyzed by use of a spectrum analyzer. A spectrum analyzer may be thought of as being composed of many filters, separating complex waves into discrete sinusoids. A sound spectrum is a graph plotting the frequency components of a given sound on the horizontal axis, and the sound intensity for each frequency in decibels on the vertical axis (Strong & Plintnik 1992). When evaluating respiratory sounds in exercising horses using a spectrum analyzer, it is most useful to evaluate inspiratory and expiratory sounds separately.

A third characteristic of sound is its intensity (amplitude of the sound wave). Because the intensity of upper airway noise made by exercising horses is often part of the owner's complaint, this characteristic of upper airway noise is also important to analyze.

Spectrogram analysis is a sound analysis technique that evaluates time, frequency, and sound-intensity characteristics simultaneously. A spectrogram is a three-dimensional plot of frequency on the vertical axis, time along the horizontal axis, and sound intensity in the third dimension. Sound intensity is often plotted in terms of color, or on a gray scale. This powerful technique has been commonly used to study human voice and biologic sounds such as bird songs, and also to evaluate upper respiratory sounds made by exercising horses (Wollemann & Olaszy 1977, Kent 1993, Heaton et al 1995, Cable et al 2002).

The source of upper airway sounds in exercising horses has had limited study to date. In humans, the sound of speech is generated by vibrations of the vocal fold (i.e. turbulent airflow in the upper airway). The sound is then modified as it passes through the vocal tract. In the vocal tract, resonant frequencies are emphasized resulting in bands of sound called formants (Kent 1993).

Methods of Sound Recording

One of the earliest methods of recording respiratory sounds during exercise in horses involves a radio-stethoscope (Attenburrow 1978a,b). Using this technique, a microphone is placed over the ventral wall of the trachea at any point between the fourth and ninth tracheal ring. The microphone is glued to the skin to prevent the skin and microphone from rubbing and thus generating friction sounds. The advantages of this technique are that it is simple, easily used in the field and probably excludes recording extraneous sounds associated with exercise, such as footfall and wind noise. The disadvantage of this technique is that it is difficult for the clinician to relate to tracheal sounds, as a human observer does not hear these sounds directly.

Another method involves placing a small microphone in the nasopharynx (Cable et al 2002). This technique protects the microphone, and the recording of extraneous sounds related to exercise is avoided. However, the nasopharyngeal position of the microphone is slightly more invasive, and there may be interference associated with bumping of the microphone on the walls of the nasopharynx. Furthermore, air rushes past the microphone in this position, creating vibrations and unwanted iatrogenic sounds. Similar to the radio-stethoscope, it is difficult to relate to pharyngeal sounds as the human observer normally hears sounds emanating from the nostrils.

Respiratory sounds in exercising horses have been recorded at the nostrils using a facemask incorporating airflow transducers, an endoscope, and a microphone (Franklin et al 2003). This technique has the advantage that upper airway endoscopy, measurement of upper airway flow mechanics, and sound recording can be performed simultaneously. It is possible, however, that the presence of the mask and flow measurement equipment alters sound recordings. A further disadvantage is that this methodology is not suitable for field use.

In our laboratory we also record respiratory sounds in exercising horses at the nostrils (Derksen et al 2001, Brown et al 2004). A unidirectional microphone with a cardioid pickup pattern is attached via a flexible wand to a cavison (Fig. 17.1). This type of microphone centers the sound pickup toward the front of the microphone, and helps reduce extraneous sounds such as footfall and track noise that originate behind and to the side of the microphone. The microphone is covered with a windscreen, and is placed equidistant between the horse's nostrils, approximately 4 cm from the horse's nose. In this way, the microphone is as close to the source of sound to be recorded as possible, while avoiding placement of the

Fig. 17.1. Cavison-mounted microphone used to record respiratory sounds in exercising horses.

Fig. 17.2. Spectrogram of respiratory sounds in a normal galloping horse. Timing is on the abscissa, frequency on the ordinate. The color indicates sound intensity, with light colors representing high intensity sounds and dark color representing low intensity sounds. Exhalation is indicated by an arrow. The upper trace represents the complex sound wave before separation into frequency and intensity.
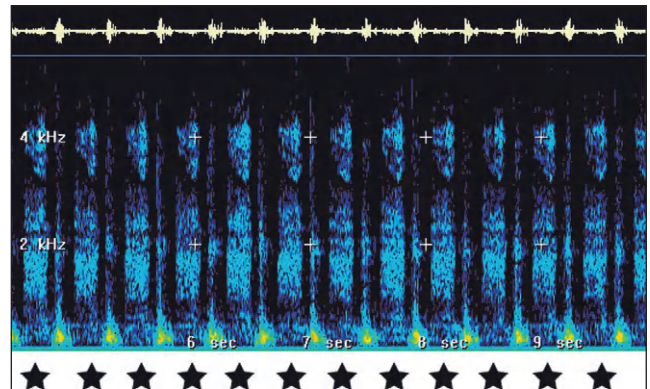
Fig. 17.3. Spectrogram of respiratory sounds in a galloping horse with experimentally induced laryngeal hemiplegia. Notice the bands of inspiratory sound (formants) centered at approximately 0.3, 1.7, and 3.7 kHz. Inspiration is indicated by a star.

microphone in the respiratory air stream. The microphone is attached to an audio recorder with a compression circuit. This kind of recorder automatically adjusts the gain, decreasing the recording of extraneous noises. The advantages of this technique are that it is easily used in the field and it records respiratory sounds that are also heard by human observers.

Simply listening to the audiotape of respiratory sounds made by exercising horses is revealing. The listener can appreciate factors such as respiratory rate, and consistency, the number of swallows, the frequency of stride lead changes, and whether or not abnormal respiratory sounds are present. Concurrent listening to the audiotape and viewing the spectrogram is an effective method of sound evaluation.

Sound Spectra of Upper Airway Sounds in Exercising Horses

Upper airway sound spectrograms of exercising horses vary depending on the measurement technique. The subsequent spectrogram descriptions will be of respiratory sounds recorded at the external nares.

In normal exercising horses, expiratory sounds predominate and occur throughout exhalation (Fig. 17.2). Sound intensity increases as speed increases. During a fast canter or gallop, the sound intensity of expiratory sounds is approximately 2.5 times that of inspiratory sounds. Most of the sound intensity occurs at frequencies below 4 kHz, with energy concentrated at frequencies below 0.8 kHz (Derksen et al 2001, Franklin et al 2003, Brown et al 2004).

Horses with RLN or experimentally induced laryngeal hemiplegia produce a loud inspiratory noise, often with the same sound intensity as normal expiratory sounds (Fig. 17.3) (Franklin et al 2003). This noise contains higher frequencies than normal expiratory sounds, and is characterized by three frequency bands called formants. These formants are centered at approximately 0.3, 1.7, and 3.7 kHz (Derksen et al 2001, Brown et al 2003). The highest frequency formant has the least sound intensity,

and is not present in all RLN-affected horses. Human hearing is most acute between 2 and 4 kHz. The sound intensity of the format centered at about 0.3 kHz is in a region where human hearing is less acute, and therefore this lower frequency noise does not appear prominent. In contrast, the formant centered at about 1.7 kHz has significant sound intensity, and is in the range of acute human hearing. Therefore this sound formant contributes most to the perception of a high-frequency whistle or roar associated with RLN. The spectral pattern just described is characteristic of horses with RLN or experimentally induced laryngeal hemiplegia (Derksen et al 2001, Brown et al 2003, Franklin et al 2003). It is possible that this spectral pattern is indeed unique to RLN. If this is the case, spectrum analysis of upper airway sounds in exercising horses could be used as a definitive diagnostic tool for this condition.

The source of the inspiratory noise in RLN-affected horses is as yet unclear. It has been reported that the resonant frequency of the lateral ventricle is approximately 1.9 kHz, suggesting that the second formant may arise from air moving across the open ventricle during inhalation (Attenburrow 1982). This suggestion requires further study.

Dorsal displacement of the soft palate is an intermittent condition in exercising horses; therefore the abnormal noise associated with the condition is also intermittent (Franklin et al 2003). In some horses, the abnormal sound can be heard throughout the exercise period, while in other horses the soft palate displaces near the end of the exercise, or as the horse pulls up. In horses with dorsal displacement of the soft palate, expiratory sounds are abnormal. The loud expiratory noise is characterized by rattling, which is visualized on the spectrogram in the time domain as bars of sound with a periodicity of about 32 ms (Fig. 17.4). Most of the sound energy is below 4 kHz, but ranges up to approximately 10 kHz (Derksen et al 2001). In some horses

with dorsal displacement of the soft palate, respiratory sounds are normal, whereas in others an inspiratory noise similar to the noise described for exhalation is also heard. In these cases, the inspiratory noise is often less loud than the expiratory noise. During exercise, affected horses often swallow frequently, presumably trying to maintain the soft palate in its normal position.

The source of the upper airway noise in horses with dorsal displacement of the soft palate is probably vibrations of the dorsally displaced soft palate (Franklin et al 2004). The rate and amplitude of soft palate vibration are functions of the airflow velocity, the compliance of the dorsally displaced soft palate, and the geometry of the individual horse's upper airway. Combined, these factors explain why some horses with dorsal displacement of the soft palate make no noise, whereas in others the noise is also apparent during inhalation. Concurrent listening to the recorded respiratory sounds made by a horse with dorsal displacement of the soft palate, and viewing the spectrogram is an effective way to diagnose the condition.

RLN and dorsal displacement of the soft palate are the most common upper airway conditions of horses. Furthermore, both conditions can be experimentally reproduced (Derksen et al 1986, Holcombe et al 1998). Consequently noises associated with these conditions have been studied more than those of the less common conditions. However, upper airway conditions such as pharyngeal collapse, epiglottic entrapment, aryepiglottic fold collapse and rostral soft palate collapse are also associated with respiratory noise during exercise. While the associations between specific conditions and their corresponding spectrogram patterns must still be made, in the future it is likely that each upper airway condition associated with noise production will have a well-described "voiceprint," allowing a presumptive spectrogram-based diagnosis of the condition in the field.

Surgical procedures and noise reduction in horses with RLN

When selecting from the many surgical options for treating RLN, veterinary surgeons must weigh the ability of the surgical procedure to improve upper airway function and/or reduce the associated respiratory noise, while minimizing the likelihood and severity of postoperative complications. Thus, the surgical procedures selected will vary depending on the intended use of the horse after surgery, the expectations of its owners regarding noise reduction, and the risk tolerance of the owner for postoperative complications.

Surgical procedures used to treat RLN include prosthetic laryngoplasty, and various combinations of laryngeal ventricle and vocal cord excision, often combined with laryngoplasty, and nerve-muscle pedicle grafting (Marks et al 1970, Ducharme & Hackett 1991, Russell & Slone
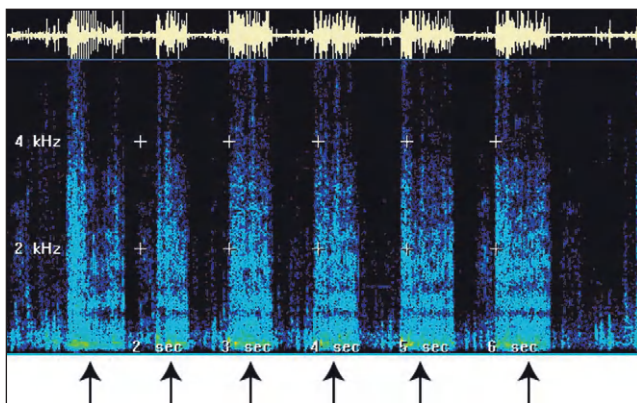
Fig. 17.4. Spectrogram of respiratory sounds in a galloping horse with experimentally induced dorsal displacement of the soft palate. Note the vertical bars ("rattling") during exhalation. Exhalation is indicated by an arrow.

1994, Tetens et al 1996, Hawkins et al 1997, Fulton et al 2003). The source of the RLN-associated noise is not clearly understood. Therefore, the efficacy of surgical procedures to reduce the noise is difficult to predict. Several studies have addressed this issue. In one study, bilateral ventriculocordectomy effectively reduced respiratory noise in horses with experimentally induced laryngeal hemiplegia (Brown et al 2003). Respiratory noises associated with the condition were almost completely eliminated, although spectrum analysis revealed some residual abnormal respiratory sounds (Fig. 17.5). Up to 90 days were required before the effect of surgery on noise reduction materialized.

In recent years, endoscopically guided laser surgery has become popular in the upper airway. The advantage of this technique is that general anesthesia is avoided, thus reducing the risk to the horse and the cost to the owner. In a recent study we evaluated unilateral laser cordectomy, and concluded that the procedure does not effectively reduce upper airway noise in horses with experimentally induced laryngeal hemiplegia. Unilateral laser cordectomy results in removal of the left vocal cord, but not the laryngeal ventricle. This study suggests therefore that the laryngeal ventricle rather than the vocal cord may be the source of laryngeal hemiplegia-associated noise.

The most commonly used surgical technique to treat RLN is prosthetic laryngoplasty, because this procedure returns upper airway flow mechanics to baseline values (Tetens et al 1996). Furthermore, the success of this surgery for returning horses to racing has been well documented (Russell & Slone 1994, Hawkins et al 1997). Prosthetic laryngoplasty reduces upper airway noise in horses with laryngeal hemiplegia, but is not as effective as bilateral ventriculocordectomy in this regard (Brown et al 2004). Additionally, respiratory noise reduction occurs more rapidly than with bilateral ventriculocordectomy (Brown et al 2004).

Reduction of respiratory noise "as discerned by the critical ear" is often used to determine the surgical success of prosthetic laryngoplasty (Marks et al 1970). This method of evaluation is distinctly subjective in nature, and assumes a tight correlation between respiratory noise and airway obstruction. In fact, in horses with experimentally induced laryngeal hemiplegia, the correlation between residual airway obstruction following prosthetic laryngoplasty and residual noise is weak (Brown et al 2004). Therefore, the residual noise during exercise cannot be used as a predictor of improvement in upper airway function in individual horses following laryngoplasty. As long as the affected arytenoid cartilage is stabilized by the prosthetic laryngoplasty, the degree of arytenoid abduction obtained does not affect upper airway flow mechanics. Interestingly, in one study it was found that the greater the arytenoid abduction, the louder the residual respiratory noise (Brown et al 2004). Explanation of this observation will require a better understanding of the source of the respiratory noise.

REFERENCES

Attenburrow DP 1978a. The development of a radio-stethoscope for use in the horse at rest and during exercise. Equine Veterinary Journal 10: 14–17

Attenburrow DP 1978b Respiratory sounds recorded by radio-stethoscope from normal horses at exercise. Equine Veterinary Journal 10: 176–179

Attenburrow DP 1982 Resonant frequency of the lateral ventricle and saccule and whistling. In: Snow DH, Persson SGB, Rose RJ (editors) Equine Exercise Physiology. Burlington Press, Cambridge, pp.27–32

Brown JA, Derksen FJ, Stick JA et al 2003 Ventriculocordectomy reduces respiratory noise in horses with laryngeal hemiplegia. Equine Veterinary Journal 35: 570–574

Brown JA, Derksen FJ, Stick JA et al 2004 Effect of laryngoplasty on respiratory noise reduction in horses with laryngeal hemiplegia. Equine Veterinary Journal 36: 420–425

Cable CS, Ducharme NG, Hackett RP et al 2002 Sound signature for identification and quantification of upper airway disease in horses. American Journal of Veterinary Research 63: 1707–1713

Derksen FJ, Stick JA, Scott EA et al 1986 Effect of laryngeal hemiplegia and laryngoplasty on airway flow mechanics in exercising horses. American Journal of Veterinary Research 47: 16–20

Derksen FJ, Holcombe SJ, Hartmann W et al 2001 Spectrum analysis of respiratory sounds in exercising horses with experimentally induced laryngeal hemiplegia or dorsal displacement of the soft palate. American Journal of Veterinary Research 62: 659–664

Ducharme NF, Hackett RP 1991 The value of surgical treatment of laryngeal hemiplegia in horses. Compendium on Continuing Education for Practicing Veterinarians 13: 472–475

Franklin SH, Usmar SG, Lane JG et al 2003 Spectral analysis of respiratory noise in horses with upper airway disorders. Equine Veterinary Journal 35: 264–268

Fig. 17.5. Mean percentage improvement of inspiratory sound intensity in laryngeal hemiplegia-affected galloping horses 90 days after treatment with bilateral ventriculocordectomy (VC), prosthetic laryngoplasty (PL) and unilateral laser cordectomy (LC). Note that respiratory noise reduction is most effective after VC, and least effective after LC.

Franklin SH, Price C, Burn JF 2004 The displaced equine soft palate as a source of abnormal respiratory noise during expiration. Equine Veterinary Journal 36: 590–594

Fulton IC, Stick JA, Derksen FJ 2003 Laryngeal reinnervation in the horse. Veterinary Clinics of North America; Equine Practice 19: 189–208

Hawkins JF, Tulleners EP, Ross MW et al 1997 Laryngoplasty with or without ventriculectomy for treatment of left laryngeal hemiplegia in 230 racehorses. Veterinary Surgery 26: 484–491

Heaton JT, Farabaugh SM, Brauth SE 1995 Effect of syringeal denervation in the budgerigar (*Melopsittacus undulatus*): the role of the syrinx in call production. Neurobiology of Learning and Memory 64: 68–82

Holcombe SJ, Derksen FJ, Stick JA et al 1998 Effect of bilateral blockade of the pharyngeal branch of the vagus nerve on soft palate function in horses. American Journal of Veterinary Research 59: 504–508

Kent RD 1993 Vocal tract acoustics. Journal of Voice 7: 97–117

Marks D, Mackay-Smith MP, Cushing LS et al 1970 Use of a prosthetic device for surgical correction of laryngeal hemiplegia in horses. Journal of the American Veterinary Medical Association 157: 157–163

Martin BB Jr, Reef VB, Parente EJ et al 2000 Causes of poor performance of horses during training, racing, or showing: 348 cases (1992–1996). Journal of the American Veterinary Medical Association 216: 554–558

Morris EA, Seeherman HJ 1990 Evaluation of upper respiratory tract function during strenuous exercise in racehorses. Journal of the American Veterinary Medical Association 196: 431–438

Pasterkamp H, Kraman SS, Wodicka GR 1997 Respiratory sounds: advances beyond the stethoscope. American Journal of Respiratory and Critical Care Medicine 156: 974–987

Raphel CF 1982 Endoscopic findings in the upper respiratory tract of 479 horses. Journal of the American Veterinary Medical Association 181: 470–473

Russell AP, Slone DE 1994 Performance analysis after prosthetic laryngoplasty and bilateral ventriculectomy for laryngeal hemiplegia in horses: 70 cases (1986–1991). Journal of the American Veterinary Medical Association 204: 1235–1241

Strong WJ, Plintnik GR 1992 Music, Speech and Audio. Soundprint, Provo, UT.

Tetens J, Derksen FJ, Stick JA et al 1996 Efficacy of prosthetic laryngoplasty with and without bilateral ventricu-locordectomy as treatments for laryngeal hemiplegia in horses. American Journal of Veterinary Research 57: 1668–1673

Williams W 1874 Respiratory sounds. In: The Principles and Practices of Veterinary Medicine. MacLachlan and Steward, Edinburgh, pp.451–462

Wollemann M, Olaszy G 1977 Spectrogram analysis of different alarm calls in gulls and waders. Agressologie 18: 97–102

18 Sinoscopy of the Paranasal Sinuses

Henry Tremaine

Introduction

Sinoscopy (or direct sinus endoscopy) is a minimally invasive method for visualization of the equine paranasal sinuses. Sinoscopy enables examination of most of the structures within the paranasal sinuses, the collection of intra-sinus biopsies and enables some surgical procedures to be performed on the sinuses of standing sedated horses. Sinoscopy can be performed using a rigid arthroscope (Ruggles et al 1993, Chan & Munroe 1995) or much more effectively (and safely for the operator) using a flexible fiberoptic or video endoscope (Worster & Hackett 1999, Tremaine & Dixon 2001). In one study, sinoscopy was diagnostically useful in 70% of the cases where it was used (Tremaine & Dixon 2001).

Technique

The horse should be sedated and restrained in stocks. Head supports are useful to keep the head still during creation of the trephine opening and subsequent sinoscopy. Sinoscopy is performed via a trephine hole in the frontal or maxillary bones, through which the endoscope (or arthroscope) can be inserted to allow inspection of the frontal, dorsal conchal caudal maxillary, or rostral maxillary sinuses.

The landmarks for the trephine hole are as follows (Fig. 18.1):

- Frontal sinus endoscopy: the trephine opening is made 1.5 cm lateral to the midline between a line joining the caudal margins of the eye and a line joining the rostral canthi.
- Caudal maxillary sinus endoscopy: a trephine hole is created 1.5 cm ventral to the most ventral point of the ventral orbital rim.
- Rostral maxillary sinus endoscopy: the trephine hole is usually created 2 cm dorsal to the rostral aspect of the facial crest, and this site should be guided by prior radiography. Rostral maxillary sinoscopy is only possible using this approach in older (>10 years of age) horses, whose cheek teeth have erupted sufficiently to prevent the reserve dental crowns impeding the passage of the endoscope.

Fig. 18.1. Areas for trephination for sinoscopy for examination of the frontal (1), caudal (2) and rostral (3) maxillary sinuses.

To perform sinoscopy, the skin and subcutis at the trephination site are desensitized with 1–2 ml of local anesthetic and a 1.5-cm longitudinal skin and periosteal incision is made before the periosteum is bluntly reflected. A 1–1.5-cm diameter Galt or Horsley's trephine or modified drill bit (Figs 18.2 and 18.3) can be used to create the trephine hole, taking care to avoid damage to deeper structures (especially if performing rostral maxillary trephination). Trephination is usually accompanied by minimal hemorrhage. Any sharp or loose bone fragments are removed from the edge of the trephine hole by rotating the trephine firmly around its margins, to obtain a smooth edge and so avoid damaging the delicate rubber sheath on the bending section of the endoscope insertion tube. When using a flexible endoscope (Figs 18.4 and 18.5), the trephine hole should be made sufficiently wide (e.g. 3–4 mm wider than endoscope body) to prevent endoscope damage.

Using the frontal bone approach (Fig. 18.4), the endoscope can be passed into the caudal maxillary sinus via the frontomaxillary opening (Fig. 18.6) (and vice versa for a caudal maxillary approach) (Fig. 18.7). Structures which can normally be observed using this approach include the interiors of the frontal and dorsal conchal sinuses, the frontomaxillary ostium (aperture) (Fig. 18.6),

Fig. 18.2. (A) Modified woodwork drill bits (top and bottom) or Galt trephine (center) are suitable for creating the trephine opening (trephine hole) for sinoscopy. (B) Close-up of modified woodwork drill bits (left) and Galt trephine (right).

Fig. 18.3. Creating a sinoscopy portal into the left frontal sinus using a modified 10-mm diameter drill bit. At this stage, the trephine should be directed more rostrolaterally to allow ease of entry of the endoscope into the caudal maxillary sinus. Reproduced with the permission of Prof. P.M. Dixon.

Fig. 18.4. An 8-mm flexible endoscope has been inserted into the left frontal sinus and is now being guided down to the frontomaxillary opening. Reproduced with the permission of Prof. P.M. Dixon.

Fig. 18.5. Sinoscopy of the left caudal maxillary sinus using a narrow (9-mm) diameter flexible endoscope.

the intra-sinus (sinusal) (i.e. laterodorsal) aspect of the ethmoturbinates, the ventral conchal bulla, the infraorbital canal, the apices of the fifth and sixth cheek teeth (Triadan 110–111, 210–211) (Fig. 18.8), the maxillary septum and possibly, the opening of the nasomaxillary ostium and the ostium of the sphenopalatine sinus. In the older horse, it may be easier to endoscopically examine the ostia of the sphenopalatine sinuses via maxillary sinoscopy.

Rostral maxillary sinoscopy may be possible via a maxillary trephine hole, at a site rostral to the maxillary septum. Sinoscopy of the ventral conchal sinus and possibly of the rostral maxillary sinus can also be performed via a frontal sinus sinoscopy and then using bone rongeurs to perforate the conchal bulla. In younger horses, the tall reserve crowns of the cheek teeth restrict the size of the conchomaxillary aperture and prevent passage of the endoscope between the rostral maxillary and the ventral conchal sinuses. The applications for rostral maxillary sinoscopy are limited as the reserve crowns of the cheek teeth (107–8, 207–8) occupy most of the rostral maxillary sinus in all except older horses. This feature severely restricts the ability to effectively maneuver an endoscope within this compartment and also risks causing damage to cheek teeth reserve crowns or apices during sinus trephination.

Sinoscopy of the rostral maxillary sinus has fewer applications than caudal maxillary sinus sinoscopy, but structures which potentially can be observed using this approach include the lumen of the rostral maxillary and
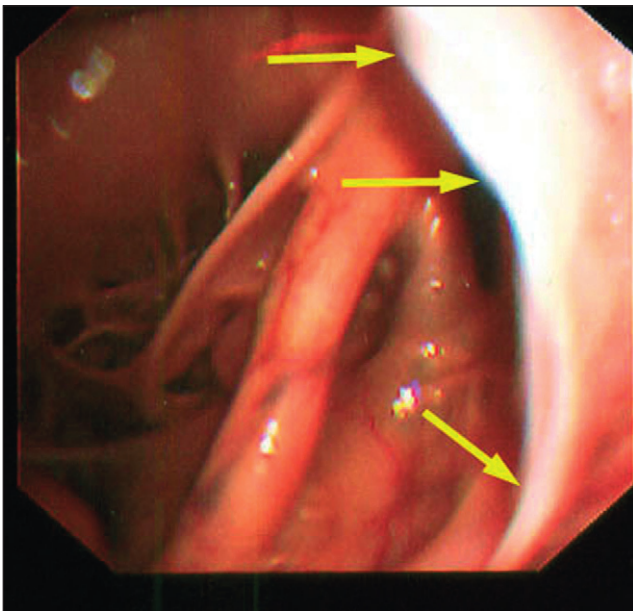
Fig. 18.6. Endoscopic view via a frontal sinus trephine hole showing the margin of the wide frontomaxillary ostium (arrows) and the underlying normal caudal maxillary sinus containing the infraorbital canal. Note that the mucosa is thin, its blood vessels are of normal prominence and exudate is absent from within the sinus lumen.
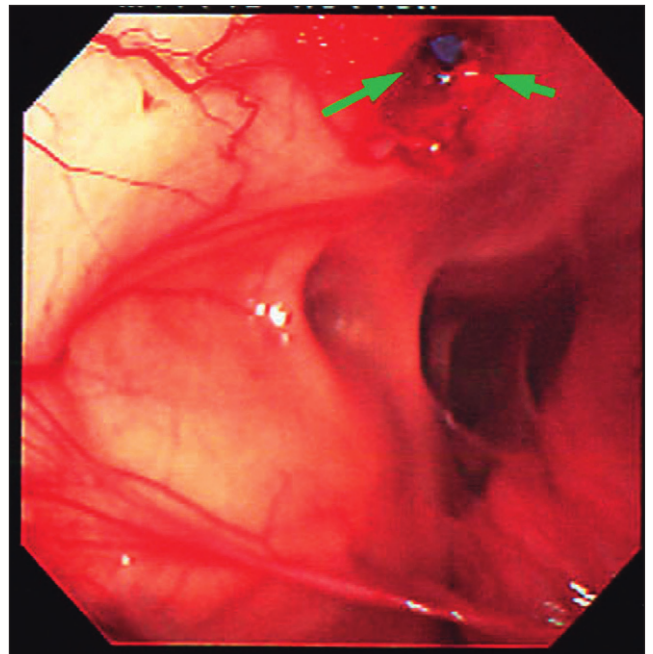
Fig. 18.7. Endoscopic view of the frontal sinus obtained by passing an endoscope from the caudal maxillary sinus through the frontomaxillary sinus opening. Note the trephine opening also present in the frontal sinus (arrows).

ventral conchal sinuses, the infraorbital canal, and usually the alveoli of the third (caudolateral root) and fourth maxillary cheek teeth (Triadan 108–9, 208–9).

Where sinus surgery is later proposed, the sinoscopy portal (which is also the site where an irrigation catheter is to be placed postoperatively) should be located distant from the proposed margins of the bone flap, to lessen the risk of dehiscence of that aspect of the surgical wound.

Biopsy instruments or probes can be inserted through a second portal under endoscopic guidance or the sinoscopy portal can be enlarged with rongeurs to allow insertion of a Ferris-Smith or intervertebral rongeur alongside the endoscope. The rongeurs can then be used to obtain biopsies, remove bone fragments, or to break down the caudal aspect of the bulla of the ventral conchal sinus to create a portal for insertion of the scope into the ventral conchal sinus and possibly the rostral maxillary sinus or to remove inspissated pus from these sites.

Applications

Sinoscopy is indicated when clinical examination or ancillary diagnostic tests including nasal endoscopy, radiography, scintigraphy or computed tomography indicate a lesion within the paranasal sinuses which merits direct visualization to obtain further diagnostic information. When large volumes of exudate are present in the paranasal sinuses, lavage of the sinuses for a few days, before attempting sinoscopy may enable visualization in greater detail. The technique has been of value in the diagnosis and treatment of cases with primary sinusitis which have accumulations of liquid (Fig. 18.9) or inspissated pus (including in the ventral conchal sinus) (Fig. 18.10), dental secondary sinusitis, sinus cysts (Figs 18.11 and 18.12), progressive ethmoidal hematoma, sinus mycosis (Fig. 18.13), sinus neoplasia (Fig. 18.14), and facial fractures (Ruggles et al 1993, Tremaine & Dixon 2001).

Sinoscopic Findings

Careful orientation of the endoscope is necessary to appreciate the intra-sinus topographical anatomy. Normal sinus mucosa is pink in color and small blood vessels can be viewed through its thin epithelium. Small volumes (few milliliters) of serous or mucoid respiratory secretions are occasionally present in the sinus. During normal sinoscopy of the frontal sinus, the dorsolateral aspect of the ethmoturbinates is visible ventral to the trephine hole. Directing the endoscope in a rostral direction reveals the dorsal conchal sinus, but this orientation of the endoscope is difficult to achieve if the trephine opening is positioned too rostral in the frontal bone. By directing the endoscope ventrally and slightly laterally (abaxially), the wide fronto-maxillary opening (Fig. 18.6) is visible. The endoscope can be directed ventrally through this ostium to enter the caudal maxillary sinus.
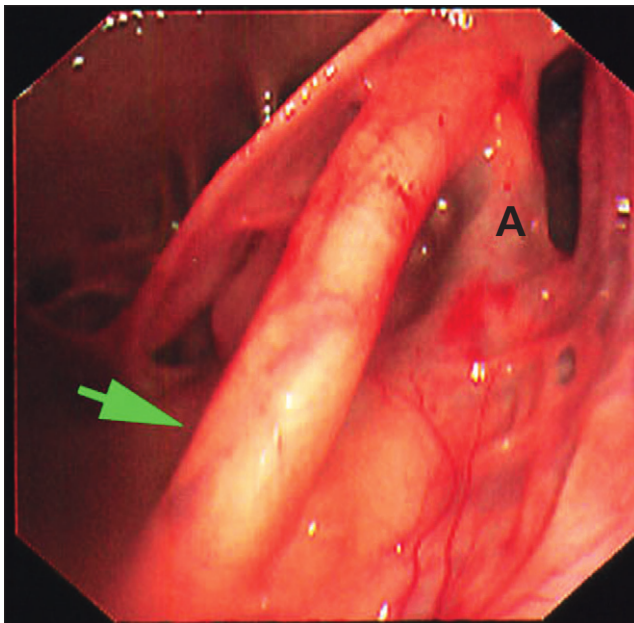
Fig. 18.8. Close-up view of the caudal maxillary sinus shown in Fig. 18.6. Note the infraorbital canal (arrow), rostral root of the apex of the fifth maxillary cheek tooth (210) (A) and some intra-sinus septa, that can be variable in their presence and appearance.
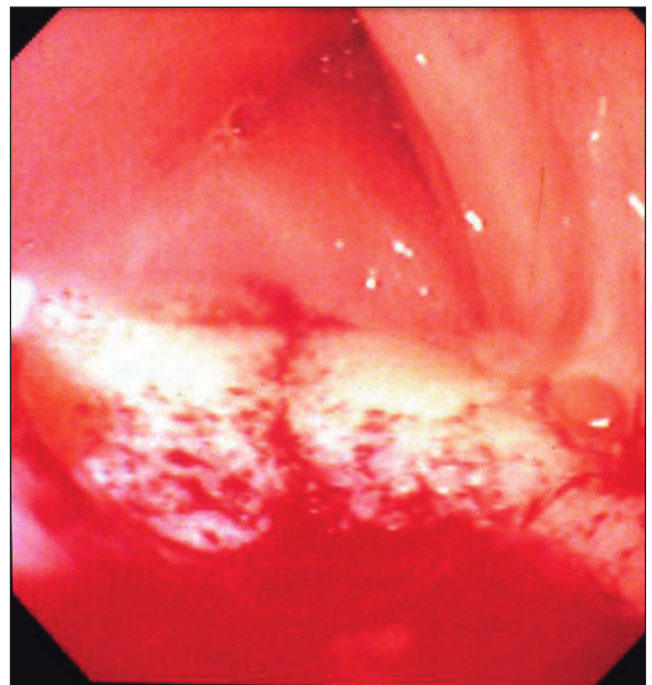
Fig. 18.9. Sinoscopy of the caudal maxillary sinus of a horse with primary sinusitis. This image shows thickening of the sinus mucosa and pus on the floor of the sinus. The small amount of hemorrhage that occurred during sinoscopy of the frontal sinus has flowed down to the caudal maxillary sinus. Reproduced with the permission of Prof. P.M. Dixon.
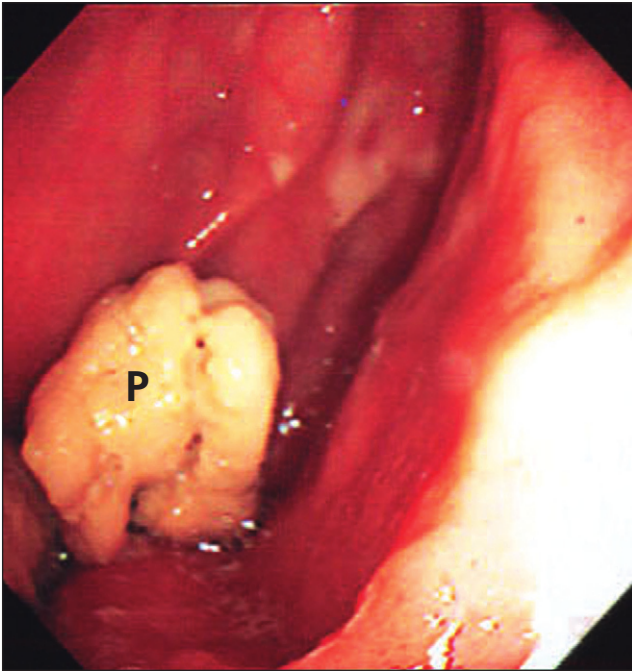
Fig. 18.10. Endoscopic view of the rostral maxillary sinus of a horse with sinusitis showing inspissated exudate (P).
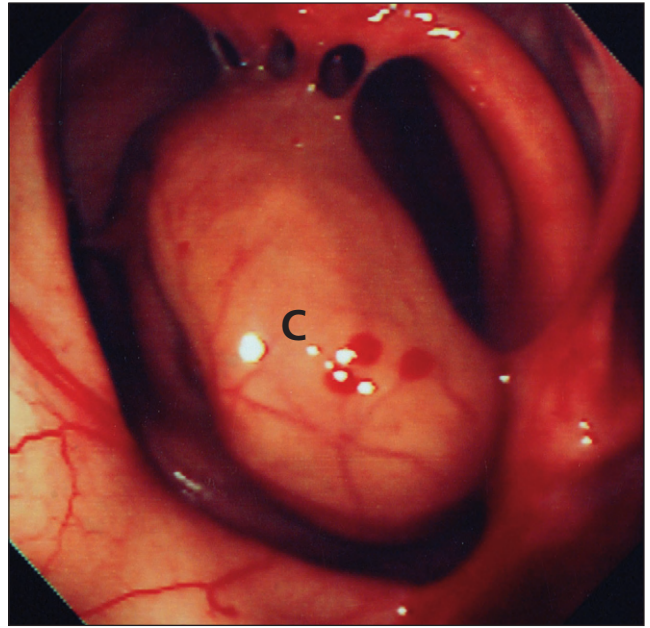
Fig. 18.11. Endoscopic view of a small, mucosa-covered cyst-like lesion in the caudal maxillary sinus (C) with some adhesions to the adjacent (thickened) sinus mucosa.
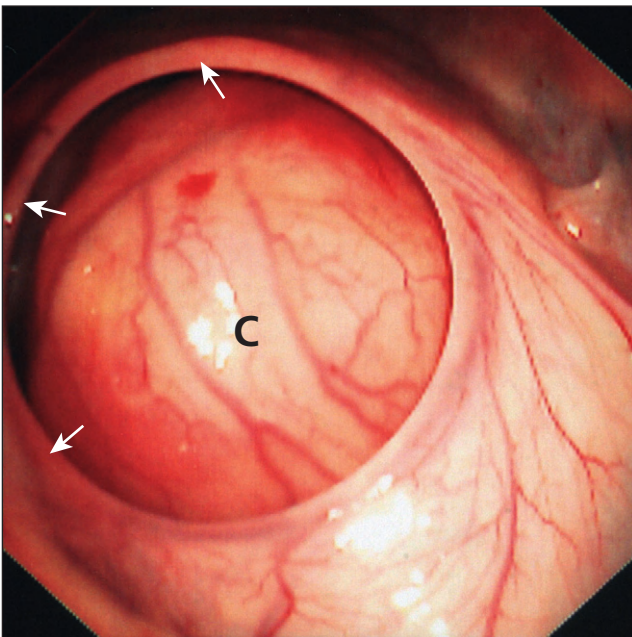
Fig. 18.12. A spheroid cystic (C) lesion in the caudal maxillary sinus causing almost complete obstruction of the frontomaxillary ostium (arrows). More rostrally in the caudal maxillary sinus, the ventral conchal bulla can have a similar appearance. Horses between 3 and 5 years old can also develop dental eruption cysts over the caudal maxillary cheek teeth leading to smaller lesions of similar appearance.
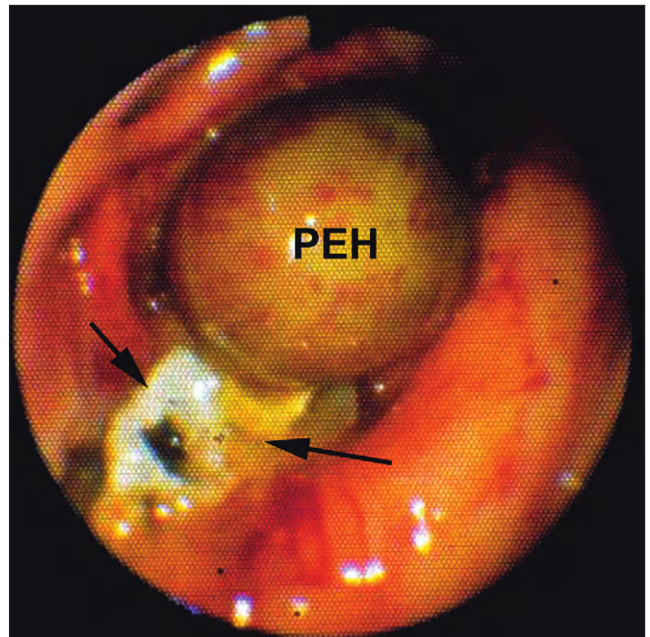
Fig. 18.13. Sinoscopic image of a progressive ethmoid hematoma (PEH) at the caudomedial aspect of the caudal maxillary sinus. Note also the adjacent mycotic plaque (arrows) that was secondary to the ethmoid hematoma and the adjacent inflamed sinus mucosa. Reproduced with the permission of Prof. P.M. Dixon.
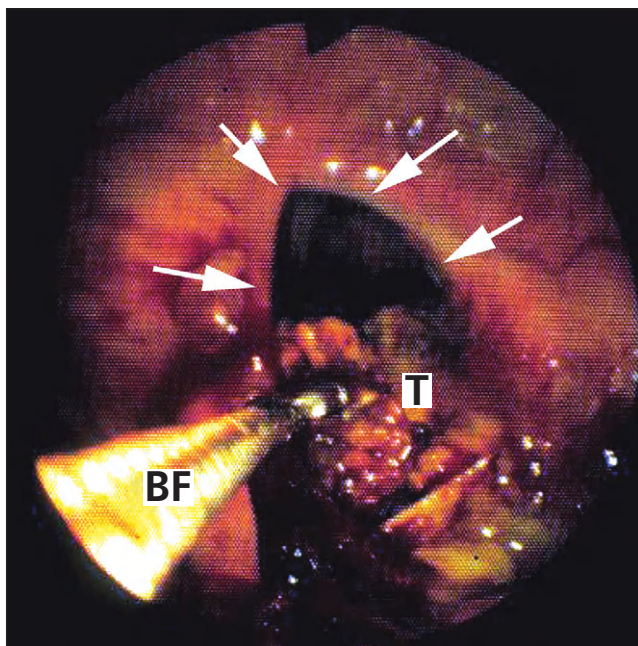
Fig. 18.14. Sinoscopy showing the frontomaxillary ostium (arrows) of a horse with sinusitis (thickened, inflamed mucosa with hemorrhagic exudates) and an irregular soft tissue mass protruding dorsally from the caudal aspect of the caudal maxillary sinus. A trans-endoscopic biopsy (BF) is being performed; this identified a carcinoma (T) that was later shown to originate in the sphenopalatine sinus. Reproduced with the permission of Prof. P.M. Dixon.

In the caudal maxillary sinus, the lateral aspect of the ethmoturbinates can be observed. Centrally in the sinus, the infraorbital canal and the underlying alveoli containing the apices and roots of the sixth (Triadan 111, 211) maxillary cheek teeth can be seen curving in a caudal direction. The alveoli of the fifth cheek teeth (Triadan 110, 210) lie more rostrally in the maxillary sinus and the maxillary septum marking the rostral limit of the caudal maxillary sinus often traverses the rostrolateral root of the fifth (Triadan 110, 210) cheek tooth. Ventral and axial to the frontomaxillary opening is the conchal bulla, formed by the thin, cartilaginous, convex caudodorsal aspect of the ventral conchal sinus.

In cases with sinusitis, sinoscopic visibility can be impaired by the presence of large volumes of purulent exudates. Accumulation of purulent material in the ventral aspects of the sinus in combination with thickening and hyperemia of the sinus mucosa are consistent with sinusitis (Fig. 18.9). The absence of any underlying lesion such as dental infection or cyst is supportive of a diagnosis of primary sinusitis. In some cases of chronic primary

sinusitis, inspissated pus can be observed in the caudal maxillary sinus (Fig. 18.10).

In cases of dental sinusitis, the alveolus over an infected apex may be swollen and hyperemic and may have a sinus tract or overlying granuloma. In many cases of dental sinusitis, these features may not be visible because of widespread mucosal swelling or the presence of pus in the sinus.

With sinus cysts, which have not expanded to fill the sinuses, an expanding rounded mass covered by apparently normal sinus mucosa can be observed and some cysts may obstruct the frontomaxillary ostium. Penetration of the cyst lining, which may be variably thickened or fibrous, using a lance catheter will yield the honey-colored exudate which is typical of this lesion.

Sinonasal mycoses have been reported as primary lesions, or secondary to intra-sinus growths and following sinus surgery. Such lesions, which are usually radiographically obscure, may be visible sinoscopically (Fig. 18.13).

Intrasinus progressive ethmoidal hematoma when viewed sinoscopically has a typical multicolored appearance and blood and serosanguineous fluid may be observed in the floor of the sinuses (Fig. 18.13).

Sinus neoplasms are variable in appearance and may be accompanied by sinusitis and destruction of the conchal bones. Fibro-osseous lesions may appear as firm, rounded masses that are resistant to attempts to biopsy using transendoscopic biopsy forceps.

Recurrence of clinical signs such as low-grade nasal discharges, malodorous discharges, and facial swelling occur occasionally after sinus surgery. Sinoscopy enables a minimally invasive assessment of such complications to assist with planning treatment.

Rare complications of sinoscopy are temporary periosteal reaction or osteomyelitis at the trephine site, cellulitis of overlying soft tissues, and subcutaneous emphysema.

REFERENCES

Chan C, Munroe G 1995 Endoscopy of the equine paranasal sinuses. In Practice 17: 419–422

Ruggles AJ, Ross MW, Freeman DE 1993 Endoscopic examination and treatment of paranasal sinus disease in 16 horses. Veterinary Surgery 22: 508–514

Tremaine WH, Dixon PM 2001 A long-term study of 277 cases of equine sinonasal disease. Part 1: Details of horses, historical, clinical and ancillary diagnostic findings. Equine Veterinary Journal 33: 274–282

Worster AA, Hackett RP 1999 Equine sinus endoscopy using a flexible endoscope: diagnosis and treatment of sinus disease in the standing sedated horse. Proceedings of the American Association of Equine Practitioners 45: 128–130

19

Computed Tomography of the Equine Upper Respiratory Tract

Martin J Philipp

Computed tomography (CT) is a well-established diagnostic technique in human medicine. Since the first reports of CT in the horse in the mid-1980s (Diehl & Cordey 1983, Allen et al 1987, O'Callaghan et al 1987), CT has proved to be a valuable tool in diagnosing disorders of the equine head, proximal cervical vertebral column and distal limbs. However, the application of CT in the horse is still limited by the requirement for special heavy-weight-bearing CT tables, general anesthesia, and by the limited gantry opening of current equipment, in addition to the high cost and thus limited availability of this equipment.

The word "tomogram" derives from the Greek words *tomos* and *gramma*, meaning cut or slice and record. The tomogram is thus a record of slices. CT is an imaging technique that is based on the attenuation of X-rays within the patient, similar to radiography. However, CT provides cross-sectional images with superior resolution to radiographs, thus avoiding problems associated with superimposition of structures, which is a major limitation of conventional radiography of the equine head.

The Principles of Computed Tomography

Acquisition and image reconstruction

Acquisition of CT images requires the rotation of an X-ray tube around the patient, during which time multiple X-ray projections are recorded. The X-ray projections are formed by scanning a thin cross-section of the object with a narrow X-ray beam. The width of the beam is set by the examiner and varies between 1 and 10 mm. Thinner slices result in better spatial resolution but they also result in longer acquisition time. A slice thickness of 5 mm has proven to be a good setting for imaging the equine head. A slice overlap of 1 mm further facilitates multiplanar and three-dimensional reconstruction.

The transmitted radiation is measured with radiation-sensitive detectors based on either scintillation crystals or xenon gas ionization chambers. A computer processes the projections collected during a 360° rotation to construct an attenuation image of the patient. As in conventional radiography, attenuation of the X-ray beam depends on the energy of the X-ray, the depth of the tissues being imaged, physical density, electron density, and the effec-

tive atomic number of individual tissues within the field of examination.

Image reconstruction is performed by back projection, iterative methods or analytical methods, which are described in detail in the literature. The reconstructed image is displayed on a matrix of squares or "pixels". Each of these pixels has a specific degree of blackness representing the degree of attenuation in a given position within the patient's tissues. As a result of the elongation of the X-ray beam and the setting of the detector window, each pixel represents a voxel – a small block of tissue that has been imaged. Thus a pixel is a picture element, while a voxel is a volume element. All acquired attenuation information within a voxel is averaged and then displayed as a pixel with a certain level of gray shade.

Voxel values are normalized to the linear attenuation coefficient of water and are expressed as Hounsfield units (HU). Water is represented by the zero value; air has a value of −1,000 HU; and cortical bone has a value of >3,000 HU. Within the body, different tissues and organs have characteristic HU values.

Gray-scale values of the image can be manipulated to fit the site and type of tissue examined and this is known as the window technique. For every gray-scale image the window width (WW) and the window level (WL) are defined. Window width describes the range of HU assigned to the gray scale of the image. Window level describes the HU value represented by the middle gray-scale value. Typical settings for soft tissue imaging are WL 50 and WW 200. HU values between −150 and +250 are shown as shades of gray, with values under −150 shown as black and values over +250 shown as white. A typical bony window setting is WL +500 and WW 1,500. Teeth are imaged at WL +1,000 and WW 4,000.

CT images are acquired in a transverse plane. With the help of computers and appropriate software programs, any other plane can be reconstructed later for viewing. Raw data may be processed and presented in many different ways, allowing enlargement of objects, edge enhancement and digital subtraction of images to produce native images and post-contrast images, calculation of histograms, and measurement of distance, surface, volume, and angles. Most CT units can also perform three-dimensional reconstructions, including surface reconstruction and

volumetric reconstruction. In surface reconstruction, the examiner may obtain a shaded view of the "surface" corresponding to a certain HU number (e.g. cortical bone). In volumetric reconstruction, the computer will display the pixel corresponding to certain HU values (e.g. muscle) as translucent, consequently allowing the adjacent pixel also to be displayed. This allows the examiner to see the relationship of adjacent tissues (e.g. bone and muscle). Highly sophisticated CT units also produce color coding of defined HU intervals. Many tissues and organs in the body have specific HU numbers. This allows volumetric reconstruction and distinction of multiple organs and their position in relation to each other within the body.

Newer CT techniques allow for faster imaging and for coverage of a larger length of field. These techniques include the use of spiral CT and multi-channel or multi-slice CT. In spiral CT, the patient is moved through the gantry while the X-ray tube and detectors function continuously, and an orange peel-like transverse image is created. The pitch of the scan is defined by the speed with which the patient is moved through the gantry. In multi-slice CT, multiple rows of detectors are installed and more than one slice can be imaged at once. Both of these techniques significantly reduce scanning time.

Data are stored on electronic devices such as magneto-optical (MO) disks or CD ROMs and selected images may be printed as hard copies. Storage of raw data allows subsequent reconstruction to be performed.

Positioning of equine patients

CT imaging in the horse must always be performed under general anesthesia to facilitate precise positioning and to avoid movement of the patient during image acquisition. Acquisition time is largely dependent on slice thickness settings and the type of CT-scanner used. The time required for a head scan can vary from approximately 30 seconds using a multi-slice spiral scanner to 30 min using a planar single-slice scanner.

Many protocols have been used for induction and maintenance of general anesthesia in horses. Both inhalation anesthesia and intravenous anesthesia may be used in CT. The different protocols used, and their relative merits, are described in detail elsewhere in the literature on veterinary anesthesia. The size and weight of horses means that custom-made CT tables are necessary. These tables may be coupled in a fixed manner to the small animal or human table that is provided with the CT system or may be separate units. However, as the table must allow very precise and repeatable positioning of the patient in the gantry, it must be sturdy, of sophisticated construction to allow fixation of the patient in different positions, and have sufficient padding to prevent injuries resulting from prolonged recumbency of the patient.

Fig. 19.1. Horse in dorsal recumbency with fixation of its head with tape and padding. An endotracheal tube is in place for administration of anesthetic gases. Reproduced with the permission of the section of diagnostic imaging/radio-oncology, Vetsuisse faculty of the University of Zürich, Switzerland.

For CT examination of the head and the proximal cervical vertebral column, the patient is positioned in dorsal recumbency (Fig. 19.1). The patient is fixed and held in position with radiolucent padding and tape or rope. Care must be taken to avoid overextension of the patient's neck during anesthesia, as this may lead to laryngeal paralysis (Dixon et al 2001). Precise axial positioning of the horse is crucial for acquisition of diagnostic images. The image plane should be perpendicular to the patient's axis and to the hard palate. Good patient fixation is also needed to minimize motion artifact induced by breathing.

Contrast media

Intravenous contrast media can be used to provide direct visualization of perfused tissues. Perfused tissue shows up as hyperdense areas in contrast to non-perfused tissues, which remain the same density. This technique is valuable if the clinician wishes to know if an imaged area is perfused or not, for example to differentiate a fluid-filled cystic mass from a soft-tissue neoplasm with vascularization extending throughout the mass (Fig. 19.2). After performing the initial (native) CT scan, the same sequence is repeated following administration of contrast medium. Essentially the same contrast media can be used in CT imaging as in conventional radiography and the risks and precautions are the same. For intravenous administration of contrast media for CT studies, tri-iodinated, water-soluble compounds are used. They are administered as an intravenous bolus at a dose of 370 mg iodine/kg body weight. They are distributed by the vascular system and later excreted by the urinary system. Perivascular leakage
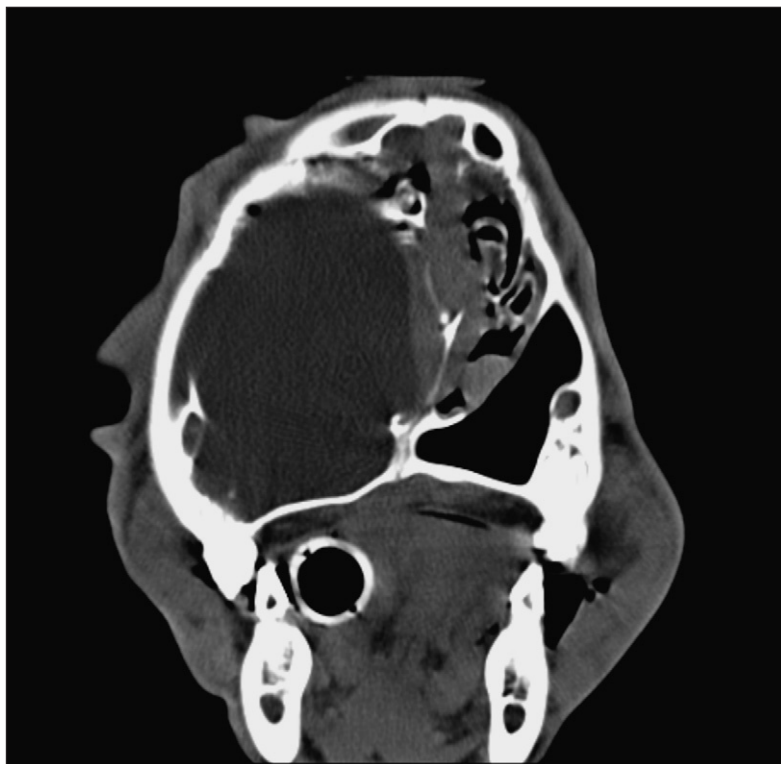
Fig. 19.2. Transverse CT image of an equine skull at the level of Triadan 09 (fourth cheek tooth) following administration of intravenous contrast medium. This image shows a fluid-filled mass within the right maxillary sinus that is causing marked displacement of the medial sinus wall and nasal septum to the left and gross distortion of the nasal concha bilaterally. Note the hyperdense rim surrounding the intra-sinus mass and its fluid-filled, hypodense center. Thickening of the overlying maxillary bone is present with irregularity of its surface. Diagnosis: paranasal sinus cyst. Reproduced with the permission of the section of diagnostic imaging/radio-oncology, Vetsuisse faculty of the University of Zürich, Switzerland.

should be avoided, as most ionic preparations are locally irritant. Renal vascular effects and renal damage induced by contrast media are well studied in humans and dogs (Porter 1993). Maintaining the patient's hydration status can minimize these adverse renal effects. Although extremely rare, severe side effects such as cardiac arrest and anaphylaxis have been described following administration of tri-iodinated, water-soluble compounds. Contrast agents with low osmolarity may have fewer adverse effects. Additionally, in equine imaging, cost is the main limitation in the use of intravenous contrast media.

Role of CT in the Evaluation of the Equine Upper Respiratory Tract

Clinical indications for CT scanning of the equine head are nasal discharge, swellings of the nasomaxillary and pharyngeal regions, epiphora, and nasal obstruction, especially when other ancillary techniques such as endoscopy and radiography are not diagnostic. CT allows precise presurgical evaluation of the size and location of upper respiratory tract lesions and so allows optimal planning of surgical procedures. CT may also be used in a postsurgical setting to ensure correct and complete repulsion of teeth, especially if the extracted tooth is fractured (Fig. 19.3).

CT of the equine head allows superior visualization of bony structures, soft tissue structures, and paranasal cavities. Detailed cross-sectional CT anatomy of the equine head has been described elsewhere (Morrow et al 2000, Smallwood et al 2002). The bony structures of the equine head are always visualized with CT. The nasal cavity and the adjoining paranasal sinuses have good image contrast because of the adjacent air and bone; thus paranasal sinuses and their communications are always identifiable in CT (Arencibia et al 2000, Lattimer 2002). The size and shape of the communications of nasal and paranasal cavities in normal horses show some degree of variation. The size of the conchomaxillary, nasomaxillary, and sphenomaxillary openings are more consistent than the frontomaxillary opening, which can vary between horses (Probst et al 2005). The frontomaxillary opening lies in a dorsal plane and provides drainage from the conchofrontal sinuses to the caudal maxillary sinus. The location of the frontomaxillary opening varies depending on the age of the horse. In younger horses the opening extends from the second to the fourth cheek teeth (Triadan 07–09). In older horses, the opening extends from the fifth cheek tooth (Triadan 10) to 20 mm past the last cheek tooth (Triadan 11) (Probst et al 2005). This should be taken into consideration when removing diseased teeth in horses (Kainer 1993).
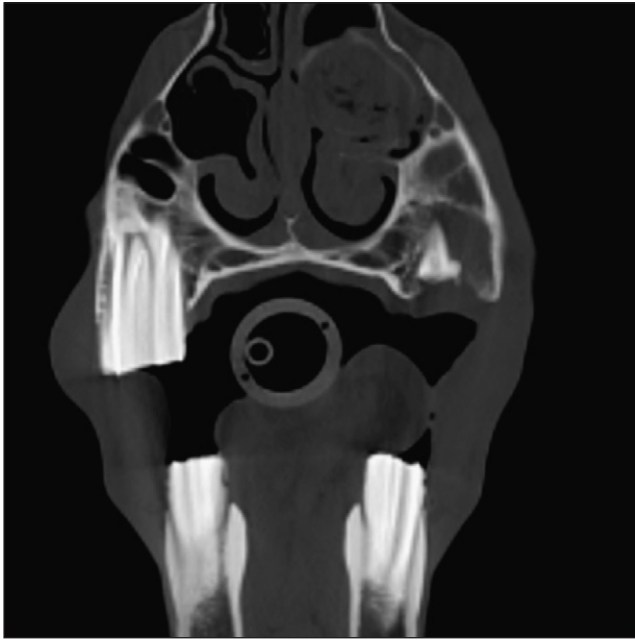
Fig. 19.3. CT of equine skull following oral extraction of Triadan 08 (third cheek tooth). A fragment of the extracted tooth is present at the ventromedial aspect of the distorted alveolus. Secondary inflammation with gas inclusion and mucosal thickening is also present in the ventral conchal sinus. Reproduced with the permission of the section of diagnostic imaging/radio-oncology, Vetsuisse faculty of the University of Zürich, Switzerland.

Normal cheek teeth have hyperdense enamel with slightly less dense dentine and cementum. The pulp appears hypodense, with the pulp canal being more opaque near the occlusal surface because of secondary dentine filling. Once formed, the tooth roots are homogeneous (contain only cement), the overlying sinus is air-filled and normally has only a thin (<5 mm) respiratory mucosa lining it, and the maxillary bone is of homogeneous appearance with a smooth surface and a sharply defined facial crest. The rostral cheek teeth (Triadan 06–08) are somewhat longer (8.5–10 cm in length) than the caudal cheek teeth (Triadan 09–11) (5–8.5 cm in length) (Henninger et al 2003). The fourth cheek tooth (09) is consistently shorter than the neighboring teeth (because it erupted first) and is often positioned close to the rostral aspect of the facial crest.

When evaluating CT scans of young horses, we must appreciate the significant changes that occur in the cross-sectional anatomy of the head as the foal matures. The paranasal sinuses expand during postnatal development. Deciduous and permanent teeth show remarkable changes in their eruption and development, and fibrous sutures between individual bones of the skull may now be recognized. The CT anatomy of the head of the foal has been described in detail by Smallwood et al 2002.

CT has an important role in the diagnosis of disorders of the equine head, including fractures, osteitis, dental disease, progressive ethmoid hematoma, maxillary sinus cyst, neoplasia of the nasal cavity, intracranial neoplasia, abscesses, temporohyoid arthropathy, and disorders of the ear canal such as otitis media and interna (Tietje et al 1996, Vink-Nooteboom et al 1998, Gardelle et al 1999, Henninger et al 2003). The few larger clinical publications on CT of the equine head include descriptions of the CT features of cholesterol granulomas of the choroid plexus (Vink-Nooteboom et al 1998), and dental alveolitis and sinusitis (Henninger et al 2003).

Sinusitis

Investigation of paranasal sinusitis is undoubtedly the most common indication for CT examination of the horse's head and CT has proven to be a powerful tool for evaluation of underlying dental disease or intra-sinus masses. Henninger et al (2003) recently described CT findings in horses with maxillary sinusitis. Evidence of sinusitis on CT scans includes thickening of the sinus mucosa (>5 mm thick) and fluid accumulation within the sinus. In that study, the rostral maxillary sinus was found to be more frequently affected than the larger caudal maxillary sinus (Henninger et al 2003). Early in the course of sinusitis, changes have been found in the ventral aspect of the rostral maxillary sinus (Henninger et al 2003). The frontomaxillary aperture may be markedly narrowed or completely obstructed in some cases of sinusitis (Henninger et al 2003). Displacement of the infraorbital canal is another common feature of maxillary sinusitis, and commonly occurs in combination with thickening and sclerosis of its bony wall. In chronic sinusitis, inflammatory polyps may be seen within the sinuses. Chronic sinus inflammation also leads to remodeling of the maxillary bone, with thickening, endosteal sclerosis and irregular surfaces, or less commonly, bony destruction.

An early sign of apical infection of the caudal maxillary cheek teeth (within the maxillary sinuses) is the appearance of a small, soft tissue mass around the tooth apex, that represents an apical granuloma (Fig. 19.4). Later in the course of disease, a periapical gas inclusion and sometimes fragmentation of the affected tooth apex (or root if it is formed) may indicate apical (tooth root) infection (Fig. 19.5). Widening of the periodontal space indicating destruction of the periodontal ligament is also usually seen in advanced dental disease. Dental disease is often accompanied by areas of lucency or opacification in the adjacent alveolar bone (Fig. 19.6) and remodeling of the maxillary bone is seen in severe periapical disease. The maxillary bone may show thickening, endosteal sclerosis, and an irregular surface in the presence of chronic inflammation. The facial crest is involved in around 50% of severe cases of maxillary cheek tooth infection (Henninger

Fig. 19.4. This CT shows an apical infection of Triadan 10 (fourth cheek tooth). There is apical granuloma formation, and periapical gas inclusion, within a distended and disrupted alveolus that has some focal calcified material deposition (reactive cementoma or new bone formation). Note the opacity of the adjacent ventral and dorsal conchal sinuses as a result of secondary (dental) sinusitis. Reproduced with the permission of the section of diagnostic imaging/radio-oncology, Vetsuisse faculty of the University of Zürich, Switzerland.
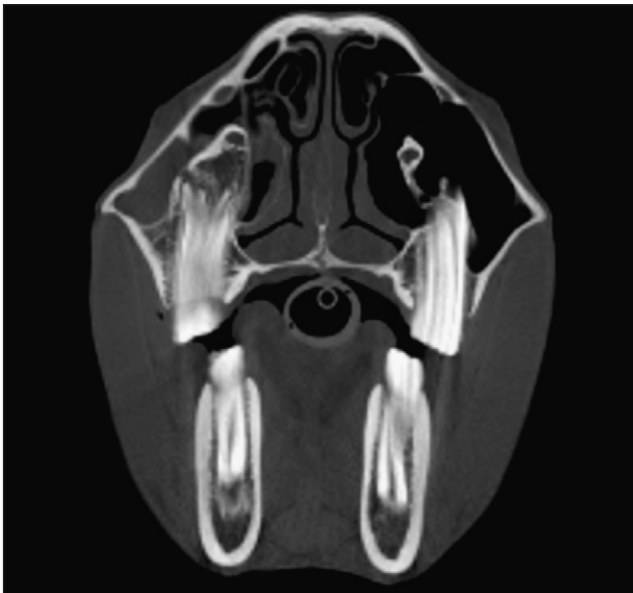
Fig. 19.5. This CT image shows chronic apical infection of Triadan 109 (fourth upper right cheek tooth). There is gross destruction of the apex, with loss of the roots. Irregular widening of the periodontium is present as a result of destruction and even total loss of alveolar bone, except at the apical area, where it appears distended. Spread of infection has caused displacement and partial destruction of the infraorbital canal. There is also thickening of the mucosal lining of the maxillary and ventral conchal sinuses. Reproduced with the permission of the section of diagnostic imaging/radio-oncology, Vetsuisse faculty of the University of Zürich, Switzerland.
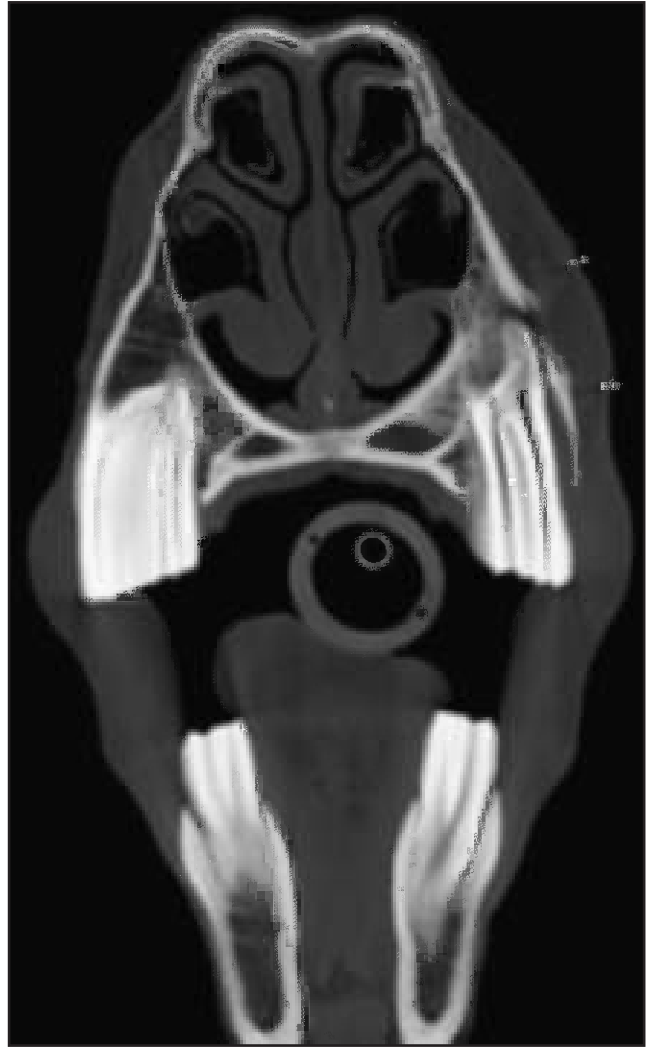
Fig. 19.6. This CT image shows extensive changes to Triadan 208 (third left upper cheek tooth) including narrowing and loss of density of this tooth, widening of the periodontium on its lateral aspect and absence of its alveolus on the medial aspect. There are also gas inclusions in the dental pulp canals, destruction of the maxillary bone, facial swelling and sinus tract formation. Metal skin clips were used for positioning. Diagnosis: apical infection. Reproduced with the permission of the section of diagnostic imaging/radio-oncology, Vetsuisse faculty of the University of Zürich, Switzerland.

et al 2003). Facial swelling and sinus tracts are seen more often in cases involving the rostral three maxillary cheek teeth. CT has been reported to positively identify infected teeth where radiography has been negative or equivocal.

Progressive ethmoidal hematoma

In CT, progressive ethmoidal hematoma presents as a soft tissue mass (around 30 HU) originating from the ethmo-turbinates (Fig. 19.7). In most cases the ethmoturbinates are enlarged or have extensive displacement or destruc-tion. The mass may extend from the rostral aspect of the

Fig. 19.7. This CT image shows a soft tissue mass in the frontal sinus that has arisen from the dorsolateral aspect of the adjacent ethmoturbinates. Partial destruction of the affected ethmoid is present. Diagnosis: progressive ethmoidal hematoma. Reproduced with the permission of the section of diagnostic imaging/radio-oncology, Vetsuisse faculty of the University of Zürich, Switzerland.

ethmoturbinates into the ipsilateral nasal meatus or caudally into the nasopharynx. These lesions may also extend from the lateral, dorsal, and ventral aspect of the ethmoturbinates into the caudal maxillary, frontal, and sphenopalatine sinuses, respectively. Destruction of the nasal septum has also been described (Tietje et al 1996). Thickening of bony structures in contact with the mass and fluid accumulation in the sinuses are other common findings. After application of intravenous contrast media, mild uniform enhancement of the progressive ethmoidal hematoma is seen.

Paranasal sinus cyst

The CT appearance of paranasal sinus cysts is of a uniform mass of about 10–20 HU. Most paranasal sinus cysts develop within the caudal maxillary sinus and less commonly in the rostral maxillary or frontal sinuses. Mineralization of the cyst wall (Lane et al 1987) may appear as dense spikes surrounding the cyst on the CT image. Extensive midline deviation of the medial wall of the sinus, and distortion of adjacent structures is common. Dental distortion and malocclusion have been described (Tucker & Farrell 2001). Fluid accumulation in sinuses outwith the cyst is common because of obstruction of normal sinus drainage. In longstanding cases, the surrounding bone may be thickened and have a roughened surface. The thinning of adjacent bone beneath expanding lesions in some cases may be the result of local secretion of prostaglandins (Tremaine & Dixon 2001). Rarely, bone destruction may occur. After intravenous administration of

contrast media, only peripheral enhancement of the mass is seen (i.e. uptake by the two layers of respiratory mucosa and underling stroma of the cyst wall) and the fluid-filled lumen of the cyst remains unchanged (Fig. 19.2).

Neoplasia

The CT appearance of neoplasm of the upper respiratory tract is highly variable, depending on the nature, extent, and location of the neoplasm. Adenocarcinomas and squamous cell carcinomas often present as highly aggressive masses with extensive destruction of surrounding structures. Their CT appearance is highly irregular with very variable HU counts. Fluid-filled areas and areas of mineralization may also be present. Depending on the tissue of origin, the mass may also penetrate and destroy the nasal septum and invade the contralateral nasal cavity. Destruction of the hard palate and invasion of the oral cavity may occur but many carcinomas may have extended from the oral cavity into the sinuses. Following intravenous administration of contrast media, excessive and often highly irregular enhancement of the tumor mass is seen.

Dentigerous cysts

Dentigerous cysts or temporal cysts are congenital abnormalities that comprise epithelium-lined cavities variably containing elements of dental origin. They are usually located on the temporal bone and discharge via a tract along the pinna. Other locations on the equine head have been rarely reported, including locations within the paranasal
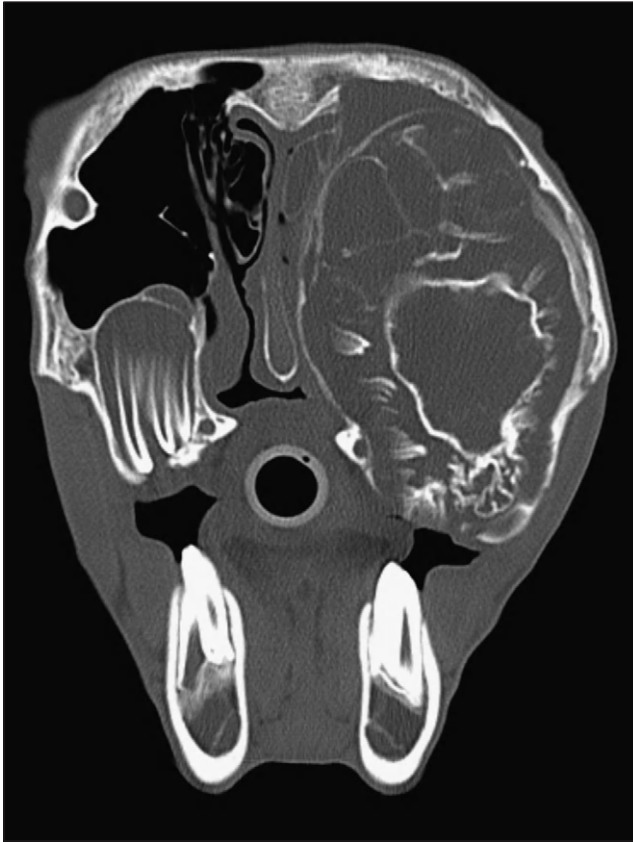
Fig. 19.8. Transverse CT image of an equine skull at the level of Triadan 09. An irregular mass fills the entire left maxillary sinus and compresses its medial wall off the nasal septum. The intra-sinus mass contains multiple tooth-like structures. There is also some fluid accumulation in the dorsal part of the maxillary sinus, probably as a result of impaired sinus drainage cause by the above mass. Diagnosis: dentigerous cyst. Reproduced with the permission of the section of diagnostic imaging/radio-oncology, Vetsuisse faculty of the University of Zürich, Switzerland.

Fig. 19.9. Fracture of vertical ramus of the right hemimandible. There is extensive soft tissue swelling in the pharyngeal region with displacement and compression of the guttural pouch (hematoma) Reproduced with the permission of the section of diagnostic imaging/radio-oncology, Vetsuisse faculty of the University of Zürich, Switzerland.

sinuses. The CT appearance of dentigerous cysts is highly variable. They present as fluid-filled structures of varying size, possibly containing calcified dental structures with very high HU counts (3000–4000 HU) (Fig. 19.8). The degree of distortion of adjacent structures such as temporal bone, ethmoturbinates, nasal septum or maxillary bone depends on the size and location of the cyst. Obstruction of the sinus and secondary sinusitis may occur. After intravenous administration of contrast media, peripheral enhancement is usually seen, but the cyst itself does not enhance.

Diseases of the pharynx, larynx, and guttural pouch

CT evaluation of the pharynx and larynx is somewhat limited by the fact that the patient is under general anesthesia and usually has an endotracheal tube *in situ*.

The presence of the endotracheal tube also prevents assessment of the spatial relationship of pharyngeal and laryngeal structures, unless there is marked asymmetry or soft tissue swelling present in this area.

In CT images, the guttural pouches appear as two symmetrical gas-filled structures, but their walls are not visualized. The stylohyoid bone is clearly seen as a bony structure with smooth and regular outlines, dividing each pouch into a medial and lateral compartment. The left and right guttural pouches can be seen to meet in the midline and form a thin septum. The auditory tube can be seen as a gas-filled structure passing in a caudal direction just ventral to the pterygoid bone. Interbreed differences in the size and extension of the two guttural pouch compartments have been reported (Sasaki et al 1999). The presence of a horizontal fluid–gas interface within the guttural pouches indicates guttural pouch hemorrhage (trauma, mycosis), mucus accumulation or empyema (usually following *Streptococcus equi* infection) (Fig. 19.9). The HU count of this fluid may give some indication of the type of fluid present within the pouch (whole blood 50 HU, blood clots 50–80 HU, mucus 10–25 HU). The retropharyngeal areas should be carefully examined for signs of soft tissue masses representing lymphadenopathy.

Fractures of the stylohyoid bone are easily recognized as a sequel to trauma, mycotic osteopathy (Tucker & Farrell 2001) or temporohyoid osteoarthropathy (Walker et al 2002). In the latter cases, CT has proven to be a powerful diagnostic tool.

Fractures of the skull

Diagnosis of fractures of the equine skull may be extremely difficult using conventional radiography. This is mainly the result of superimposition of multiple bony structures over the site of interest. Fractures of any part of the head and the cranial cervical vertebral column may be visualized by CT. The usefulness of CT in the diagnosis of skull fractures in the equine patient has been well documented (Ramirez 1998, Gardelle et al 1999). In cases where there is only minimal fracture displacement, or where incomplete fractures have occurred, it is advised to set slice thickness at no greater than 3 mm and to allow for overlap of slices of at least 1 mm. This allows for better spatial resolution in planar reconstruction and three-dimensional reconstruction.

REFERENCES

Allen JR, Barbee DD, Boulton CR et al 1987 Brain abscess in a horse: diagnosis by computed tomography and successful surgical treatment. Equine Veterinary Journal 19: 552–555

Arencibia A, Vazquez JM, Rivero M et al 2000 Computed tomography of normal cranioencephalic structures in two horses. Anatomy Histology and Embryology 29: 295–299

Diehl M, Cordey J 1983 Bone densitometry: using the axial Isotom tomograph on healthy and diseased navicular bone in horses *in vitro*. Berliner and Munchener Tierarztliche Wochenschrift 96: 305–307

Dixon PM, McGorum BC, Railton DI et al 2001 Laryngeal paralysis: a study of 375 cases in a mixed-breed population of horses. Equine Veterinary Journal 33: 452–458

Gardelle O, Feige K, Geissbuhler U et al 1999 Possibilities for computer tomography of the equine head based on two cases with a fracture of the base of the skull. Schweizer Archiv fur Tierheilkunde 141: 267–272

Henninger W, Frame EM, Willmann M et al 2003 CT features of alveolitis and sinusitis in horses. Veterinary Radiology and Ultrasound 44: 269–276

Kainer RA 1993 Clinical anatomy of the equine head. Veterinary Clinics of North America; Equine Practice 9: 1–23

Lane JG, Longstaffe JA, Gibbs C 1987 Equine paranasal sinus cysts: a report of 15 cases. Equine Veterinary Journal 19: 537–544

Lattimer JC 2002 Equine nasal passages and sinuses. In: Thrall DE (editor) Textbook of Veterinary Diagnostic Radiology, 4th edn. WB Saunders, Philadelphia, pp.87–98

Morrow KL, Park RD, Spurgeon TL et al 2000 Computed tomographic imaging of the equine head. Veterinary Radiology and Ultrasound 41: 491–497

O'Callaghan MW, Pascoe JR, Tyler WS et al 1987 Exercise-induced pulmonary haemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. IV. Changes in the bronchial circulation demonstrated by CT scanning and microradiography. Equine Veterinary Journal 19: 405–410

Porter GA 1993 Effects of contrast agents on renal function. Investigative Radiology 28 (Supplement 5): S1–S5; discussion S6

Probst A, Henninger W, Willmann M 2005 Communications of normal nasal and paranasal cavities in computed tomography of horses. Veterinary Radiology and Ultrasound 46: 44–48

Ramirez O, Jorgensen JS, Thrall DE 1998 Imaging basilar skull fractures in the horse: a review. Veterinary Radiology and Ultrasound 39: 391–395

Sasaki M, Hayashi Y, Koie H et al 1999 CT examination of the guttural pouch (auditory tube diverticulum) in Przewalski's horse (*Equus przewalski*). Journal of Veterinary Medicine and Science 61: 1019–1022

Smallwood JE, Wood BC, Taylor WE et al 2002 Anatomic reference for computed tomography of the head of the foal. Veterinary Radiology and Ultrasound 43: 99–117

Tietje S, Becker M, Bockenhoff G 1996 Computed tomographic evaluation of head diseases in the horse: 15 cases. Equine Veterinary Journal 28: 98–105

Tremaine WH, Dixon PM 2001 A long-term study of 277 cases of equine sinonasal disease. Part 1: details of horses, historical, clinical, and ancillary diagnostic findings. Equine Veterinary Journal 33: 274–282

Tucker RL, Farrell E 2001 Computed tomography and magnetic resonance imaging of the equine head. Veterinary Clinics of North America; Equine Practice 17: 131–144

Vink-Noooteboom M, Junker K, van den Ingh TS et al 1998 Computed tomography of cholesterinic granulomas in the choroid plexus of horses. Veterinary Radiology and Ultrasound 39: 512–516

Walker AM, Sellon DC, Cornelisse CJ et al 2002 Temporohyoid osteoarthropathy in 33 horses (1993–2000). Journal of Veterinary Internal Medicine 16: 697–703

20

Thoracoscopy and Lung Biopsy

Joel Lugo and John F Peroni

Introduction

Thoracoscopy is a minimally invasive endoscopic surgical procedure that allows video-assisted examination of the thoracic cavity (Landreneau et al 1993). The procedure is performed by inserting a rigid endoscope and additional operative instrumentation through the intercostal spaces. A unilateral pneumothorax is induced after perforation of the thoracic wall and the thoracic anatomical structures are observed after collapse of the ipsilateral lung.

In 1985 Mackey and Wheat tested the technique on 10 horses using a 130° rigid endoscope and then used thoracoscopy in five horses with pleuropulmonary disease (Mackey & Wheat 1985). The authors advocated using thoracoscopy as an adjunctive diagnostic procedure to provide a more accurate prognosis. A subsequent report described eight clinical cases and six normal horses (Mansmann & Bernard-Strother 1985). These authors combined a description of the normal thoracic anatomy with a series of clinical cases of chronic thoracic disease. In contrast with the study by Mackey and Wheat, these authors used a 110-cm long flexible, fiberoptic endoscope, which proved useful for the examination of the most ventral aspect of the chest cavity.

Thoracoscopy in the horse has since gained popularity. In 1998, 28 clinical cases were described that had been diagnosed and treated using thoracoscopy (Vachon & Fischer 1998). The procedures were performed in the standing horse and also under general anesthesia for the diagnosis and treatment of thoracic neoplasia, pleuropneumonia (adhesion breakdown and intrathoracic drain placement), thoracic abscessation, pericarditis, and diaphragmatic hernia.

Indications

Thoracoscopy is most efficiently employed as a complementary procedure to traditional diagnostic imaging methods, such as ultrasonography and radiography (Ahmed & Jones 2004). The identification and investigation of pulmonary, pleural, and mediastinal disease is greatly enhanced by an unobstructed and direct view of the intrapleural structures but, most importantly, thoracoscopy presents the clinician with the option of converting from a diagnostic to a therapeutic procedure.

An added benefit is that thoracoscopy can be easily accomplished in the standing horse, thereby limiting the risks associated with combining open thoracic surgery and general anesthesia.

The advantages of thoracoscopy in assessing pleuritis and pneumonia were elucidated in a report of 28 cases (Vachon & Fischer 1998). In horses affected by pleuropneumonia, clinicians were able to view the cranioventral regions of the thorax, thereby allowing accurate placement of thoracic drains without compromising pulmonary or cardiac structures and facilitating debridement of necrotic lung tissue and resection of affected pulmonary segments. As evident from this clinical report, thoracoscopy is most often employed in those chronic cases of pleuropneumonia that are unresponsive to standard antimicrobial therapy and pleural drainage. In these cases, thoracoscopy allows the aggressive debridement of nonviable lung tissue, the evaluation and resection of pleural adhesions, and the establishment of the most appropriate site for ventral drainage. The procedure can also be performed in selected cases of acute pleuropneumonia, when breakdown of immature adhesions and establishment of drainage are necessary, and in cases in which an endoscopically guided thoracic lavage may aid in resolution of acute pleuritis. Care should be taken to avoid using the technique on horses with acute pleuropneumonia, because the inflammatory response associated with the procedure or possible iatrogenic hemorrhage may disturb the pleural environment enough to allow exacerbation of a low-grade infection.

The investigation of thoracic neoplasia is another indication for thoracoscopy. The procedure has allowed the ante-mortem diagnosis of thoracic neoplasia and the collection of tissue biopsy samples for histological diagnosis. Both flexible and rigid endoscopes have been used to diagnose pleural metastatic invasion by gastro-esophageal squamous cell carcinoma, hemangiosarcoma and cholangiocellular carcinoma (Ford et al 1987, Rossier et al 1990, Mueller et al 1992).

Other reported thoracoscopy-assisted procedures are diaphragmatic hernia repair (Malone et al 2001), assessment of the thoracic portion of the esophagus for obstruction and stricture evaluation, and the investigation of the nature and location of thoracic wounds (Peroni 2003).

Equipment and Technique

The equipment used for thoracoscopy in the horse is the same as that required for laparoscopy, including an endoscope coupled to a video camera. Commonly a 58-cm long, 10-mm diameter rigid endoscope is used (30° Hopkins endoscope). The endoscope is attached to a video camera and light source. The video camera used is a standard endoscopic camera that is commonly used for arthroscopy and laparoscopy. A 300-watt xenon light source is recommended. The endoscope is inserted through the chest wall via a trocar/cannula unit, which can be disposable or non-disposable and of variable length (Fig. 20.1). Screw-in cannulae are safer to insert into the chest because they do not require a trocar, thereby eliminating iatrogenic pulmonary trauma. Importantly, the cannula must be equipped with a side port with a stopcock to allow attachment of tubing for insufflation and suction. Insufflation is not usually required for thoracoscopy; however a CO_2 insufflation (e.g. laparoflator) may be employed to distend the thorax and improve visualization of thoracic contents of foals, which have a more compliant chest wall than adults. A CO_2 laparoflator may also be employed to facilitate surgical exposure during thoracoscopy performed under general anesthesia. A suction unit is mandatory to re-establish the normal subatmospheric pleural pressure following completion of thoracoscopy. Additional trocar/cannula units of varying length and size may be used to introduce accessory instrumentation in the thorax or to change the insertion point of the endoscope.

The use of a rigid endoscope is generally preferred for intrapleural procedures although a flexible endoscope has been used (Mackey & Wheat 1985). The advantage of rigid endoscopy is the ease with which the endoscope can be moved within the thorax. Flexible endoscopes tend to follow gravity when placed in the chest and invariably fall toward the most ventral region of the thorax. As often occurs during upper airway endoscopy, flexible endoscopes require at least two individuals to advance the unit and simultaneously maneuver the camera located in the tip of the endoscope. In contrast, with the rigid endoscope the surgeon is in control of both position and field of view. The 58-cm long endoscope with its 30° optical viewing angle is ideally suited for a broad view of the thorax and also allows the delivery of high-intensity xenon light for optimizing detail. One advantage of the flexible fiberoptic endoscopes may be the fact that the tip of the endoscope can be maneuvered 360°, which may be convenient in closed spaces such as walled-off pleural abscesses.

In preparation for thoracoscopy, horses are usually premedicated with flunixin meglumine (1 mg/kg q 12 h) and systemic broad-spectrum antibiotics. In elective thoracoscopy, prophylactic perioperative antibiotic therapy may include an aminoglycoside and β-lactam antibiotic combination administered for 24 h. In the standing horse, thoracoscopy can be completed safely using local anesthesia and systemic analgesia/sedation (either xylazine or detomidine combined with butorphanol). Detomidine is a selective α_2-adrenergic receptor agonist favored over xylazine for thoracoscopy because its effects are of longer duration and profoundly sedative (Daunt et al 1993). Detomidine can be safely administered as a continuous infusion and has proven to be safe both in normal horses and in horses with recurrent airway obstruction undergoing thoracoscopy and lung biopsy (Peroni et al 2000, Lugo et al 2002).
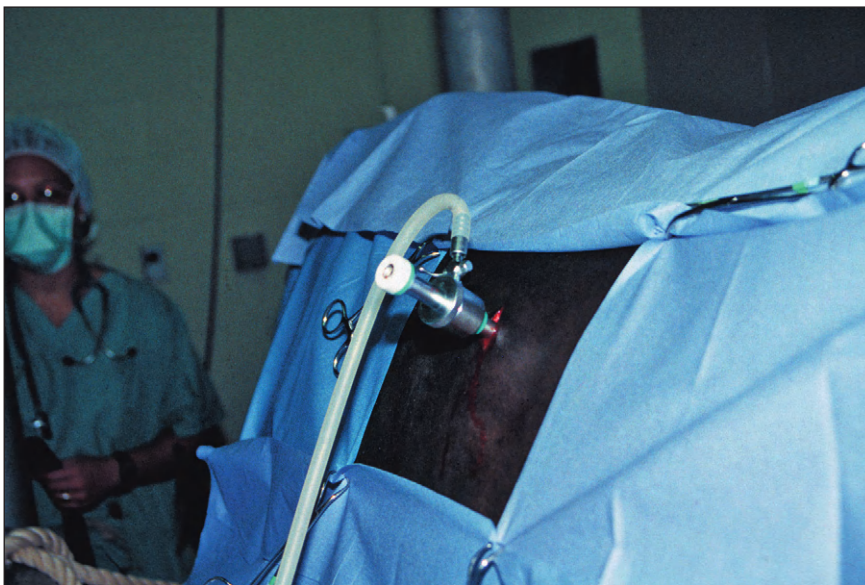
Fig. 20.1. Cannula placed in the dorsocaudal aspect of the left thorax during thoracoscopy performed in the standing horse. Suction tubing is shown attached to the side port of the cannula.

Horses are restrained in stocks and the hemithorax is clipped over an area extending from the caudal aspect of the shoulder to the last rib and from the dorsal thorax to the ventral thorax at the level of the elbow joint. This area is aseptically prepared. Local anesthetic is infused subcutaneously into the site of the endoscope portal, either at the 8th, 10th, or 12th intercostal space, just ventral to the serratus dorsalis muscle. The authors consider that entering the thorax via the 10th and 12th intercostal spaces is most effective to facilitate comfortable exploration of the thorax. An ipsilateral pneumothorax is induced by making a 2-cm skin and subcutaneous tissue incision and inserting a stainless steel, blunt cannula into the pleural space. Upon cannula placement a characteristic sound of air rushing into the chest can be heard with each breath, indicating the development of a pneumothorax. Following removal of the cannula, a sharp 10-mm diameter trocar/cannula unit is inserted through the skin incision into the thoracic cavity to serve as the endoscope portal. The rigid endoscope is connected to a video camera and light source and is then placed through the portal to explore the hemithorax.

Approximately two-thirds of the thoracic cavity, including major portions of the lungs, mediastinum, thoracic aorta, esophagus, diaphragm, and sometimes the base of the heart/pericardium can be visualized (Klohnen & Peroni 2000). The cranioventral region of the thoracic cavity is more difficult to observe because the collapsed lung tends to fall cranially, thereby obstructing the view of the most ventral region of the chest. During the exploratory phase of thoracoscopy it may be necessary to make additional accessory portals to facilitate introduction of additional operative instrumentation needed to complete procedures such as lung biopsy (see below). When placing multiple portals, the rules of appropriate triangulation technique used in arthroscopy and laparoscopy should be followed to maximize hand–eye coordination and efficient instrument manipulation.

Upon completion of thoracoscopy, the normal subatmospheric pleural pressure is re-established by applying suction to the pleural space via sterile tubing connected to the endoscopic portal unit. First, all cannulae are removed with the exception of the endoscope portal and, while suction is applied, it is useful to leave the endoscope in the dorsal thorax to observe complete lung inflation over about 15–20 seconds. Skin incisions are closed with a simple interrupted pattern using non-absorbable suture material or staples.

Thoracoscopy is generally performed in the standing horse. Most procedures can be accomplished safely in this manner and the decision to proceed with general anesthesia should be carefully weighed. Horses with pleuropulmonary disease are not the best candidates for general anesthesia; however, in certain cases it may be necessary to explore the ventral lung surfaces, ventral thoracic cavities,

and lateral surface of the heart. In clinical practice, general anesthesia has been used to accomplish window pericardectomy in horses with constrictive pericardial effusion and also exploration of thoracic wounds involving the ventral aspect of the chest (Vachon & Fischer 1998).

Complications of Thoracoscopy

Thoracoscopy is well tolerated and has been associated with minimal cardiopulmonary complications in healthy horses (Peroni et al 2000). Despite the relatively low incidence of complications, the clinician should be aware of the consequences of inducing a pneumothorax, especially in horses with underlying lung disease. Even in horses with severe pneumonia, however, the incidence of intra- or post-operative problems is low.

Creation of an iatrogenic pneumothorax is a necessary feature of thoracoscopy and horses should be carefully monitored during the procedure, particularly if lung disease has significantly impaired ventilation–perfusion matching. Lung collapse can further exacerbate gas exchange and the surgeon should undertake precautionary measures that may reduce the risk of hypoxemia. The lung can be gradually collapsed by intermittently opening and closing the teat cannula used to create the pneumothorax. Transient exacerbation of pulmonary compromise, evidenced by tachypnea, can be alleviated by re-inflation of the lung (Vachon & Fischer 1998). In addition, the degree of lung collapse can be varied during the procedure by applying suction to the pleural space. It is important to regulate the degree of pneumothorax depending upon the anatomical region to be inspected and the overall cardiopulmonary status of the horse (Peroni et al 2001). Nasal insufflation with 100% oxygen may be an additional useful procedure to implement during thoracoscopy.

Although rare, other possible complications include lung perforation upon entry into the chest and intra-pleural hemorrhage. Pulmonary injury can be avoided if appropriate measures are taken such as using guarded or screw-in type cannulae and, most importantly, ensuring that adequate lung collapse has been achieved before entering the pleural space. The most common reason for hemorrhage is perforation of an intercostal blood vessel. The intercostal artery and vein are located along the caudal edge of each rib and inadvertent perforation of these structures can be prevented by careful insertion of the cannula along the cranial edge of the rib. In horse with chronic pleuritis, mature fibrous adhesions may form between the visceral and parietal pleurae, which mechanically limit lung collapse during creation of the pneumothorax. Preoperative knowledge of this complication is important and can be accomplished via a careful ultrasound examination. Depending on the location of fibrin tags and adhesions the surgeon may choose to strategically place the endoscope portal so that the most

unobstructed view of the chest can be obtained. Typically, adhesions form in the mid and lower thorax; therefore, a more dorsally placed endoscope can facilitate exploration.

Lung Biopsy

Despite the numerous diagnostic modalities available for investigation of equine thoracic diseases, accurate diagnosis and prognosis of some pleuropulmonary diseases often require direct evaluation of the pulmonary tissue. Transendoscopic bronchial pinch lung biopsy (Fig. 20.2) (Mair 1992) and percutaneous lung biopsy (Schatzmann et al 1974, Raphel & Gunson 1981, Savage et al 1998) have been used in horses. More recently, thoracoscopically guided pulmonary wedge resection has been described (Lugo et al 2002). The most common indications for lung biopsy include suspicion of infiltrative disease, neoplasia and interstitial disease, and for pulmonary research (Nyman et al 1991, Naylor et al 1992, Savage et al 1998). Lung biopsy should be performed with care or avoided in horses with pulmonary abscesses and bacterial pneumonia and pleuropneumonia, because the infectious process may be disseminated to other areas of the lung and pleural cavity.

Fig. 20.2. Transendoscopic bronchial pinch biopsy. Reproduced with the permission of P.M. Dixon, University of Edinburgh.

Transendoscopic pinch biopsy

A technique for transendoscopic biopsy of the airway mucosa has been described (Mair 1992, Buechner-Maxwell et al 1996). We were unable to find studies that evaluated this procedure in a controlled manner and compared histological and microbiological results with those obtained via more common diagnostics techniques.

After the horse is restrained in stocks and sedated (xylazine or detomidine), a >150-cm endoscope is passed through the ventral meatus of the nasal cavity to the level of the bifurcation of the trachea into a main-stem bronchus. Twenty to thirty milliliters of local anesthetic diluted in 25 ml saline is instilled through the endoscope biopsy channel to provide topical anesthesia of the bronchial mucosa. The endoscope is then passed into a bronchus and wedged. The bronchial tissue sample is obtained using endoscopic biopsy forceps, which are introduced through the operating channel of the endoscope. Multiple biopsy samples can be obtained. The biopsy sample is immediately placed in 10% formalin and/or in media for bacterial and fungal culture.

Percutaneous lung biopsy

The percutaneous technique described by Raphel and Gunson (1981) has become the standard method for lung biopsy in horses because it is reasonably safe, easily performed, and inexpensive. The technique is performed with the horse standing, sedated, and restrained in stocks. A 10-cm square of skin is clipped between the seventh and ninth intercostal space, approximately 10 cm above a horizontal line to the point of the elbow. Alternatively, if a specific site of lung tissue is to be sampled, ultrasonographic and radiographic evaluation may help to localize the site of interest. The skin, subcutaneous tissue, and intercostal muscles are infiltrated with local anesthetic solution and the skin is surgically prepared. A Tru Cut biopsy needle (Travelonol Laboratories, Deerfield, IL, USA) 15 cm long with a 2-cm specimen notch is inserted through a stab incision made just cranial to the rib. The biopsy needle is rapidly advanced into the lung and the tissue sample is collected. Biopsy samples are immediately placed in formalin for fixation. Because of the elastic nature of the lung, the chamber of the biopsy needle is seldom filled; therefore, multiple samples are usually collected. Following biopsy, horses should be rested for 2–3 days. The main drawback of this procedure is that frequently the samples obtained are small and may not be of diagnostic value. Complications described following percutaneous lung biopsy include sudden collapse, epistaxis, pneumothorax, and fatal pulmonary hemorrhage (Costa et al 2000). Indeed, in a survey reported by Savage et al (1998), 33 of 44 respondents had seen a complication at least once, and approximately 3% of the horses died after the procedure.

Thoracoscopically guided wedge resection lung biopsy

Thoracoscopically guided wedge resection lung biopsy is safe, well tolerated, and provides a minimally invasive method for lung biopsy in horses, avoiding the complications
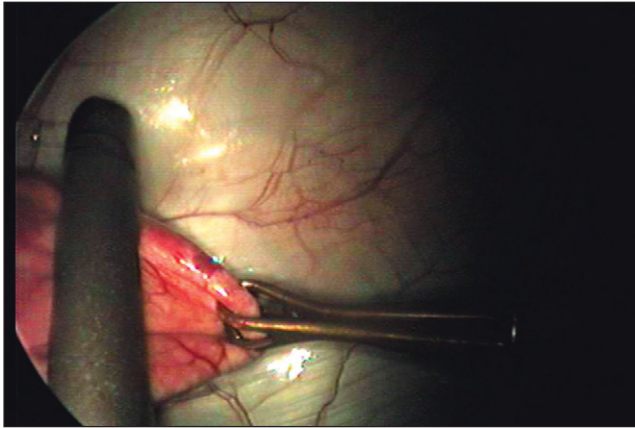
Fig. 20.3. A wedge resection of the lung being performed.

seen with other techniques. Unlike percutaneous lung biopsy, this method of lung biopsy provides a tissue sample that is adequate for histologic examination. With this technique, the sample obtained contains few artifacts, increasing the reliability of lung biopsies for both clinical and research purposes. Other advantages are that the specimen obtained can comprise both normal and diseased tissue, and that complications reported with other techniques can be prevented. Furthermore, if a larger tissue sample is desired, multiple wedge resections can be performed. The tissue sample is generally obtained from the caudodorsal aspect of the caudal lung lobe and is thus useful for diagnosis of peripheral lung lesions or diffuse pulmonary diseases.

Lung biopsy via thoracoscopy is performed with the horse standing and using chemical restraint as described above. After the endoscope is placed in the pleural cavity, a site for lung biopsy is selected. One accessory instrument portal is placed one or two intercostal spaces cranial and 15 cm ventral to the endoscope, and the other instrument portal is usually placed through the 15th intercostal space approximately 10 cm ventral to the endoscope portal. The caudal aspect of the caudal lung lobe is grasped with endoscopic forceps, and using commercially available endoscopic stapling equipment, the wedge resection sample is obtained (Fig. 20.3). The sample of tissue is withdrawn and the biopsy site is evaluated for hemostasis. The normal subatmospheric pressure of the pleural cavity is re-established by removing the air via suction and the skin incisions are closed as described above.

REFERENCES

Ahmed N, Jones D 2004 Video-assisted thoracic surgery: state of the art in trauma care. Injury 35: 479–489

Buechner-Maxwell V, Christmann M, Murray M et al 1996 A transendoscopic biopsy of the horse's airway mucosa. Journal of Equine Veterinary Science 16: 375–379

Costa LR, Seahorn TL, Moore RM et al 2000 Correlation of clinical score, intrapleural pressure, cytologic findings of bronchoalveolar fluid, and histopathologic lesions of pulmonary tissue in horses with summer pasture-associated obstructive pulmonary disease. American Journal of Veterinary Research 61: 167–173

Daunt DA, Dunlop CL, Chapman PL et al 1993 Cardio-pulmonary and behavioral responses to computer-driven infusion of detomidine in standing horses. American Journal of Veterinary Research 54: 2075–2080

Ford TS, Vaala WE, Sweeney CR et al 1987 Pleuroscopic diagnosis of gastroesophageal squamous cell carcinoma in a horse. Journal of the American Veterinary Medical Association 190: 1556–1558

Klohnen A, Peroni JF 2000 Thoracoscopy in horses. Veterinary Clinics of North America; Equine Practice 16: 351–362

Landreneau RJ, Mack MJ, Keenan RJ et al 1993 Strategic planning for video-assisted thoracic surgery. Annals of Thoracic Surgery 56: 615–619

Lugo J, Stick JA, Peroni J et al 2002 Safety and efficacy of a technique for thoracoscopically guided pulmonary wedge resection in horses. American Journal of Veterinary Research 63: 1232–1240

Mackey VS, Wheat JD 1985 Endoscopic examination of the equine thorax. Equine Veterinary Journal 17: 140–142

Malone ED, Farnsworth K, Lennox T et al 2001 Thoracoscopic-assisted diaphragmatic hernia repair using a thoracic rib resection. Veterinary Surgery 30: 175–178

Mansmann RA, Bernard-Strother S 1985 Pleuroscopy in horses. Modern Veterinary Practice 66: 9–17

Mair T 1992 Diagnostic techniques of the lower respiratory tract diseases. In: Robinson NE (editor), Current Therapy in Equine Medicine, 3rd edn. WB Saunders, Philadelphia, pp.302–303

Mueller PO, Morris DD, Carmichael KP et al 1992 Antemortem diagnosis of cholangiocellular carcinoma in a horse. Journal of the American Veterinary Medical Association 201: 899–901

Naylor JN, Clark EG, Clayton HM 1992 Chronic obstructive pulmonary disease: usefulness of clinical signs, bronchoalveolar lavage, and lung biopsy as diagnostic and prognostic aids. Canadian Veterinary Journal 33: 591–598

Nyman G, Lindberg R, Weckner D et al 1991 Pulmonary gas exchange correlated to clinical signs and lung pathology in horses with chronic bronchiolitis. Equine Veterinary Journal 23: 253–260

Peroni JF 2003 Laparoscopic assessment of abdominal trauma in horses. Compendium on Continuing Education for the Practicing Veterinarian 24: 490–494.

Peroni JF, Robinson NE, Stick JA et al 2000 Pleuropulmonary and cardiovascular consequences of thoracoscopy performed in healthy standing horses. Equine Veterinary Journal 32: 280–286

Peroni JF, Horner NT, Robinson NE et al 2001 Equine thoracoscopy: normal anatomy and surgical technique. Equine Veterinary Journal 33: 231–237

Raphel CF, Gunson DE 1981 Percutaneous lung biopsy in the horse. The Cornell Veterinarian 71: 439–448

Rossier Y, Sweeney CR, Heyer G et al 1990 Pleuroscopic diagnosis of disseminated hemangiosarcoma in a horse. Journal of the American Veterinary Medical Association 196: 1639–1640

Savage CJ, Traub-Dargatz JL, Mumford EL 1998 Survey of the large animal diplomates of the American College of Veterinary Internal Medicine regarding percutaneous

lung biopsy in the horse. Journal of Veterinary Internal Medicine 12: 456–464

Schatzmann U, Straub R, Gerber H et al 1974 Percutaneous lung biopsy in the horse. Veterinary Record 94: 588–590

Vachon AM, Fischer AT 1998 Thoracoscopy in the horse: diagnostic and therapeutic indications in 28 cases. Equine Veterinary Journal 30: 467–475

21 Post-mortem Examination

Kenneth C Smith

Introduction

Careful and comprehensive post-mortem examination is a fundamental method of diagnosis for many cases of equine respiratory disease, and is also critical in clinico-pathological audit. As expertise in equine medicine, surgery, and diagnostic imaging progresses, so it is important that pathology advances at a similar rate, to capitalize on technological advances. Classical techniques of veterinary pathology (morbid anatomy and histopathology) are now augmented and enhanced by the armory of modern molecular diagnostics [immunophenotyping, *in situ* hybridization and polymerase chain reaction (PCR)], and it is likely that techniques such as tissue microarray analysis will also form part of the routine toolbox of the veterinary pathologist in coming years (Bodrossy & Sessitsch 2004). Such novel techniques rest upon a foundation of sound post-mortem technique and tissue sampling, which are outlined in this chapter.

Method of Post-mortem Examination

Selection of post-mortem method

Several techniques of equine post-mortem examination have been described (Rooney & Robertson 1996). The method used is one of personal preference for the pathologist, but should in any event permit full examination of all of the body organs in a standardized and reproducible manner. To gain maximal information from a post-mortem examination it is preferable wherever possible that the carcass be referred to an experienced equine pathologist with access to full laboratory facilities as soon as possible after death or euthanasia. The pathologist should be given access to all the clinical and laboratory data relating to the horse before commencing the post-mortem examination, and it is often of value to both individuals if the attending clinician observes all or part of the post-mortem examination. Where referral of the carcass to a specialist institute is not feasible, it is nonetheless important that the veterinary surgeon in the field undertakes a systematic and comprehensive examination, taking contemporaneous notes and photographs of any changes found. Samples should be collected for other analyses such as microbiology, toxi-

cology, histology, and immunohistochemistry. For these subsequent examinations to yield the most useful information, it is important that any changes present on dissection have been recognized and described in full. Photographs of such changes also assist the histopathologist in interpretation of microscopic changes.

Post-mortem examination of adult horses

The method outlined in this chapter is one used routinely in the author's laboratory in Newmarket for post-mortem examination of the respiratory tract, and is based upon examination of the horse supported in dorsal recumbency. The method commences after external examination of the carcass, removal and examination of the abdominal viscera, and removal of the head by transection of the atlanto-occipital joint (Rooney & Robertson 1996).

Examination of the diaphragm and pleura

Following removal of the abdominal contents, the diaphragm is incised to test for subatmospheric intrapleural pressure. Where pneumothorax is present, the expected in-rush of air into the thoracic cavity upon incision of the diaphragm does not occur. Pneumothorax in horses is a rare disorder but can be acquired secondary to traumatic damage to the trachea, esophagus, lungs, chest wall or diaphragm. If a diaphragmatic defect is present then it is important to determine whether this is congenital, acquired or a post-mortem change. Congenital diaphragmatic hernias generally involve the dorsal diaphragmatic quadrant in foals, have smooth edges, and may be associated with visceral herniation. Acquired and presumably post-traumatic diaphragmatic hernias, by contrast, have ragged edges with hemorrhage and often occur on the left side of the diaphragm. Post-mortem rupture of the diaphragm can occur in horses that are examined several hours after death, as a consequence of post-mortem gastrointestinal tympany (usually involving the large bowel). Post-mortem rupture is not accompanied by hemorrhage, although post-mortem discoloration of tissue often necessitates microscopic examination of the torn diaphragmatic edges to make an absolute distinction.

After assessing diaphragmatic integrity, the central portion of the diaphragm is removed by incising around

the periphery and the thoracic contents are inspected *in situ* from the caudal aspect of the cavity. In the majority of horses the integrity of the caudal mediastinum maintains a barrier between the left and right sides of the thoracic cavity so that if the diaphragm is incised in an anti-clockwise fashion from right to left then air enters only the right pleural cavity until the incision is continued beyond the midline to incise the mediastinum. Pneumothorax may thus be defined as left- or right-sided. Some horses have fenestrations within the caudal mediastinum that allow air movement between the left and right sides, resulting in bilateral pneumothorax. These fenestrations are difficult to detect on dissection, and will be missed unless careful observation of air movement is made on incision around the diaphragm.

A sample of pleural fluid can be obtained aseptically as the diaphragm is removed. In an adult horse, there is a small volume (usually 5–15 ml) of clear yellow fluid in each side of the pleural cavity. This fluid becomes progressively discolored red as a result of hemolysis if post-mortem examination is delayed. Excessive fluids, changes in fluid color or consistency, or the presence of pleural adhesions, are indications for collection of pleural fluid for cytological and bacteriological analysis. Pleural fluid should be collected into:

- an ethylenediaminetetraacetic acid (EDTA) container for cytological assessment
- a container with an equal volume of neutral buffered formalin for cytology if any delay in analysis is anticipated
- a sterile container for protein measurement and bacteriology.

Cytological and bacteriological analyses of pleural fluid are discussed in Chapter 9. Inflammation of the pleural surfaces (pleuritis) may occur in pleuropneumonia, giving rise to thickening and discoloration of the pleurae, with accumulation of exudate in the pleural cavities. It is often difficult to recover microorganisms from pleural fluid collected from horses that have already received antibiotic therapy, and pleural fluid smears demonstrating exudative inflammatory change should be scrutinized carefully for microorganisms using appropriate special stains. Mesothelioma is rare in horses, as in other domestic animals. Published reports of mesothelioma describe massive pleural effusion, in which malignant cells may be recognized on cytology, with multifocal, shaggy, plaque-like thickenings of pleural surfaces (Colbourne et al 1992, Stoica et al 2004).

Examination of the trachea

Following collection of pleural fluid, the thoracic organs are removed by separating the trachea and esophagus from the neck, having incised through the trachea and esophagus below the larynx, bluntly dissected the trachea and esophagus from the cervical muscle and connective tissue, and transected the brachiocephalic blood vessels

and soft tissue attachments in the thoracic inlet. The lungs and heart together with the anterior mediastinum (including the thymus if present) are removed through the diaphragmatic opening and via the abdominal cavity, by passing the trachea/esophagus back through the thoracic inlet, having released the heart within the pericardial sac from its ventral attachments. The volume of fluid in the pericardial sac is noted, and, if appropriate, cytology or microbiology samples are collected as described for pleural fluid samples, before the pericardial sac is detached. Once the thoracic viscera have been exteriorized into the abdominal cavity, the aorta is transected at the diaphragm and the thoracic viscera are drawn backwards by pulling on the trachea and freeing any other soft tissue attachments. The parietal surface of the pleura is then inspected, and the ribs are checked for evidence of injury. Fractured ribs are unusual in adult horses, but common in foals as a consequence of birth trauma, classically affecting the fourth to ninth ribs and carrying an attendant risk of laceration to the contained thoracic organs by the fractured rib ends (Schambourg et al 2003).

In some cases, lateral exposure of the chest may be preferred, after removing the rib cage by sawing through the ribs at their cranial and caudal extremities, to permit detailed dissection of thoracic organs *in situ*. This technique is of value in cases where congenital anomalies or other lesions affecting blood vessels or other structures of the thoracic inlet and anterior mediastinum are suspected (particularly anomalies of the first and second ribs and associated blood vessels) (K.E. Whitwell, personal communication).

The trachea is inspected for evidence of deformity and then opened by cutting along the groove between the cartilages on the dorsal aspect down to the main-stem bronchi, and then continuing the incision along the main bronchial divisions. The mucosal surfaces and lumina of the trachea and main-stem bronchi are then inspected, and materials are collected for microbiology (including virus isolation, PCR, aerobic and anaerobic bacterial culture) and histology, as guided by the clinical history and macroscopic findings. If endoscopic examination of the airways was performed before death, the post-mortem appearance of the airways should be correlated with the endoscopic findings. Apart from tracheal collapse in ponies and donkeys, primary lesions affecting the trachea are uncommon.

Examination of the lungs

As the lungs are such large organs, and the distribution of lesions within the lungs often correlates with different types of insult, care needs to be taken that all lobes of each lung receive adequate examination by inspection of color changes, palpation for changes in consistency and serial incision of the parenchyma for evidence of focal lesions. Areas of suspected lung consolidation should be tested for flotation in water or formalin. It is good practice to collect samples for routine histology from each of the lung

lobes (left and right apical, middle and caudal, and right accessory), to include both dorsal and ventral areas, and to include additional labeled samples from any areas with visible abnormalities. These samples may be collected into individual formalin-filled universal bottles, or identified within larger tubs of formalin by individually attached parcel labels. A systematic method for examination and bacteriological sampling of the different levels of the respiratory tract and the lung lobes has been described and presented in the context of the normal respiratory microflora of horses (Blunden & Mackintosh 1991).

Pulmonary edema, congestion, and accumulation of stable froth in the trachea and main-stem bronchi are common incidental findings in horses that are euthanased with barbiturates or similar compounds, and may also occur in the agonal stages of a variety of diseases that terminate in cardiac failure. Histological examination of the lungs is critical in such cases to establish the duration of the edema and congestion. This is carried out by assessment of the number and degree of vacuolation of bronchoalveolar macrophages, which may contain hemosiderin in cases of chronic cardiogenic edema ("heart failure cells") or exercise-induced pulmonary hemorrhage (EIPH). The presence of these cells is useful in aging congestive or hemorrhagic lesions. Studies on EIPH have shown that the numbers of these cells do not increase significantly within alveoli until around 1 week after the start of hard training, and that they decrease around 3 weeks after training ceases (Meyer et al 1998). Discussion of cardiac pathology is beyond the scope of this chapter, and the reader is referred to standard textbooks of equine cardiology (Darke et al 1996).

Severe viral pneumonia is rare in adult horses. As equine influenza and equine herpesvirus-1 (EHV-1) and EHV-4 are rarely fatal, and often subclinical, knowledge of the pathology of the respiratory disease is largely based upon historical or experimental studies (Jones & Maurer 1943, Kydd et al 1994a,b). Certain isolates of EHV-1 are capable of causing primary severe respiratory infection, as is the recently characterized Hendra virus (formerly equine morbillivirus) (Barclay & Paton 2000). With the exception of these highly virulent agents, horses presented for post-mortem examination following an episode of viral infection often demonstrate secondary bacterial infection by the time of necropsy. Uncomplicated viral infection of the lungs cannot be diagnosed solely by gross examination, and is often difficult to distinguish from edema of differing etiologies. Histological examination of sections of lung is critical in conjunction with virus isolation and PCR in such cases.

Bacterial infections of the lung in adult horses are rarely fatal, although they are often associated with impaired respiratory performance, as exemplified by *Streptococcus zooepidemicus* and other airway infections in young race-horses in training. In a review of 45 horses with bacterial pneumonia and/or pulmonary abscesses it was found that the infection was primary in 11 horses (eight of which had

a history of long-distance travel), with the remainder having infection secondary to aspiration of food or saliva, thoracic trauma, generalized infection, airway disease, neoplasia or thrombo-embolism (Mair & Lane 1989). Opportunistic infections may occur, with pulmonary abscessation resulting from penetration of the lungs via the chest wall, mediastinum or diaphragm, or extension of infection to the lungs from the tracheobronchial lymph nodes in cases of *Streptococcus equi* infection (see Chapter 23). Cases of bacterial infection secondary to long-term corticosteroid therapy have also been recorded (Mair 1996). Further to sporadic cases of opportunist bacterial infection, there is good evidence that *Streptococcus pneumoniae* is also able to act as a primary pathogen, resulting in focal pneumonia under experimental conditions (Blunden et al 1994).

Mycobacterial infection in horses is uncommon, and it appears that the horse has a high innate resistance to tubercle bacilli (Luke 1958). Most reported infections have involved *Mycobacterium bovis* but *M. avium* and *M. tuberculosis* have also been recorded. Infection is generally by the alimentary route, and the lesions in the lungs and tracheobronchial lymph nodes are typically firm, gray, and smooth, rather than caseous. Histologically, there is initial granulomatous inflammation that progresses to fibrosis. Acid-fast bacilli may be very difficult to find, requiring multiple Ziehl–Neelsen-stained sections, and the lesions may be mistaken for sarcomas on macroscopic examination.

Verminous pneumonia may occur as a result of infection with *Dictyocaulus arnfieldi* in horses pastured with donkeys, but this and other parasitic infestations are rare in animals on routine anthelmintic therapy. With the wide-spread use of effective anthelmintics it is unusual to observe aberrant strongyle lesions affecting the lungs or vasculature of the thoracic cavity, excluding cases of neglect.

Various immune-mediated conditions may involve the lungs, including multisystemic eosinophilic epitheliotropic disease and idiopathic pulmonary eosinophilia. These conditions are sporadic, and their etiology is ill-defined, although it is speculated that host immune reactions to prior endoparasitism are important in at least a proportion of cases. The most important immune-mediated pulmonary disease affecting the lungs in adult horses is recurrent airway obstruction. Clinical and immunopathological features of this disorder are well defined (Robinson et al 1996), and the histological lesion is a generalized chronic bronchiolitis with epithelial hyperplasia, goblet cell metaplasia, smooth muscle hypertrophy, peribronchiolar fibrosis and infiltration by lymphocytes and plasma cells (Kaup et al 1990). Neutrophils are often (but not consistently) prominent within airway lumina, airway epithelium and peribronchiolar spaces, sometimes accompanied by smaller numbers of eosinophils. There is often peribronchiolar mast cell hyperplasia. This histological lesion does however overlap with other types of chronic small airway

inflammation, including inflammatory airway disease, and there is a need for an objective grading system to facilitate histological diagnosis of probable recurrent airway obstruction in horses without a full medical history.

Exercise-induced pulmonary hemorrhage occurs to some degree in the majority of horses during race training. The severity ranges from the presence of small amounts of blood in the trachea and main-stem bronchi on post-race endoscopy, to severe epistaxis that is occasionally life-threatening. Incidental lesions of EIPH are apparent on post-mortem examination of the lungs of many racehorses as gray/brown plaques and discolored subpleural foci on the dorsocaudal portions of the caudal lung lobes. Histologically these lesions correspond to areas of bronchiolitis, fibrosis, neovascularization of arterial branches, and hemosiderin accumulation in alveoli and interstitial septa.

Neoplasia affecting the lower respiratory tract of horses is uncommon. The most frequently reported primary pulmonary tumor of the horse is the granular cell tumor (myoblastoma), which occurs in older animals. It may be associated with coughing and pulmonary insufficiency, or occur as an incidental finding. The cell of origin of this type of tumor is uncertain, but current opinion favors a Schwann cell precursor (Kagawa et al 2001).

Examination of the upper respiratory tract

Examination of the upper respiratory tract is achieved as follows. The head is detached from the neck at the atlanto-occipital joint, leaving the soft tissues posterior to the guttural pouches intact. The skin is reflected from the head, permitting examination for subcutaneous bruising or skull trauma. The rostral portions of the upper and lower jaws are removed by transverse cuts (by bandsaw or handsaw) just caudal to the incisor teeth. This then enables parasagittal bisection of the head, just slightly abaxial of the midline, to minimize damage to the brainstem and midline central nervous system structures. The nasal septum is removed from the side of the nasal cavity, where it is still intact, to enable complete inspection of right and left turbinates. The remainder of the upper respiratory tract, including larynx, guttural pouches and lymphoid tissues, and the oral cavity are examined at this stage, and, if appropriate, samples are collected for microbiology and histology.

The most important condition affecting the equine larynx is recurrent laryngeal neuropathy. Atrophy of the cricoarytenoideus dorsalis muscle is recognized at post-mortem examination as reduced mass and pallor of the affected muscle, with histological changes typical of neurogenic myofiber atrophy. The pharynx may contain cysts (e.g. glossopharyngeal or subepiglottic), but these are usually incidental findings. Histological examination of the wall of the cyst may assist in determining the embryonic origin. Pharyngitis is rare in adult horses, although the majority of young horses demonstrate follicular hyperplasia of pharyngeal lymphoid tissues that is recognized as

nodular thickening of the mucosa. This change is not specific for particular etiological agents, and generally regresses with age.

A history of unilateral nasal discharge is an indication for full examination of the nasal cavity and paranasal sinuses, which requires removal of bone from the maxilla to fully expose the maxillary sinuses, and facilitate tooth apex inspection. Rhinitis in horses may be caused by viral infection (including equine rhinoviruses, EHV-1, and EHV-4), bacterial infection (particularly involving infection with *S. zooepidemicus*), fungal infection (such as *Aspergillus fumigatus, Cryptococcus neoformans* or *Rhinosporidium seeberi*), or by immune-mediated processes such as allergy. The nature of the inflammatory reaction (serous, catarrhal, purulent, ulcerative, pseudomembranous, hemorrhagic or granulomatous) is important in determining the likely etiology and duration of the condition, with acute rhinitis generally presenting as a serous exudate that progresses to become catarrhal and then purulent. In subacute to chronic rhinitis, localized mucosal hyperplasia, edema and reduced venous drainage may rarely predispose to the development of nasal polyps that present as soft mottled pedunculated masses protruding into the nasal cavity. These lesions must be distinguished histologically from neoplasms. Sinusitis may occur as an extension from purulent rhinitis or periodontal or dental apical infections, and is of particular importance in this species owing to the size and complexity of the paranasal sinuses and their limited capacity for drainage. Empyema is the usual gross finding in cases of chronic sinusitis, and histological examination and appropriate microbiology are necessary to achieve an etiological diagnosis.

A history of epistaxis should prompt close inspection of the guttural pouches for mycotic plaques, which appear as white/gray plaque-like thickening of the wall of the affected pouch, with erosion of underlying blood vessels. The causal organism, often *Aspergillus* spp., may be identified by fungal culture from the plaques and/or by direct demonstration of fungal hyphae in tissue sections using appropriate special staining. The principal differential diagnosis for epistaxis originating from the upper respiratory tract is progressive ethmoid hematoma, which represents a hemorrhagic variant of nasal polyp. This condition is recognized at post-mortem examination as a friable fleshy mass occupying the ethmoturbinate region and extending proximally into the nasal cavity or into the paranasal sinuses. Histological examination may be necessary to distinguish progressive ethmoid hematoma from rare cases of hemangiosarcoma, adenocarcinoma or other neoplasia occurring in this region of the nasal cavity.

The guttural pouches are also key areas for examination in cases of acute or chronic *S. equi* infection. Collection of swabs or cultures from the guttural pouches is important in the identification of carrier animals. Horses with clinical signs of *S. equi* infection may demonstrate abscessation of the submandibular and retropharyngeal lymph nodes,
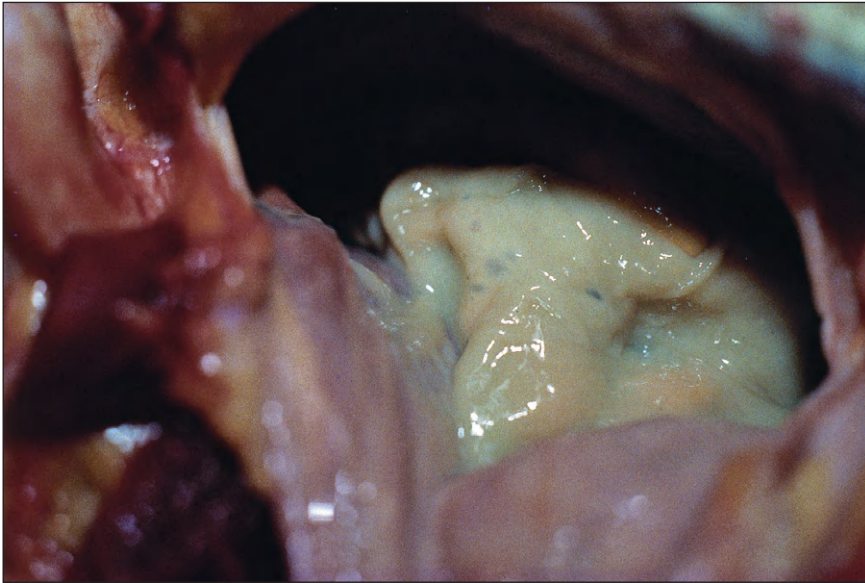
Fig. 21.1. Guttural pouch of a pony infected with *Streptococcus equi*. Note abundant purulent material filling pouch: guttural pouch empyema. Reproduced with the permission of Dr Richard Newton, Animal Health Trust, Newmarket, UK.

in association with guttural pouch empyema (Fig. 21.1). Cervical and tracheobronchial lymph nodes may also demonstrate abscessation, and clinical signs may then include upper and lower respiratory tract obstruction. Asymptomatic carriers of *S. equi* generally have nodules of inspissated caseous material, termed chondroids, within one or both guttural pouches. Bacteria or bacterial nucleic acids are generally detectable within chondroids using culture or PCR analysis.

Sinonasal neoplasms occur sporadically in horses and are generally malignant. Squamous cell carcinomas predominate, with occasional reports of other tumors such as fibrosarcoma and adenocarcinoma.

Post-mortem examination of fetuses and foals

The method outlined above is not appropriate for the examination of equine fetuses and foals up to 6 months of age, where infectious disease is a particular concern (Smith et al 2003). The respiratory tract and associated structures of fetuses and neonatal foals may be examined as follows. The carcass is placed in right-sided recumbency and the left limbs and body wall are reflected dorsally to expose the viscera. Before further handling of the tissues, virology and then bacteriology samples are taken. The abdominal and then thoracic cavities are inspected and the organs are examined individually, firstly *in situ* and then on a table. Particular attention is paid to evidence of birth trauma (ribs, limb joints, cranium, brain, cervical spine) in stillborn, late-term or neonatal foals; congenital abnormalities; sites of infection in neonates (guided by clinical history); state of aeration of lungs; viral-type lesions in the lungs of aborted fetuses or neonates; and evidence of veterinary intervention, such as administration of sub-

stances, surgical procedures, diagnostic procedures, and resuscitation attempts. EHV-1 infection in late-term fetuses and neonatal foals typically causes a constellation of lesions that includes hepatosplenomegaly, perirenal edema, and body cavity effusions. Transudative pleural effusion is a particularly important diagnostic aid that has been documented since the first reports of this pathogen in the 1930s (Westerfield & Dimock 1946, Allen et al 1999) (Fig. 21.2). Other viral infections of the fetus and neonate are equine viral arteritis and equine adenovirus, the latter particularly in cases of severe combined immunodeficiency in Arab foals (McChesney et al 1973). Bacterial infections may be pre- or post-natal, the former often being associated with placentitis and generally being associated with pathogens such as *Escherichia coli* or *S. zooepidemicus*. Histological and microbiological examination of the lungs is essential to distinguish bacterial infection in neonates from uncomplicated primary atelectasis, as occurs in cases of dystocia. Meconium inhalation within airways and alveoli is a useful indicator of intrapartum stress in such cases.

Postnatal bacterial infections occurring in the first few weeks of life may involve a similar range of organisms to those infecting foals in the first days of life. The most important bacterial infection occurring in older foals (1–6 months of age) is *Rhodococcus equi*. Post-mortem diagnosis of *R. equi* pneumonia is achieved by the recognition either of multiple caseous abscesses within the lungs of foals with the chronic form of the disease or of diffuse to multifocal suppurative pneumonia in the acute form (Yager 1987). The causal organism is a Gram-positive pleomorphic rod bacterium that is often identified within phagocytic cells on tissue sections. Foals aged between 1 and 6 months of age may also present to the pathologist with a history of acute-onset respiratory distress, and demonstrate post-mortem lesions of interstitial pneumonia.
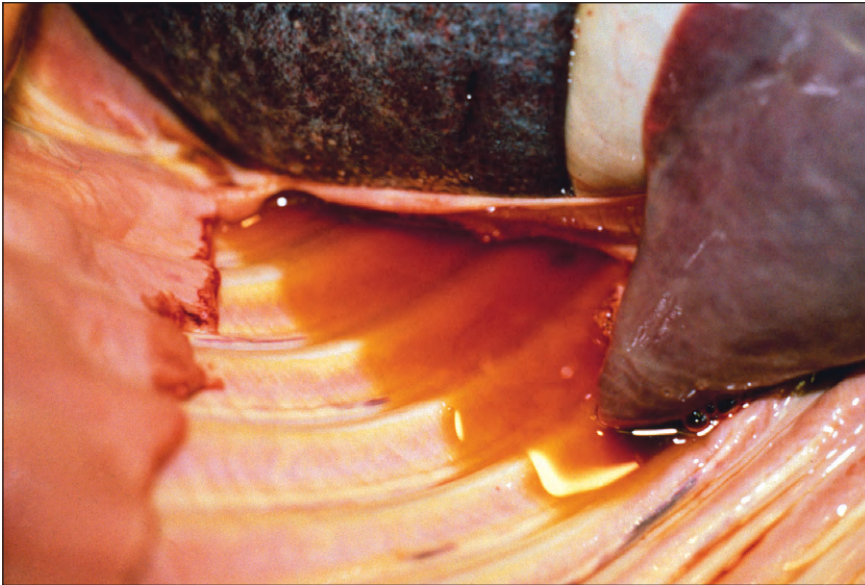
Fig. 21.2. Equine fetus aborted as a result of EHV-1 infection. Note abundant straw-colored fluid in pleural cavity.

Sections of lung demonstrate diffuse alveolitis with hyaline membrane formation and/or type 2 pneumocyte hyperplasia. A proportion of these cases are associated with *Pneumocystis carinii* infection (Ewing et al 1994, Peters et al 1994), while various theories have been advanced to explain the remainder, including viral and toxic disease.

Summary of Post-mortem Sample Collection

Table 21.1 summarizes those samples that should be collected in the comprehensive examination of the respiratory tract. The sampling protocol should be modified according to the clinical signs exhibited by the horse, and by the gross post-mortem findings. Fixation is generally by immersion in 10% neutral buffered formalin, but more specialized fixatives such as paraformaldehyde may be required for specific procedures such as *in situ* hybridization. Methods of perfusion fixation are rarely employed in adult horses, owing to technical difficulties. Samples for bacteriology and virology must be collected with good aseptic technique to avoid cross-contamination. It is good practice to retain unfixed specimens from the upper and lower respiratory tract, both in virus transport medium and frozen in sterile containers, should subsequent virological, toxicological or genetic analysis be undertaken.

Table 21.1. Suggested minimal sampling set for full post mortem examination of the respiratory tract of an adult horse		
Method	**Collection medium**	**Samples for collection**
Histopathology and immunohistochemistry*	Neutral buffered formalin	Nasal mucosa (turbinate and septum), nasopharynx, tonsil (nasal and lingual), guttural pouch, lymph node (submandibular, retropharyngeal, cervical, tracheobronchial), trachea (proximal, middle and distal), main-stem bronchus, lung (left and right apical, middle and caudal lobes, right accessory lobe)
Virology and PCR	Virus transport medium	Pooled samples from nasal and nasopharyngeal mucosa. Pooled samples from respiratory tract-associated lymph nodes. Pooled samples from left and right lungs
Bacteriology	Unfixed tissue samples or unfixed swabs	Nasal mucosa, guttural pouch, trachea, main-stem bronchus and left and right lung
Other (e.g. serology toxicology)	Unfixed tissue samples	Serum and snap-frozen samples of lung

*Some antibodies are optimized for snap-frozen rather than fixed tissue: check with laboratory in advance.
PCR = polymerase chain reaction.
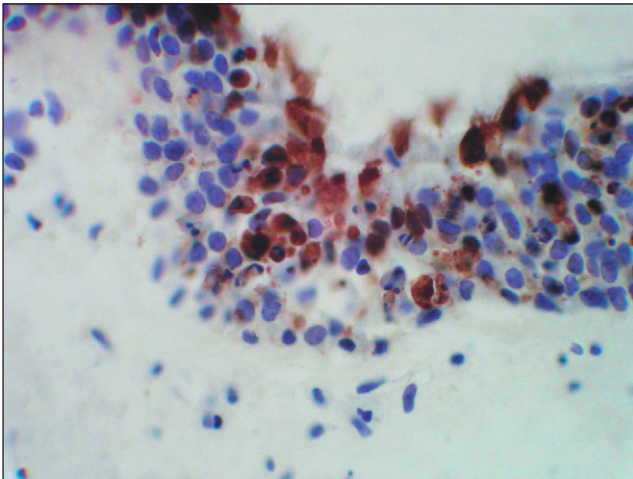
Fig. 21.3. Equine tracheal explant infected with equine influenza virus (H3N8) and immunostained for viral antigen expression using a polyclonal antiserum and indirect immunoperoxidase method with DAB as chromogen and Mayer's hematoxylin as counterstain. Epithelial cells infected with influenza stain brown. Magnification ×400.

Health and Safety in the Post-mortem Room

Health and safety considerations should be followed in the examination of horses with suspected zoonoses (use of facemasks or respirators; avoidance of mechanical saws that could aerosolize tissue debris). These guidelines should be clarified with the regulatory authorities of the appropriate country (e.g. Health and Safety Executive in the United Kingdom). For proper health and safety precautions to be followed in a post-mortem examination it is essential that the carcass be accompanied by a full medical history, including any ante-mortem laboratory results. Travel history is fundamental, and it is important that the examining pathologist is well acquainted with infectious diseases occurring in the country of origin of imported horses.

Molecular Pathology

Pathology has kept pace with other developments in veterinary diagnostics through the increasing application of new molecular techniques to post-mortem diagnosis. Of greatest value in routine diagnostic pathology are immunohistochemical methods, both for the phenotyping of inflammatory and neoplastic cells (Mair et al 1988, Kelley & Mahaffey 1998, Blunden & Gower 1999), and for the detection of infectious agents (Kydd et al 1994a,b, Lopez et al 1996) (Figs 21.3 and 21.4). The majority of these techniques are now applicable to routinely processed and fixed material but it is always worthwhile to retain a specimen of unfixed tissue for cryostat sections if the fixation stability of the target antigens is not known.

PCR techniques are also applied routinely to material collected at post-mortem examination, and these techniques can often be adapted to the examination of archived formalin-fixed paraffin-embedded tissues. Owing to the speed, economy, and sensitivity of PCR diagnosis, these methods have largely superseded routine virus isolation for infectious disease diagnosis. PCR-based techniques are of particular value in screening tissues for latent viral infections (Edington et al 1994). *In situ* hybridization is a more technically demanding technique than immunohistochemistry, and generally less used for routine diagnostic investigations. It is of value in the detection of infectious
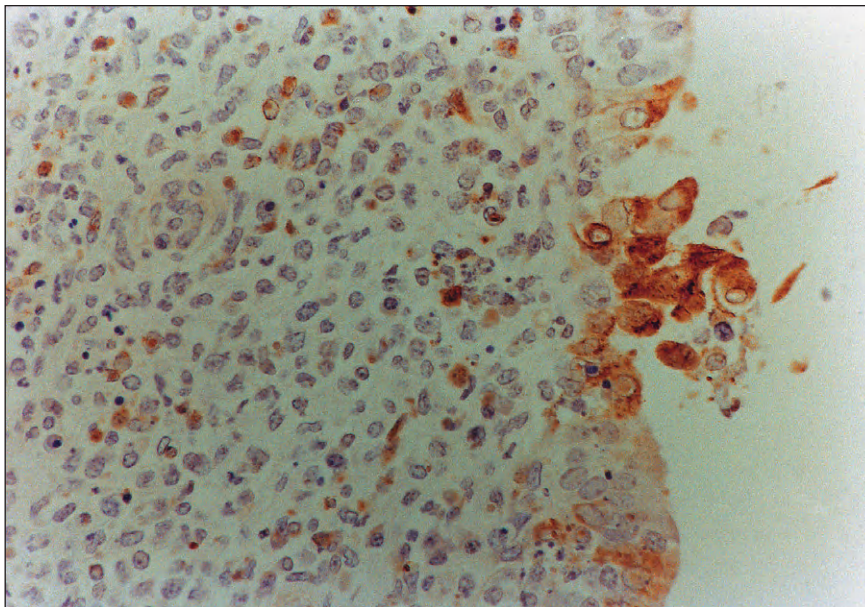
Fig. 21.4. Immunoperoxidase staining of nasal mucosa of pony following EHV-1 infection. Cells containing EHV-1 antigens are immunostained brown. Note disorganization and erosion of mucosa, with associated inflammation. Reproduced with the permission of Dr Julia Kydd, Animal Health Trust, Newmarket, UK.

agents where specific antibodies are not available or where latency results in highly restricted nucleic acid transcription and undetectable protein expression.

Conclusion

Close communication between experienced equine pathologists and respiratory clinicians is key to the successful investigation of equine respiratory disorders. A systematic post-mortem examination of the equine respiratory tract is recommended in all cases of respiratory disease that do not respond to logical therapy, and classical methods should be supplemented and extended wherever appropriate by modern molecular techniques.

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REFERENCES

Allen GP, Kydd JH, Slater JD et al 1999 Recent advances in understanding of the pathogenesis, latency and immunology of EHV-1 abortion. In: Wernery U, Wade JF, Mumford JA et al (editors) Proceedings of the Eighth International Conference on Equine Infectious Diseases. R&W Publications, Newmarket pp.129–146

Barclay AJ, Paton DJ 2000 Hendra (equine morbillivirus). Veterinary Journal 160: 169–176

Blunden AS, Mackintosh ME 1991 The microflora of the lower respiratory tract of the horse: an autopsy study. British Veterinary Journal 147: 238–250

Blunden AS, Gower SM 1999 A histological and immunohistochemical study of the humoral immune system of the lungs in young Thoroughbred horses. Journal of Comparative Pathology 120: 347–356

Blunden AS, Hannant D, Livesay G et al 1994 Susceptibility of ponies to infection with *Streptococcus pneumoniae* (capsular type 3). Equine Veterinary Journal 26: 22–28

Bodrossy L, Sessitsch A 2004 Oligonucleotide microarrays in microbial diagnostics. Current Opinion in Microbiology 7: 245–254

Colbourne CM, Bolton JR, Mills JN et al 1992 Mesothelioma in horses. Australian Veterinary Journal 69: 275–278

Darke PGG, Bonagura JD, Kelly DF 1996 Post mortem examination of the cardiovascular system. Color Atlas of Veterinary Cardiology. Mosby-Wolfe, London, pp.10–20

Edington N, Welch HM, Griffiths L 1994 The prevalence of latent Equid herpesviruses in the tissues of 40 abattoir horses. Equine Veterinary Journal 26: 140–142

Ewing PJ, Cowell RL, Tyler RD et al 1994 *Pneumocystis carinii* pneumonia in foals. Journal of the American Veterinary Medical Association 204: 929–933

Jones TC, Maurer FD 1943 The pathology of equine influenza. American Journal of Veterinary Research 4: 15–31

Kagawa Y, Hirayama K, Tagami M et al 2001 Immunohistochemical analysis of equine pulmonary granular cell tumours. Journal of Comparative Pathology 124: 122–127

Kaup FJ, Drommer W, Deegen E 1990 Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD). I: Alterations of the larger conducting airways. Equine Veterinary Journal 22: 343–348

Kelley LC, Mahaffey EA 1998 Equine malignant lymphomas: morphologic and immunohistochemical classification. Veterinary Pathology 35: 241–252

Kydd JH, Smith KC, Hannant D et al 1994a Distribution of equid herpesvirus 1 in the respiratory tract of ponies: implications for vaccination strategies. Equine Veterinary Journal 26: 466–469

Kydd JH, Smith KC, Hannant D et al 1994b Distribution of equid herpesvirus 1 in the respiratory tract associated lymphoid tissue: implications for cellular immunity. Equine Veterinary Journal 26: 470–473

Lopez JW, del Piero F, Glaser A et al 1996 Immunoperoxidase histochemistry as a diagnostic tool for detection of equine arteritis virus antigen in formalin fixed tissues. Equine Veterinary Journal 28: 77–79

Luke D 1958 Tuberculosis in the horse, pig, sheep and goat. Veterinary Record 70: 529–536

Mair TS 1996 Bacterial pneumonia associated with corticosteroid therapy in three horses. Veterinary Record 138: 205–207

Mair TS, Lane JG 1989 Pneumonia, lung abscesses and pleuritis in adult horses: a review of 51 cases. Equine Veterinary Journal 21: 175–180

Mair TS, Stokes CR, Bourne FJ 1988 Immunohistochemical study of the local humoral immune system of the equine respiratory mucosa. Research in Veterinary Science 45: 160–165

McChesney AE, England JJ, Rich LJ 1973 Adenoviral infection in foals. Journal of the American Veterinary Medical Association 162: 545–549

Meyer TS, Fedde MR, Gaughan EM et al 1998 Quantification of exercise-induced pulmonary haemorrhage with bronchoalveolar lavage. Equine Veterinary Journal 30: 284–288

Peters SE, Wakefield AE, Whitwell KE et al 1994 *Pneumocystis carinii* pneumonia in thoroughbred foals: identification of a genetically distinct organism by DNA amplification. Journal of Clinical Microbiology 32: 213–216

Robinson NE, Derksen FJ, Olszewski MA et al 1996 The pathogenesis of chronic obstructive pulmonary disease of horses. British Veterinary Journal 152: 283–306

Rooney JR, Robertson JL 1996 Respiratory system. In: Rooney JR, Robertson JL (editors) Equine Pathology, 1st edn. Iowa State University Press, Iowa, pp.23–56

Schambourg MA, Laverty S, Mullim S et al 2003 Thoracic trauma in foals: post mortem findings. Equine Veterinary Journal 35: 78–81

Smith KC, Blunden AS, Whitwell KE et al 2003 A survey of equine abortion, stillbirth and neonatal death in the UK from 1988 to 1997. Equine Veterinary Journal 35: 496–501

Stoica G, Cohen N, Mendes O et al 2004 Use of immunohistochemical marker calretinin in the diagnosis of a diffuse malignant metastatic mesothelioma in an equine. Journal of Veterinary Diagnostic Investigation 16: 240–243

Westerfield C, Dimock WW 1946 The pathology of equine virus abortion. Journal of the American Veterinary Medical Association 109: 101–111

Yager JA 1987 The pathogenesis of *Rhodococcus equi* pneumonia in foals. Veterinary Microbiology 14: 225–232

22 Viral Infections of the Equine Respiratory Tract

James Wood, Kenneth C Smith, Janet M Daly
and J Richard Newton

Introduction

This chapter describes features of the most commonly encountered equine respiratory viruses (equine influenza viruses, equine herpesviruses and equine arteritis virus) and their control, as well as those of less widespread but nevertheless important infections such as Hendra virus and African horse sickness. A few comments are made at the end of the chapter about other viruses with effects on the respiratory tract.

Outbreaks of disease

A common feature of all viruses covered here is their ability to cause outbreaks of disease as a result of their contagious nature. Despite this, clinical cases may appear in either individual animals or as outbreaks in large populations of animals, or indeed anywhere between these two extremes, depending on the specific infection, the individual level and "herd" immunity, how the animals are managed and also, critically, on chance effects.

Some insight can be gained into etiology from clinical presentation and epidemiological features but clinical cases or outbreaks of equine respiratory disease are still often attributed to infection with a virus, or with "the virus" without any specific etiological investigations. Optimal treatment and advice on management and prevention cannot be given without knowledge of specific cause and while etiological investigations can sometimes be hard to justify in individual cases, they should be encouraged when dealing with disease in groups. Our experience, and that of others, is that it is often impossible to differentiate cases of disease caused by equine influenza, equine herpesvirus, and *Streptococcus equi* var. *equi* on clinical grounds alone. Although stating the obvious, hematological investigation of outbreaks does not provide a reliable insight into etiology.

Equine Influenza Virus

Historical context

A disease of horses clinically indistinguishable from influenza and referred to as a "distemper" was described as long ago as 1732. In the 1930s German researchers comparing epidemic coughing in young racehorses with the then recently isolated swine influenza virus, showed for the first time that the disease was the result of a filterable virus and that it was experimentally reproducible. The disease had many pseudonyms, including "laryngotracheo-bronchitis", "infectious bronchitis", "infectious catarrh", "Hoppengarten cough", "Gulf Stream disease", "epizootic cough", and "Newmarket cough". Influenza became recognized as a highly contagious respiratory disease characterized by acute pyrexia and a harsh dry cough that rapidly spread both within and between groups of young horses, particularly in racing yards. There were few deaths or complications when horses were rested and recovery usually occurred in 2–3 weeks.

The first identification of the viral family causing this disease was in 1955 when it was shown that horses in a respiratory disease epidemic in Sweden seroconverted to a soluble human influenza virus antigen. Shortly afterwards, an influenza virus was isolated for the first time from coughing horses in Czechoslovakia and this prototype virus was the Prague/56 H7N7 virus (see below for description of how viruses are classified). Retrospective serological surveys demonstrated that the virus had been the cause of disease in many areas of the world. Serological and virological monitoring of epidemic respiratory disease demonstrated that this prototype virus continued to be responsible for disease outbreaks in predominantly young horses in many countries between 1957 and 1963. In January 1963 there was an outbreak of rapidly spreading acute respiratory disease among horses at several racetracks in Miami, USA. A characteristic of this outbreak that differentiated it from earlier outbreaks was that it affected all ages rather than predominantly younger horses. A novel subtype, H3N8, was isolated and was subsequently responsible for an equine influenza pandemic. A serological survey of H3N8 antibodies following the epidemic of H7N7 influenza in the UK in 1963 demonstrated that British horses were completely susceptible to the new virus and an epidemic was predicted. Despite the start of production of a vaccine, insufficient stocks were available before the disease was first seen in February 1965 on two studs in Sussex. The stud had recently received mares from France, where the disease had been present for about 1 month.

Virology

Influenza viruses are pleomorphic, spherical or filamentous virions with a diameter of 80–120 nm. They have a segmented, single-stranded RNA genome of negative sense. The eight gene segments code for two surface glycoproteins, the hemagglutinin (HA) and neuraminidase (NA), the internal matrix protein and nucleoprotein, and other structural and non-structural proteins involved in virus replication. The RNA segments are closely associated with the nucleoprotein and are surrounded by the matrix protein, which is closely associated with the lipid envelope containing the two surface glycoproteins. HA is the major surface glycoprotein making up approximately 25% of the virus protein as compared to 5% for NA. These glycoproteins are the principal determinants for cell entry in infection (HA) and for exit from the cell after virus replication (NA). Influenza virus is enveloped and, as such, does not remain infectious for long outside the host (generally <7 days even in favorable conditions) and is rapidly inactivated by sunlight and disinfectants (Hannant et al 1996).

There are three types of influenza viruses, A, B, and C, all of which are classified in the Orthomyxovirus family. The antigenic character of the nucleoprotein and matrix proteins determines the virus type. All equine influenza viruses are type A. The HA and NA define subtypes within a virus type. Among all influenza viruses, 16 HA and nine NA subtypes have been identified. In the viruses that naturally infect horses, only H7N7 and H3N8 have been recognized.

Antigenic drift occurs when mutations in the HA gene sequence result in amino acid substitutions. As with other RNA viruses, influenza virus replication is highly error-prone, and therefore newly synthesized viral genes have a high frequency of mutation. Many of these mutations are either inconsequential or are detrimental to the virus but mutations affecting the antigenic sites of the HA (and NA) can lead to the virus not being recognizable by pre-existing antibodies generated by infection or vaccination with an earlier strain. Immunological pressure, such as from vaccination, may influence antigenic drift (Mumford 1999).

Phylogenetic relationships between viruses are established either using evidence from RNA sequencing of the HA gene or by antigenic analysis using hemagglutination inhibition (HI) tests using ferret or equine sera. Historically, antigenic drift in equine H3N8 viruses has been examined in HI tests employing postinfection or postvaccination sera prepared in a number of different species. Conclusions about the antigenic relatedness of equine H3N8 viruses and the significance of observed differences with respect to the immunity induced have varied.

Antigenic differences between equine influenza strains have been examined using *R*-values calculated from reciprocal tests between pairs of viruses and matching antisera. Using this expression, *R*-values approaching 0 indicate that the viruses are antigenically unrelated and values approaching 100 indicate that viruses are antigenically indistinguishable. When this method is applied to reports of reciprocal HI tests with equine H3N8 viruses, a number of conclusions can be drawn. It is clear that the species in which the antisera are prepared has a marked effect on the apparent relatedness of two viruses. Horse sera are relatively cross-reactive, particularly when taken from repeatedly vaccinated animals whereas ferrets develop the most strain-specific antibody response. Where ferret sera have been used to examine the same pair of viruses in different laboratories, the antigenic relatedness based on *R*-values is, on the whole, very similar, suggesting that this approach is a reliable method of quantifying antigenic differences.

Minor antigenic drift has been identified within the H7N7 subtype, while more extensive drift into two distinct lineages has been observed in H3N8 viruses (Fig. 22.1) (Daly et al 1996, Lai et al 2001). As a result of this feature of influenza viruses, the formulation of human influenza vaccines is reviewed on an annual basis and in most years is changed to reflect the virus strains most representative of those in worldwide circulation.

A formal surveillance system was established for equine influenza in 1995 (Mumford 1999). An international panel of experts, including representatives from the Office International des Epizooties (OIE) and the World Health Organization (WHO) influenza reference laboratories, reviews data collected on outbreaks of influenza, vaccine performance in the field, and antigenic and genetic characteristics of new virus isolates annually. The expert surveillance panel makes recommendations on the need to update vaccine strains, which are published in the *OIE Bulletin*. The criteria used for deciding on the need to update equine influenza vaccine strains are based largely on those used for human influenza vaccine strain selection, i.e. detection of changes in the HA as characterized by HI tests using ferret and horse antisera, genetic sequencing of the *HA1* gene and vaccine breakdown in the field. Improved surveillance in the field, standardization of the potency of vaccines (see later) and the introduction of this vaccine strain selection system have enabled the development of a fast-track licensing system in the European Union (EU) for equine vaccines containing updated strains. While recommendations for equine influenza vaccines are reviewed on an annual basis, changes are not required so frequently.

Major or subtype changes in the surface glycoproteins can occur as a result of the reassortment of viral genome segments with those from other influenza viruses, or introduction from other host species, particularly aquatic birds, the major reservoir host for influenza viruses. New viruses generated by this process (frequently termed "antigenic shift") may result in pandemics because all populations will tend to be completely susceptible, as was seen with the
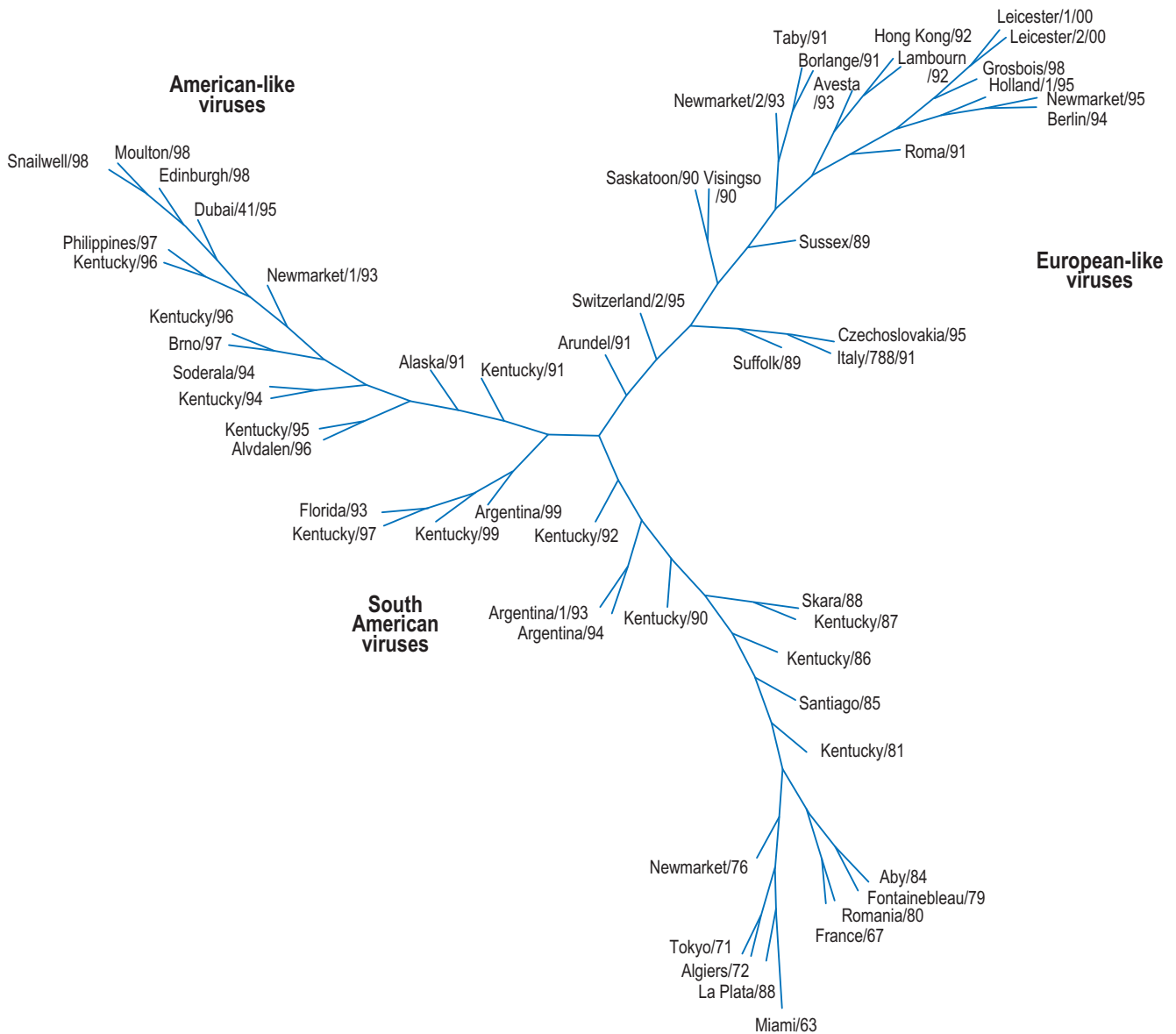
Fig. 22.1. Phylogenetic tree of equine influenza A (H3N8) viruses based on amino acid sequences of HA1 molecules.

emergence of H3N8 viruses in Miami in 1963 and with human influenza viruses on three occasions in the 20th century. An entirely avian H3N8 virus transferred to horses in Jilin province in China in 1989 (Shortridge et al 1995). The resulting outbreak was associated with a mortality of up to 20% and a morbidity of up to 80%. This compares with a mortality rate of around 2% in an outbreak caused by a conventional equine H3N8 virus elsewhere in China in 1993–4. The avian-derived H3N8 virus did not persist in the equine population and has not been detected since that time.

Recent investigations in North America have implicated an equine-derived H3N8 virus as the cause of outbreaks of severe respiratory disease in kenneled greyhounds, associated with hemorrhagic pneumonia and significant mortality (Crawford et al 2005). Transmission in the canine population is now widespread in the USA.

Clinical signs and pathogenesis

In controlled experimental infection studies in horses, the incubation period usually varies between 1 and 3 days with a range of 18 h to 5 days, with the length of the incubation period being inversely related to the virus dose (Mumford 1999). Coughing and fever are the most common clinical signs of equine influenza in naive animals, the cough being dry, harsh and initially non-productive. Coughing is frequent during the first week of infection and
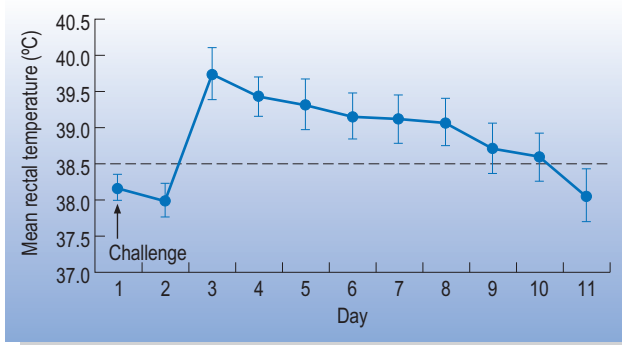
Fig. 22.2. Mean daily rectal temperatures (mean ± 95% confidence intervals around the mean) following experimental influenza virus infections in naive Welsh mountain ponies conducted at the Animal Health Trust (pyrexia is defined as >38.5°C and is indicated by the broken line).

in uncomplicated cases, given sufficient rest, will disappear within 1–3 weeks. There is usually a mild rhinitis and no obvious swelling of the submandibular lymph nodes. The nasal discharge is initially serous but frequently becomes mucopurulent as a result of secondary bacterial infection. In populations of partially immune, vaccinated horses where clinical signs are not necessarily typical of influenza, one of the most common signs is nasal discharge, which rapidly spreads among horses.

Pyrexia is a feature of natural outbreaks of equine influenza virus infection in naive horses. Experimental infections with equine influenza viruses have allowed specific clinical signs to be more precisely characterized with respect to time since infection. Daily rectal temperature data in naive ponies experimentally challenged with various H3N8 influenza viruses are shown in Fig. 22.2 (data courtesy of J. Daly and L. Spencer). In general, H3N8 infections produce more severe clinical disease with higher and more prolonged pyrexia than H7N7 infections, as H3N8 viruses appear to be more pneumotropic than H7N7 viruses. Peak temperatures in H7N7 and H3N8 infections have been reported as 40.4°C and 41.2°C, respectively. Continuous fever beyond 4 or 5 days accompanied by pronounced mucopurulent nasal discharge is attributable to secondary bacterial infection. Other signs of influenza infection may include anorexia, dyspnea, and myalgia, and there may be icterus in some cases. Cardiac damage may occur, particularly in older animals and in those that have been worked during the acute phase of the disease (Gerber 1970).

Mortality rates are usually very low in uncomplicated cases, with the exception of young foals that have not acquired maternal-derived immunity. Death in foals occurred during H3N8 outbreaks in Britain in 1965 and South Africa in 1986, particularly in foals that were born while their dams were suffering from the acute disease.

These foals developed primary viral pneumonia and they had very high temperatures (41–42°C), stopped sucking, had increased heart rates, and manifested signs of severe respiratory distress, including tachypnea (80–90 breaths per minute) and soft gurgling sounds on auscultation of the thorax. Many became weak and died 7–10 days later.

Influenza is also a cause of mortality in donkeys and mules. Necropsies performed on donkeys affected in the H3N8 outbreak in Britain in 1969 revealed acute bronchopneumonia complicated by secondary bacterial infection. Secondary complications and sequelae to equine influenza are common in stressed or neglected animals. Bacterial infections, including *Streptococcus equisimilis*, *Streptococcus zooepidemicus*, *Escherichia coli*, *Staphylococcus* spp., *Pseudomonas* spp., *Pasteurella* spp., *Aerobacter* spp., *Actinobacillus equuli*, and *Bordetella bronchiseptica*, result in purulent nasal discharges, pharyngitis, conjunctivitis, and sometimes bronchopneumonia.

During an outbreak of equine influenza in the UK during 2003, there were reports of unusually severe clinical signs among unvaccinated animals. Two influenza-infected horses developed neurological signs, and one was euthanased. Post-mortem examination of the brain of that horse revealed viral-type, non-suppurative encephalitis, and influenza virus antigen was demonstrated by the immunostaining of sections of nasal mucosa but was not demonstrated in the brain (Daly et al 2006). Experimental infection of ponies not previously exposed to equine influenza virus with a strain representative of the 2003 outbreak resulted in more severe clinical signs and higher levels of cytokines in nasal secretions than observed for earlier strains of equine influenza virus (unpublished data). In the absence of an alternative explanation, the possibility that infection with a highly pathogenic strain of equine influenza virus had given rise to neurological complications was suspected on the basis of the findings.

Outbreaks of clinically mild influenza do occur among vaccinated horses that have incomplete immunity, particularly if vaccine strains have become outdated. In such outbreaks, the mild signs may not be recognized or diagnosed as influenza. In two longitudinal studies of respiratory disease in racehorses, in Canada and the UK, influenza was associated with signs of upper respiratory tract disease (Morley et al 1999, Newton et al 2003). In other outbreaks, the first sign noted was poor training and racing performance. It is clear from experimental work that subclinical infections can occur and indeed are common under the cover of partial immunity (see later).

Hematological changes during equine influenza infections are non-specific and variable. Anemia, leukopenia and lymphopenia are often observed in the first 3–7 days with an increased neutrophil : lymphocyte ratio in the first 2 or 3 days after infection. Monocytosis may also be observed during early convalescence from about day 7 after infection.
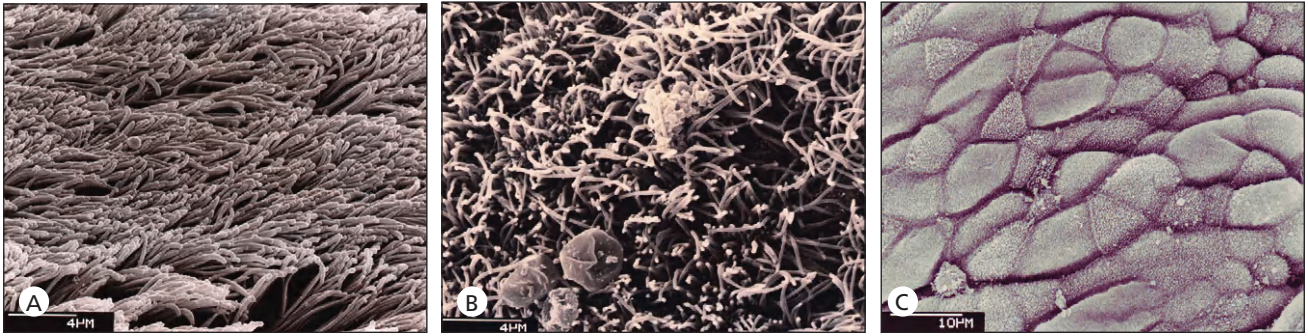
Fig. 22.3. Scanning electron micrographs of (A) normal equine tracheal ciliated epithelium, (B) equine tracheal ciliated epithelium 2 days post-influenza infection, showing disruption and loss of cilia from the tracheal epithelium, and (C) equine tracheal ciliated epithelium 6 days post-influenza infection, showing disruption and loss of cilia from the tracheal epithelium. Reproduced with the permission of A.S. Blunden and J.A. Mumford.

Equine influenza virus, as well as causing pathology of the upper respiratory tract, also extensively damages the ciliated epithelial cells lining the conducting airways. This leads to disruption of normal mucociliary clearance with consequent accumulation of mucus and bacteria in the airways and exposure of the lamina propria and irritant receptors, all leading to frequent coughing. Figure 22.3 shows disruption and loss of cilia from the tracheal epithelium at different stages of experimental influenza infection.

Diagnosis

The characteristic clinical features of equine influenza in susceptible animals (rapidly spreading disease manifested by a harsh, dry cough, high temperature, and nasal discharge) are sufficiently characteristic to permit a tentative clinical diagnosis. However, in animals that have previously experienced the infection or that have waning vaccinal immunity, it is difficult to differentiate influenza from other respiratory infections. In such situations, or where equine influenza has not occurred in the locality in the recent past, laboratory diagnosis, involving rapid antigen detection, virus isolation or serology, is required.

Specimens for virus detection and/or isolation should be collected as soon as possible after the onset of pyrexia and coughing, as the period of virus excretion may be as short as 1–2 days in animals with pre-existing immunity. Virus may be cultured from nasopharyngeal secretions collected into virus transport medium, usually by swabbing of the nasopharynx or by transendoscopic tracheal lavage. The transport medium should not contain fetal calf serum, which inhibits influenza virus growth, and samples should be submitted on ice to the laboratory as quickly as possible.

Influenza virus can be cultured in embryonated hens' eggs or in susceptible mammalian cells such as Madin–Darby canine kidney (MDCK) cells. Embryonated hens' eggs are inoculated via the amniotic (6- to 8-day-old embryos) or allantoic (8- to 12-day-old embryos) routes. Amniotic and allantoic fluids are harvested after incubation for 48–72 h at 34°C and examined for hemagglutinating activity using chick erythrocytes.

The adoption of more widespread vaccination has made the diagnosis of influenza infection less straightforward, with clinical signs being less severe, blood samples from acute cases already possessing moderate levels of serum antibody, and the quantities of live virus retrievable from the respiratory tract being greatly reduced. The development of sensitive and rapid enzyme-linked immunosorbent assays (ELISA) for the detection of influenza nucleoprotein antigen in extracts from nasopharyngeal swabs has greatly improved the ability to diagnose influenza in previously vaccinated horses (Cook et al 1988, Livesay et al 1993). In addition, ELISA may be more appropriate where submission of specimens in a good state of preservation to laboratories for virus isolation may not be practical when the distances are great or the temperatures are unfavorable for maintaining viability of the virus. The nucleoprotein is antigenically similar in all influenza A viruses and thus this assay cannot be used to differentiate H3N8 and H7N7 subtypes. Detailed subtyping of the infecting virus requires its isolation, although the identity of the HA can be determined through the examination of host serological responses. Viral antigen can also be detected directly in nasal secretions by immunofluorescence of infected cells or the viral genome can be detected and sequenced by polymerase chain reaction (PCR).

Antibody to influenza virus may be detected by its ability to inhibit the agglutination of chick erythrocytes mediated by the influenza virus HA. The HI test is subtype specific, and is much more sensitive for antibody to H7 viruses than it is to H3 virus antibody when whole virus is used in the assay. The sensitivity of the test for antibody to the H3 viruses may be enhanced by disruption of the virus

with detergent. In HI tests a fourfold or greater increase in antibody titer between acute and convalescent sera is regarded as significant and indicative of infection. Although an ELISA has been developed for the detection of serum antibodies to equine influenza, this has been of limited benefit in horses that have received multiple doses of vaccine.

Subclinical infections may occur in vaccinated horses but they do not necessarily stimulate a fourfold increase in HI antibody, and such animals may be a source of infection to other horses. Significant increases in antibody may be detected using the single radial hemolysis (SRH) test (Morley et al 1995). In that test, influenza virus is coupled to sheep erythrocytes with chromium chloride, and agarose gels are prepared containing these sensitized cells and guinea-pig complement. Equine serum, inactivated by heating at 56°C for 30 min, is introduced into wells in the gels and incubated for 18–20 h at 37°C. The diameters of the resultant zones of hemolysis are measured. The reproducibility of the assay allows the identification of infections that stimulate no more than a twofold increase in antibody. The SRH assay has also been widely used in both experimental and field studies of vaccine efficacy, and correlation between vaccine-induced SRH antibody and protective immunity from whole virus inactivated vaccines is well established (Mumford & Wood 1992, Newton et al 2000b).

Epidemiology

Equine influenza is a highly contagious disease characterized by rapid spread in susceptible populations and morbidity rates are consequently high. In one outbreak in 1965, only 11 out of 634 susceptible horses resisted the disease, giving a morbidity rate of 98.2%. Detailed analysis of outbreaks in 1963 suggested that the basic reproduction rate, or R_0, in a North American racetrack was around 10.2, consistent with its highly contagious nature (Glass et al 2002). (R_0 is a term used to quantify transmissibility and represents an estimate of the average number of secondary cases of an infection produced directly by an index on introduction into a naive population.) Despite this, detailed computer modeling, supported by subsequent clinical observations, suggests that many outbreaks often die out as a result of chance effects after only a few animals have been infected, especially in vaccinated populations. Thus, influenza should be considered even when outbreaks do not affect all the susceptible animals in a population.

Equine influenza has been reported in many parts of the world, including North and South America, the West Indies, Europe, countries of the former USSR, North Africa, the Middle East, India, Singapore, Japan, and Mongolia. The introduction of equine influenza into South Africa in 1986, India in 1987, Hong Kong in 1992, Dubai in 1995, and Puerto Rico and the Philippines in 1997 were other milestones in the transmission of this virus throughout the

world. Australia, New Zealand, and Iceland are the only countries that are known to have remained entirely free from the infection and Japan has not experienced a large epidemic since 1972. The disease is considered endemic in the USA, UK, and other European countries, based on the occurrence of almost annual outbreaks.

There are many different factors that influence the epidemiology of equine influenza. Some relate to the intrinsic properties of the virus, some to the pathogenesis of the disease, the immune system of the horse and, not least, to the equine population structure and activity of the equine industry. A key factor in the spread of equine influenza in the last two decades has been the increase in transportation of horses over long distances by air. Horses incubating the disease, or those that are clinically or subclinically infected, can introduce the infection into a susceptible population if they are not quarantined adequately. Examples of this have been seen in the UK, South Africa, Hong Kong, Dubai, Puerto Rico, and the Philippines. In some areas of the world, quarantine procedures have been virtually ignored to facilitate the international movement of horses for racing, competition and breeding purposes, particularly among thoroughbreds. In addition, inadequate immunization from vaccination leaves horses with partial immunity and can merely result in the suppression of clinical signs. This may make infections difficult to recognize clinically while still allowing virus excretion. Such horses probably play an important role in the maintenance and spread of equine influenza. However, countries such as Australia, New Zealand, Dubai, Hong Kong, and Japan all now have extremely stringent quarantine requirements, even for major competitions, to prevent the introduction of equine influenza infection to their susceptible horse populations. In most of these countries, vaccination is used in conjunction with strict quarantine and post-import testing.

Influenza viruses produce a frequent and harsh cough, which is an efficient way of spreading the virus in aerosols and over distances of many meters or more. Although it is believed that influenza is transmitted almost exclusively directly between horses, evidence from the South African outbreak in 1986 indicated that people and contaminated vehicles could transmit the virus indirectly (Guthrie et al 1999). Virus excretion in fully susceptible horses lasts between 7 and 10 days, although infected animals are rarely infectious for more than 7 days. Data from experimental infections suggests that large amounts of virus may be shed continuously over the infectious period, with $>10^3$ EID_{50} (50% egg infective dose per ml of swab extract) influenza virus being recovered from single daily nasopharyngeal swabs. Coughing may persist for longer than virus excretion, but the short incubation period also contributes to the rapid spread of infection. Long-term carriers (>10–14 days) of equine influenza are not thought to occur.

Immunity to equine influenza is short-lived, with clinical immunity lasting little more than a year. Immunity to infection may be even more short-lived, therefore, allowing re-infection to occur without clinical signs within a matter of months after a previous infection (Hannant et al 1988). There is no evidence that the occurrence of equine influenza is influenced by climatic conditions. Outbreaks of disease, particularly in the USA and some parts of Europe, are primarily related to the mixing and movement of horses and to the introduction of young stock onto racetracks where the infection may be endemic.

Features of equine influenza are similar in other equid species, such as donkeys, mules, and zebras. A high mortality rate has been reported in donkeys in some, but not all outbreaks. Indigenous equids are likely to provide important foci of susceptible animals for equine influenza viruses in regions where their widespread vaccination is impractical.

Prevention and control

Management procedures aimed at limiting the severity of disease and the spread of infection, whether on a local or international basis, require sensitive diagnostic techniques for rapid detection of clinical and subclinical infection, effective vaccines, and a coordinated strategy for the use of both. Equine influenza vaccines were first developed in the 1960s and are used widely for control of equine influenza but, in spite of intensive vaccination programs in some groups of horses, equine influenza infections remain a serious problem. While H7N7 vaccines were generally successful, the H3N8 component of inactivated vaccines has not been so effective and reasons for vaccine breakdown have been the subject of intense investigations. Research has focused on vaccine potency, the use of different adjuvants, vaccination schedules, and antigenic drift. During the last decade, progress has been made in all these areas of investigation, providing new approaches to the control of equine influenza.

Vaccine potency

The principal markers for resistance to influenza virus infection derived from whole virus inactivated or subunit vaccines are circulating antibodies specific for the HA and NA glycoproteins. Progress in assessing the protective efficacy of early vaccines was hampered by a lack of reliable methods to measure the HA content of vaccines and the host's antibody response to the HA and of a reproducible challenge method in horses. Improved methods of measuring vaccine potency, antibody responses, and protection against infection have been developed, facilitating progress in vaccine standardization and design.

Based on experience with human influenza viruses, a reliable *in vitro* potency test for the measurement of immunologically active HA in equine influenza vaccines,

the single radial immunodiffusion (SRD) test, was introduced. In addition, the SRH assay for measuring antibody to HA in equine serum is significantly more reproducible than the HI or other tests. Detailed studies of vaccine potency in horses have demonstrated a direct relationship between HA content in vaccines, measured in µg by SRD, and HA antibody levels, measured by SRH, stimulated by inactivated vaccines. Adjuvants vary in their ability to induce greater antibody levels at given levels of HA as measured by SRD.

Vaccine evaluation by experimental challenge infection of horses was slow to progress because of difficulties encountered in reproducing clinical disease. These difficulties have been overcome by using nebulized aerosols in known naive animals (Mumford & Wood 1992). This delivery system mimics a natural infection by producing infectious droplets (diameter $< 5\,\mu m$) capable of reaching the upper and lower airways and avoids a concentration of challenge inoculum at the site of sampling in the nasopharynx. Using this challenge method, a series of experiments to measure the protection afforded by inactivated virus vaccines with a variety of adjuvants and antigen presentation systems has been performed. The SRD test has been used to standardize inactivated vaccines, the SRH test has been used to measure antibody responses in the horse, and challenge infections were used to assess protection against infection and disease in animals with different defined immunological backgrounds. These studies have shown good correlation between vaccine-induced antibody levels directed against HA and protective immunity against infection with antigenically similar viruses ("homologous" viruses), with an effect of challenge dose. A threshold of SRH antibody levels required to prevent infection varied between $>120\,mm^2$ and $>154\,mm^2$, with the threshold increasing incrementally with the infectious viral dose. Field studies have validated this challenge system as being representative of field challenges (Newton et al 2000b). Vaccinated racehorses in the UK with pre-exposure SRH levels $\geq 140\,mm^2$ during outbreaks did not become infected, whereas those with lower antibody levels did. In South Africa in 1986, pre-infection SRH antibody levels of around $160\,mm^2$ were associated with a 90% protection rate.

Many commercially available equine influenza vaccines contain inactivated whole virus with an adjuvant, including mineral oil, alhydrogel and carbomer and some are based on viral subunits [e.g. ISCOMs (immunostimulatory complexes) or micelles combined with Quil A; Wood et al 1983a, Mumford et al 1988, 1994a,b,c]. Antibody responses stimulated by vaccines containing aluminum phosphate or hydroxide were more durable than those induced by aqueous vaccines of equivalent antigenic content. Even with adjuvant, mean antibody levels tended to decline to low levels by 16–20 weeks after the second and third doses of vaccine, although this again was affected by antigenic

content. In contrast, the incorporation of a polymer adjuvant was found to stimulate antibody that remained at a high level for at least 6 months after the third dose of vaccine. Similarly, vaccination with three doses of ISCOMs containing 15 μg HA resulted in the level of SRH antibody persisting at around 70 mm^2 for 15 months following the third dose.

The historical lack of standardization of vaccines from different sources, and the undemanding standards of some licensing authorities, has resulted in the use of products with inadequate potency in terms of ability to stimulate antibody to the HA (Wood et al 1983a,b, 1986, 1988). A large double-blind field trial using a commercial killed vaccine failed to demonstrate a significant difference in the rate of disease between vaccinated and unvaccinated animals in the face of a naturally occurring outbreak of disease in a population of horses stabled at a racetrack in North America (Morley et al 1999). The situation is improving with the establishment of European Pharmacopoeia international reference preparations to standardize the serological tests used in potency evaluation of vaccines, and the introduction of federal regulations on equine influenza vaccines in Europe and, more recently, in the USA. Inactivated vaccines currently available in Europe (in 2005) generally induce high peak antibody responses (>150 mm^2 SRH) with good duration after the third dose and some vaccines from North America do the same.

Natural immunity and live vaccines

Immunity provided by inactivated influenza virus vaccines is dependent on high levels of circulating antibody to HA and, in the absence of such antibody, vaccinated horses are susceptible to infection (Newton et al 1999, 2000b). In contrast, infection with influenza induces longer term immunity independent of circulating antibody against HA. For example, ponies with low or undetectable anti-HA antibodies were clinically protected from challenge infection more than 1 year after natural infection (Hannant et al 1988). This suggests an important difference in the immune response following infection compared with vaccination using inactivated virus. Additional components of the immune response that may be involved are cell-mediated immunity and mucosal antibody responses local to the site of infection.

Equine influenza virus infection has been demonstrated to generate virus-specific mucosal immunoglobulin A (IgA) and serum IgGa and IgGb responses, whereas a parenterally administered inactivated virus vaccine induced only a serum IgG(T) response. The qualitative differences between the immune responses that follow infection or vaccination with inactivated virus suggest that improvements can be made in vaccine design. Ideally, vaccines should induce broadly reactive, local and systemic, antibody and cellular immune responses, establish memory

and consequently generate a rapid anamnestic response upon field exposure to equine influenza virus. The incidence of free and cell-associated virus is thereby reduced and recovery is enhanced. Live attenuated and live vectored equine influenza vaccines that should more closely mimic natural infection have become commercially available.

One vectored product is a live recombinant vaccine, based on a canarypox vector that expresses the HA genes of equine influenza viruses. The recombinant virus undergoes an abortive infection in mammalian cells so that no progeny viruses are made but the expressed viral antigens are processed endogenously and presented as peptides via major histocompatibility complex class I by the host cell in the same manner as occurs in natural infection. Canarypox-vectored vaccines induce cellular immune responses to human immunodeficiency virus and probably do the same for equine influenza, although the data to demonstrate this are only just becoming available with the advent of new assays.

A cold-adapted, temperature-sensitive, modified live virus equine influenza vaccine, delivered intranasally, is commercially available in the USA (Chambers et al 2001, Lunn et al 2001, Townsend et al 2001). The safety and efficacy of the vaccine has been demonstrated in experimental studies but the vaccine does not provide sterile immunity, particularly 6 months after a single dose. There is no correlation between concentrations of serum antibody induced by vaccination and protection against infection, but anamnestic responses can be demonstrated at 7 days post infection. Primed animals have some circulating antibody but serum antibody cannot be used as a measure of efficacy of such live virus vaccines. The ability to provide alternative correlates of immunity has lagged behind the development of these alternative vaccination strategies.

Optimizing vaccination schedules

The early vaccination schedules for inactivated virus vaccines required two primary doses 4–6 weeks apart followed by annual booster doses. Where vaccination is compulsory in major European thoroughbred racing authorities, the current minimum requirements are of a primary course of two doses 4–6 weeks apart and a 6-month booster followed by annual boosters. Extensive experience and mathematical models validated against experimental and field data have demonstrated that vaccination dramatically reduces both the incidence and size of epidemics, with larger outbreaks of equine influenza being exceptional amongst groups of vaccinated animals but with substantial seasonal fluctuation associated with levels of antibody declining after boosters (Fig. 22.4) (de la Rua-Domenech et al 1999, Glass et al 2002, Park et al 2003). Thus the vaccination policy ensures a sufficient level of overall herd immunity to prevent large-scale
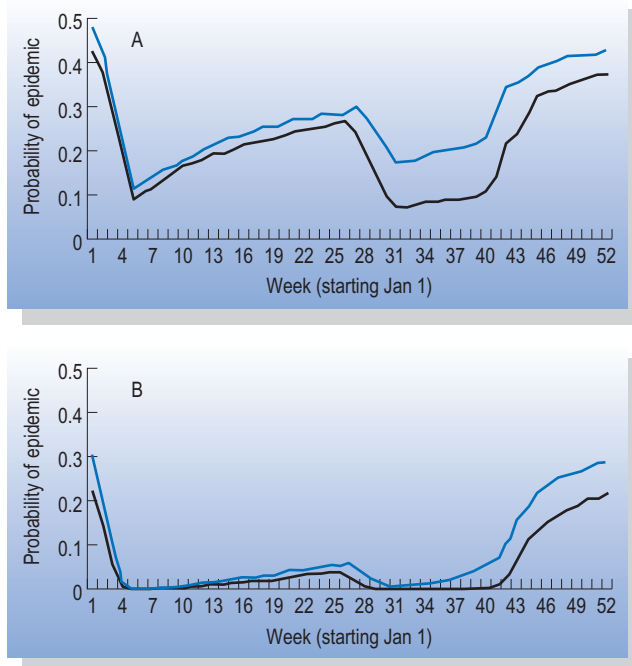
Fig. 22.4. Comparison of the probability of (A) a 3% outbreak or larger, and (B) a 10% outbreak or larger under the commonly used vaccination policy (black line) and the alternative 6-monthly vaccination strategy (blue line). Reproduced with permission from Park et al (2003).

Fig. 22.5. Mean Suffolk/89 SRH responses in four cohorts of yearlings and one cohort of horses in training following booster vaccination in 1996. Reproduced with permission from Newton et al (2000b).

Fig. 22.6. The scatter plot of mean SRH antibody levels (*y*) plotted against time since last vaccination (*x*) for 140 thoroughbred yearlings. Reproduced with permission from Newton et al (2000a).

outbreaks that are likely to lead to cancellation of race meetings and other equestrian events. In contrast, a program of three doses followed by annual vaccination probably does not provide sufficient immunity to protect all young horses from the disease or individual training yards from small outbreaks of influenza. In particular, horses following this regimen will have low antibody titers for several months between their second and third vaccinations. Also, SRH antibody levels in yearling thoroughbreds on studs in Newmarket declined below a protective level within 4 months of a booster vaccination (Fig. 22.5) (Newton et al 2000b). Importantly, this also coincided with the autumn sales, a recognized risk period for transmission of influenza in young thoroughbreds.

Later observations in yearlings entering training yards in Newmarket confirmed that antibody levels at this time were influenced by both time elapsed since the last vaccination and the total number of vaccines that had been previously administered (Fig. 22.6) (Newton et al 2000a).

Additional 6-month booster vaccination benefits horses that may be at high risk during this interval. Intensive vaccination regimens, involving booster doses every 30–60 days, have been practiced in the USA but too frequent administration of a potent vaccine could be detrimental. Using a mathematical model to assess the risk of an outbreak occurring in a thoroughbred population in a typical flat-racing training yard, increasing the

frequency of vaccination in horses aged 2 years and upwards to include 6-monthly boosters would offer a significant increase in protection over annual vaccination, particularly in the latter half of the year (Park et al 2003).

Timing of the age at first vaccination may be critical to the subsequent development of antibody. Maternal antibody generally inhibits the development of neonatal antibody synthesis, and it has often been assumed that these antibodies have decayed to an insignificant level by 3–4 months. The temptation is to vaccinate elite stock before the loss of maternal antibodies to avoid any window

of susceptibility. Foals born to mares vaccinated during the gestation period have high levels of maternal antibody within 2 days of birth. It has been suggested that not only does vaccination in the face of maternal antibody interfere with the development of active immunity but that repeat vaccination in the face of maternal antibodies could induce tolerance (Cullinane et al 2001). It is recommended that mares should be vaccinated against equine influenza in the last 4–6 weeks of pregnancy to ensure the transfer of protective levels of antibody in the colostrum, and that foals should not be vaccinated until their maternal antibodies have waned (i.e. not until 6 months of age or they are seronegative).

Vaccine strain selection

Surveillance of antigenic drift is a cornerstone of global influenza control programs based on vaccination. Epidemiological investigation of disease outbreaks also provides important information on vaccine efficacy and the need to update vaccine strains. Field studies, backed up by detailed experimentation, have confirmed the need for inclusion of antigenically relevant strains in vaccines (Daly et al 1996, 2003, 2004, Newton et al 1999). When antigenic drift is occurring, cross-protection between vaccine and challenge viral strains will diminish over time.

Experimental studies have demonstrated that increasingly higher levels of protective immunity are required from outdated vaccines to achieve minimal protection (Yates & Mumford 2000, Daly et al 2004). Generally, after antigenic drift has occurred, transmission between inadequately vaccinated animals will occur before clinical signs are observed. When circulating field strains become sufficiently different from vaccine strains, they can cause clinical disease irrespective of the frequency of administration and response to vaccination, as was seen in Europe in 1989 (Livesay et al 1993).

The widespread epidemic that was caused by a variant of the H3N8 subtype in the USA and Europe during 1979–80 first raised the possibility that antigenic drift could play a major role in the epidemiology of equine influenza. During the 1989 outbreak of influenza in the UK, only horses with very high levels of vaccine-induced antibody were protected against infection, raising the possibility that there had been significant antigenic changes in the 1989 isolate that prevented its neutralization by antibody stimulated by vaccines containing Miami/63, Fontainebleau/79 or Kentucky/81. Sequencing of the *HA1* gene and antigenic analysis suggested that there were significant differences between a representative 1989 strain and the vaccine strains in use at the time. The hypothesis was tested by vaccinating groups of ponies with monovalent vaccines containing either of the vaccine strains or a 1989 strain and experimentally challenging them with a 1989 virus. Although all vaccines provided clinical protection, vaccine

efficacy in terms of ability to eliminate virus excretion correlated directly with the degree of antigenic relatedness between vaccine and challenge strain. Following a meeting of OIE and WHO experts on newly emerging strains of equine influenza, it was recommended that equine influenza vaccines be updated to include a 1989 isolate, and that efforts be made to increase surveillance and virus characterization.

Phylogenetic analysis of HA sequences revealed that equine H3N8 viruses, which had been evolving as a single lineage, apparently diverged into two distinct lineages during the mid-1980s (Fig. 22.1) (Daly et al 1996, Lai et al 2001). Viruses in one lineage were predominantly isolated from horses in Europe, whereas viruses in the other lineage were predominantly from horses on the American continent. It was apparent, however, that American lineage viruses had been introduced into Europe on at least one occasion. The genetic divergence of American and European lineage viruses was reflected in their antigenic reactivity, raising the question of the potential importance of geographical variations in antigenic character for vaccine efficacy.

Field observations have supported the hypothesis that antigenic differences between viruses of the American and European lineages are sufficient to have an adverse effect on vaccine efficacy. In contrast to outbreaks described above, in which high levels of antibody were protective in outbreaks with viruses homologous to those in vaccines, during an outbreak caused by an American lineage virus in 1998, when the vaccines used contained only European lineage viruses, a quarter of horses with antibody levels higher than $140\,mm^2$ became infected (Newton et al 1999). The importance of continued antigenic drift in causing vaccine breakdown has been evidenced by the increasing number of isolations of "American-like" H3N8 viruses from horses in Europe that have been immunized with vaccines containing only "European-like" strains. Further vaccination and experimental challenge studies in ponies suggested that vaccines containing virus from the American lineage may not be as effective in protecting against infection as the homologous vaccine against challenge with virus from the European lineage, but differences were not marked.

Data from these experiments, in particular those that quantitatively describe protective antibody thresholds, have been used to set the parameters for mathematical models. These models have demonstrated how transmission of equine influenza was markedly enhanced in the presence of antigenic drift. Small changes observed experimentally at the individual pony level can have large effects when scaled up to the population level in mathematical models (Park et al 2004).

Experts of OIE/WHO have recommended that if future equine vaccines are to be effective then they should contain representatives from both the American and the European lineages.

International control

The ever-increasing international movement of horses for competition and breeding purposes presents a challenge with regard to the control of equine influenza. Several explosive outbreaks of equine influenza attributable to the introduction of infected animals into susceptible indigenous populations have been described during the last 20 years. As a result of economic and competitive issues, it is desirable for the disruption to training programs caused by quarantine to be kept to a minimum when horses are moved. There is, therefore, a reliance on surveillance of influenza in the population that the animals are leaving and on the effectiveness of vaccines to prevent viral shedding. When these measures fail, and subclinically infected horses shedding virus are transported, the short quarantine periods that are often used fail to prevent introduction of infection.

Regulations relating to the movement of animals based on the use of improved diagnostic techniques and vaccination policies that recognize the limitations of current products are now in place. The Code Commission of the OIE recommends that importing countries that are free of equine influenza should require that all horses traveling from endemic areas are fully vaccinated and have received their last booster dose within 2–8 weeks of travel. A simple additional measure that can be implemented is the screening of antibody using the SRH assay, which can identify potentially susceptible animals that require re-vaccination to boost their antibody levels before traveling. The advent of more rapid diagnostic tests for equine influenza means that animals can be screened for virus shedding while still in quarantine at their destination before being released into potentially susceptible local populations.

Treatment of clinical cases

During the febrile stage of influenza, horses may be treated with non-steroidal anti-inflammatory drugs while antibiotics such as penicillin may be useful to combat secondary infections. Horses recovering from equine influenza, even after mild or subclinical disease, should be given adequate rest before training is resumed to avoid subsequent chronic respiratory disease (Gross et al 1998, 2004). As a guide, the number of weeks of rest that is recommended is the same as the number of days that the animal suffered from pyrexia, with a minimum of 2 weeks rest and slow resumption of work thereafter. Although antiviral therapy with matrix inhibitors amantadine and cimantadine has been used in experimentally infected horses, the risk of fatal side effects, impracticality and prohibitive expense all mean that it is not used routinely (Rees et al 1997, 1999). The newer neuramidase inhibitors have not been tested in horses. Because of the availability of rapid diagnosis and longer duration of outbreaks in large groups of horses, it has become common practice for mass vaccination to be implemented in the face of an outbreak, particularly in racing centers with large numbers of young, valuable horses. Anecdotal reports suggest that this is effective in reducing adverse clinical effects of influenza infection, probably by both stimulating immunity before infection and reducing the amount of virus shed.

Equine Herpesvirus-1 and -4

Historical context

Equids are host to at least 11 taxonomically grouped herpesviruses. Asinine herpesviruses 1, 2, and 3 (Browning et al 1988, Kleiboeker et al 2002), which principally infect donkeys, are synonymous with equine herpesviruses (EHV) 6, 7, and 8. Six of the 11 are classified as Alphaherpesvirinae (EHV-1, -3, -4, and -9, and asinine herpesviruses 1 and 3) and five as Gammaherpesvirinae (EHV-2 and -5, and asinine herpesviruses 2, 4, and 5). EHV-9 was originally named gazelle herpesvirus 1 (GHV-1) (Roizmann et al 1992).

The association between EHV-1 infection and abortion was first recognized in Kentucky in 1932 (Dimock et al 1936). At that time, the virus was termed equine influenza virus because of the influenza-like symptoms that it produced, and it was not until 1954 that it was shown that herpesviral respiratory disease in young horses (equine rhinopneumonitis) and equine abortion in pregnant mares were caused by the same agent, identified as EHV-1. In 1959, major antigenic differences between EHV-1 strains were noted by workers in Japan, and meticulous observations of herpesviral disease in the closed pony herd maintained at the Pirbright research station in the UK in the 1970s subsequently led to the concept that the EHV-1 strains associated with abortion and respiratory disease were different virus subtypes (Burrows et al 1984b). Restriction endonuclease fingerprinting subsequently demonstrated that the two subtypes were genetically distinct viruses that were renamed EHV-1 (formerly subtype 1, associated with abortion) and EHV-4 (formerly subtype 2, associated with respiratory disease). These differences in clinical outcome are not absolute though: EHV-1 can also cause respiratory disease, and EHV-4 occasionally causes abortion. Certain strains of EHV-1 also cause neurological disease, ranging from mild ataxia to quadriplegia (Allen et al 1986).

EHV-2 and EHV-5 are closely related gammaherpesviruses. EHV-2 is considered to be ubiquitous in horses, and it is therefore difficult to associate the presence of virus with specific clinical syndromes. Nonetheless, studies in the USA, Europe and Australia have shown an increased prevalence of EHV-2 in foals with clinically apparent lower respiratory tract disease than in normal foals (MJ Murray et al 1996), in addition to demonstrating the virus in samples from foals with keratoconjunctivitis (Collinson et al

ENVELOPE:
viral glycoproteins

TEGUMENT:
structural and *trans*-activating
proteins

CAPSID:
packages viral genome
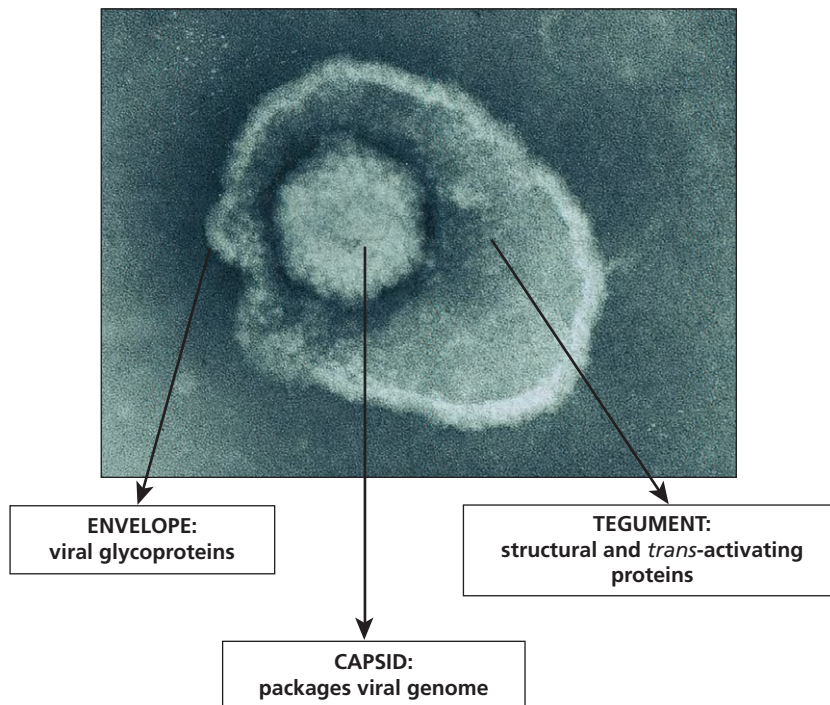
Fig. 22.7. Ultrastructural features of an EHV-1 virion. Reproduced with the permission of Dr Nick Davis-Poynter.

1994). Respiratory tract infection with EHV-2 has also been suggested as a predisposing factor for *Rhodococcus equi* infection in foals, perhaps through immunosuppression (Nordengrahn et al 1996). Molecular studies have also suggested that EHV-2 may have a role in reactivating EHV-1 and EHV-4 infections from latency (Edington 1992). More detailed investigation of EHV-2 and EHV-5 in experimental challenge studies will however be necessary to establish the importance of these viruses as respiratory pathogens in the field.

The asinine herpesviruses have not been studied in the same detail as the other equine herpesviruses, and their classification is in flux, but it is likely that major aspects of the pathogenicity, latency and immunogenicity of the asinine viruses are similar to those of their equine counterparts. Thus, asinine herpesviruses 4 and 5 are gammaherpesviruses that have been associated with interstitial pneumonia in young donkeys and would be expected to behave in a similar manner in their natural hosts to EHV-2, whereas asinine herpesvirus 3 is an alphaherpesvirus that shares some sequence homology with EHV-1 and is primarily associated with upper respiratory tract disease. Closer collaboration between researchers dealing with donkeys and horses will be necessary to establish whether cross-infection with equine and asinine herpesviruses is important in causing clinical disease. We have isolated what appeared to be both asinine and equine herpesviruses from donkeys suffering severe clinical disease.

Virology

EHV-1 and EHV-4 are alphaherpesviruses of the varicellovirus subfamily, being most closely related to the virus that causes chickenpox and shingles in people. The virus particle consists of an inner crystalline DNA nucleocapsid, surrounded by an amorphous tegument layer composed of structural and *trans*-activating proteins, and an outer envelope bearing major immunogenic glycoproteins (Fig. 22.7). In common with other enveloped viruses such as influenza, EHV-1 and EHV-4 are quickly inactivated in the environment by sunlight and disinfectants (Allen et al 1986, Crabb & Studdert 1995).

Both EHV-1 and EHV-4 have been completely sequenced (Telford et al 1992, 1998). Each virus has 76 genes, and there is considerable sequence homology between the two viruses, with the degree of amino acid identity for individual viral proteins ranging from 55 to 96%. This has two important practical outcomes: diagnostic tests for EHV-1 and EHV-4 often cross-react, and there is immunological cross-protection between the two viruses.

Recent exploitation of sequence data for EHV-1 by workers in Newmarket (Nugent et al, submitted) has enabled grouping of EHV-1 strains into six major strain groups (Fig. 22.8) on the basis of sequence variability of a single gene (*ORF68*). The six major strain groups show a degree of geographical restriction, with group 2 viruses being the most widespread, and occurring in North and South America, Europe and Australia. Group 5 viruses predominantly occur in North America, and viruses
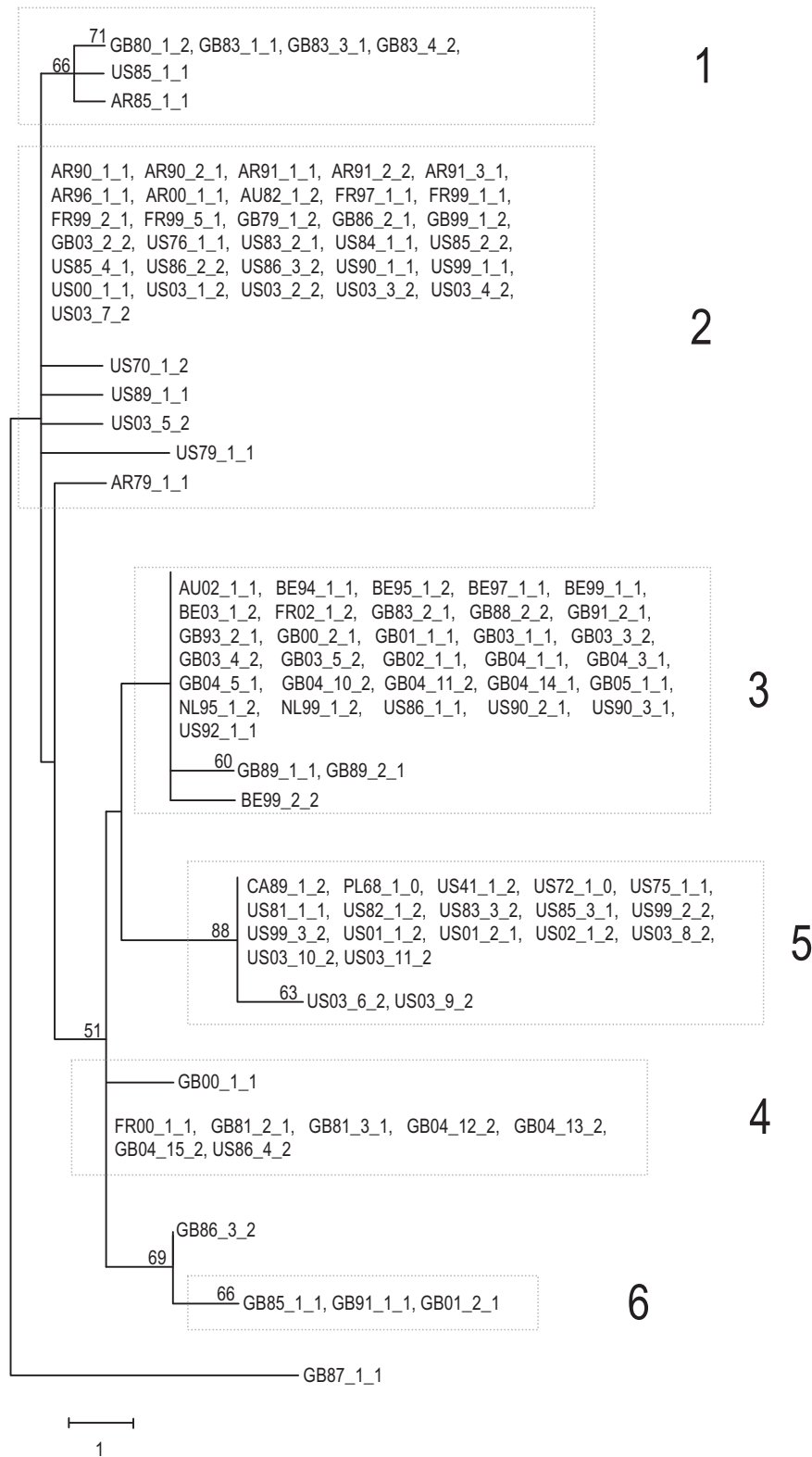
Fig. 22.8. A phylogram of ORF68 DNA sequence for 106 EHV-1 isolates, classified into six major strain groups, is shown. The unrooted tree was constructed using MEGA version 2.1 (Kumar et al 2001), using the neighbor-joining method (distance according to the number of nucleotide differences). Bootstrap values above 50 are shown (from a total of 100 iterations). Clusters of isolates within each of the six major strain groups are indicated (boxes). The scale bar denotes one nucleotide difference. Isolates were coded according to their country of origin (C), year of outbreak (Y), a unique identifier for the outbreak (x: 1, 2, 3 etc) and the pathogenic features of the outbreak (p: 0 – attenuated vaccine strain; 1 – non-neuropathogenic; 2 – neuropathogenic) according to the scheme CCYY_x_p. Countries of origin are designated AR (Argentina), AU (Australia), BE (Belgium), CA (Canada), FR (France), GB (Great Britain), NL (Netherlands), PL (Poland) and US (USA).

in groups 3, 4, and 6 predominantly occur in Europe. Furthermore, molecular epidemiological studies for additional variable genes has shown a significant association between a particular genetic mutation (of the DNA polymerase) and strains of EHV-1 isolated from outbreaks of neurological disease. These are important breakthroughs, which will revolutionize our understanding of EHV-1 molecular epidemiology, pathogenesis, and prevention, as well as facilitating more targeted diagnostic tests and control procedures.

Pathogenesis of herpesviral respiratory disease

Since uncomplicated herpesviral respiratory disease is rarely fatal, most information on the pathogenesis of the respiratory disease has been obtained from experimental studies performed as part of long-term vaccine research programs in the UK and USA. Both EHV-1 and EHV-4 initially infect and replicate in epithelial cells of the upper respiratory tract following inhalation of infectious aerosols or contact with infected fomites. EHV-1 can also infect the conjunctival epithelium, presumably via aerosol contact. Initial steps in infection of the respiratory tract are rapid: in primed adult ponies, occasional infected epithelial cells can be detected by immunoperoxidase staining in the nasopharynx, trachea, and bronchi as early as 12 h after experimental intranasal infection (Kydd et al 1994b). As a result of this primary replication, infectious virus may be shed in nasal mucus transiently (2–4 days) in animals with previous exposure to EHV-1 but shedding can persist for at least 15 days post infection (p.i.) in naive animals (Gibson et al 1992). Virus then spreads quickly (within 48 h) to monocytes and large and small lymphocytes in the sinuses of the respiratory tract-associated lymph nodes (Kydd et al 1994a). Progression of infection over days 4–6 p.i. involves the formation of multiple erosions in the nasal mucosa and nasopharynx, with viral antigen expression in degenerating epithelial cells, local lymphocytes, monocytes and endothelial cells of nasal blood vessels. Infection of epithelial cells, endothelial cells and leukocytes in the lungs can be observed from day 2 to day 13 p.i., with a peak on day 9 when occasional mural non-occlusive thrombi may occur in the pulmonary interstitium. Infection of such diverse cell types leads ultimately to leukocyte infection and progression to cell-associated viremia, thereby disseminating virus to sites of secondary replication including the pregnant uterus. Viremia develops from day 3 p.i. in both primed and naive animals but in the latter persists for up to 21 days (Scott et al 1983). The viremia primarily involves T cells (of CD5/CD8 phenotype) (Lunn et al 1991), and the interaction between infected circulating lymphocytes and endothelial cells in the central nervous system and genital tract is fundamental in determining whether respiratory infection is succeeded by neurological

or abortigenic disease. Simultaneously, in the initial stages of nasal and conjunctival epithelial infection, EHV-1 gains access to neurons of the trigeminal nerve and reaches the trigeminal ganglion by 48 h p.i., before or synchronously with the onset of viremia (Slater et al 1994). EHV-1 is generally cleared from the respiratory tract within 3 weeks after primary infection and after 1–2 weeks following subsequent infections.

The detailed pathogenesis of EHV-4 infections has not been elucidated but primary infection results in nasopharyngeal shedding for 7–10 days and for a shorter time (≤ 5 days) following secondary infection or recrudescence. It is likely that the initial steps in EHV-4 infection of the respiratory tract are similar to those in EHV-1 infection, but in contrast to EHV-1, dissemination generally terminates at the local lymph nodes. Most EHV-4 isolates do not infect endothelial cells or cause cell-associated viremia and therefore are not associated with abortion and neurological disease. Exceptionally, however, cell-associated viremia can be detected and more virulent isolates of EHV-4 also have a capacity to infect endothelial cells in fetuses and foals (Matsumura et al 1992, Blunden et al 1995). EHV-4 infections are generally cleared from the respiratory tract within 7–20 days after first infection and within 2–7 days in subsequent infections.

Clinical signs of respiratory disease

Experimental studies have shown a short incubation period for EHV-1 respiratory disease, which is usually 1–3 days duration. In the field, the incubation period may be longer (up to 10 days), although the relative contributions of infectious virus dose, pathogenicity and host immunity in determining incubation period are not known (Ostlund 1993). Young horses infected with EHV-1 show clinical signs of upper respiratory tract infection (rhinitis or pharyngitis), with some animals developing concurrent tracheobronchitis. Older horses, which are partially immune as a result of earlier infections with EHV-1 or EHV-4, generally show a reduced duration and severity of respiratory tract disease, which is often subclinical. Indeed, the first sign of EHV-1 infection in mature horses may be abortion or neurological disease (Fig. 22.9).

Useful information on the outcome of EHV-1 infection in young horses has been gained from work with specific pathogen-free foals, which are immunologically naive (Gibson et al 1992). If infected with EHV-1 by intranasal inoculation, specific pathogen-free foals show a biphasic pyrexia, with peaks of rectal temperature on days 1–2 p.i. and on days 6–7 p.i. The total duration of pyrexia can be 8–10 days, and the foals are depressed and anorexic over this period. There is a nasal and ocular discharge, which is initially serous but becomes mucoid and mucopurulent over the first week of infection. The discharge is associated with viral rhinitis and conjunctivitis that

Fig. 22.9. A recumbent horse exhibiting typical signs of severe paralytic EHV-1.

become complicated by secondary bacterial infection. Viral infection of regional lymph nodes results in lymphadenopathy, which is most readily detected as swelling of the submandibular lymph nodes. Retropharyngeal lymph node enlargement is also present in most cases, but is not detectable on palpation. The lymph node enlargements are maximal over days 7–10 p.i. and may be persistent over several weeks. If infection spreads to involve the lower respiratory tract in young foals then clinical signs may be more severe: the foals are markedly depressed, tachypneic and dyspneic, lose interest in the mare and may stop suckling. EHV-4 causes upper respiratory tract disease which is clinically indistinguishable from that caused by EHV-1 and, on occasion, can also cause bronchopneumonia (Coignoul et al 1984a). Since both EHV-1 and EHV-4 infections principally cause clinical signs ascribable to upper respiratory tract disease, coughing is not usually a prominent clinical sign (this is in contrast to influenza infection, where coughing is common). Where coughing does occur secondary to herpesviral infection it is often suspected that suboptimal stable air quality or inadequate rest following pyrexia may be contributory factors.

As well as the disease described above, EHV-1 and EHV-4 have been associated with around 5% of cases of inflammatory airway disease in young racehorses. There is no evidence that the low proportion of cases associated with EHV infection take any longer to resolve than the other 95% of cases of inflammatory airway disease – which have an average duration of around 2 months. The economic losses associated with respiratory disease are related not only to the lost training days during the acute stages of infection, but also to the longer term effects on performance. Some infections can be so mild (for reasons discussed above) that no signs whatsoever, even in horses competing, are observed. For example, EHV-1 has been isolated from samples taken from horses 48 h prior to them winning races.

Treatment of herpesviral respiratory disease using antiviral compounds is rarely reported, largely because of the lack of published data on the efficacy of these compounds in horses, and the fact that the productive phase of viral infection has often ceased by the time the clinical signs are recognized. There are occasional reports of the use of acyclovir in the treatment of young foals with EHV-1 respiratory disease or EHV-2-associated ocular disease (MJ Murray et al 1998), but these studies have generally been uncontrolled, and the number of animals involved has been too small for meaningful statistical analysis.

Diagnosis

EHV-1 and EHV-4 respiratory disease may be diagnosed by detection of virus in nasopharyngeal swabs collected in the acute phase of disease, particularly over the first few days of infection when the horse is pyrexic. Virus may be demonstrated by isolation on tissue culture or detection of specific viral DNA by PCR (Sharma et al 1992). Tracheal washes collected during the early stages of infection may also demonstrate degenerative changes in epithelial cells, with viral antigen detectable by immunofluorescence. However, in practice, since clinical signs are generally mild and often subclinical, diagnosis is often retrospective and based upon serology. In the 1950s and 1960s assays were developed to measure serum antibodies with virus neutralizing (VN) and complement fixing (CF) activity *in vitro* (Doll & Bryans 1962, Thomson et al 1976). These diagnostic

techniques demonstrated seroconversions of both VN and CF antibodies starting approximately 2 weeks after field or experimental infection with EHV-1. VN antibodies are long-lived, persisting for up to 1 year after infection and largely type-specific (i.e. EHV-1 or EHV-4), being directed primarily against the major envelope glycoproteins B and C (gB and gC). In contrast CF antibodies are short-lived, usually becoming undetectable by 3 months after infection. The CF antibody test is therefore useful, particularly when applied to pairs of sera collected at least 14 days apart, as an indicator of recent EHV-1 infection. However, the epitopes recognized by CF antibodies are cross-reactive with both EHV-1 and EHV-4 proteins, so this response is inaccurate for differentiation of type. A type-specific ELISA using fusion proteins expressing variable regions of a different envelope glycoprotein, gG, has been developed and applied extensively to epidemiological studies of EHV-1 and EHV-4 field infection in Australia (Foote et al 2004).

Since mortality associated with EHV-1 or -4 respiratory disease is low, it is unusual to make this diagnosis by post-mortem examination of adult horses, unless as part of the investigation of herpesviral neurological disease. Adult horses presenting with hind limb ataxia and incontinence suggestive of EHV-1 infection may be diagnosed in life by detection of circulating virus-infected leukocytes in heparinized blood samples, and will generally have high CF antibody titers to both EHV-1 and EHV-4 in samples collected soon after the onset of neurological disease (Edington et al 1986). Post-mortem diagnosis generally requires immunohistochemistry to demonstrate viral antigen expression at sites of vasculitis in the central nervous system. Antigen has often been cleared from the respiratory tract by the time that lesions are established in the central nervous system.

The pathologist will often be required to investigate EHV-1 as a cause of pneumonia in neonatal and suckling foals (Prickett 1970, Smith et al 2003). Foals infected with EHV-1 show hyperemia, vesiculation, necrosis, and ulceration of the mucosa of the upper respiratory tract, with miliary dark-red foci in the lungs. Microscopically, these lesions correspond to foci of necrotizing to exudative rhinitis and terminal bronchiolitis or alveolitis. Viral inclusion bodies may be detected in degenerating epithelial cells of the upper and lower respiratory tract, broncho-alveolar macrophages and lymphoid cells within mucosa-associated lymphoid tissue and local lymph nodes. These respiratory tract lesions may occur in primary post-natal infection of foals, and also form part of the multi-systemic viral lesions seen in aborted fetuses or sick neonatal foals with the congenital form of the disease. In the case of the abortigenic disease, diagnosis is achieved by detailed post-mortem examination of the aborted fetus and placenta, detecting infectious virus, DNA or antigens by a combined application of virus isolation, PCR and immunohistochemistry.

Epidemiology

Host range and international distribution of infection

The natural host range and reservoir of infection for EHV-1 and EHV-4 are generally restricted to members of the Equids, although for EHV-1, non-equine species, for example onager and deer, may be involved. The full host range for EHV-4 has not yet been elucidated. EHV-1 and EHV-4 have a worldwide geographical distribution, are ubiquitous in the USA, UK, and Australia and are considered significant equine pathogens in these countries. In common with influenza, the international movement of horses for racing, breeding, and competition has facilitated the global spread of these viruses.

Prevalence of infection

Both EHV-1 and EHV-4 infections become established early in life in most equids. These viruses, along with EHV-2, appear on stud farms every breeding season, with young horses generally acquiring infection as foals, often while still under the cover of maternal immunity. Indeed, recent studies in Australia have demonstrated shedding of EHV-4 by foals as young as 11 days (Foote et al 2004). Frequent episodes of re-infection or reactivation of latent virus occur during the first 12–18 months of life, when associated clinical signs of respiratory disease may be recognized. Both serological and PCR-based studies suggest that the majority of horses then harbor EHV-1 and EHV-4 throughout life, and experience repeated re-infections or reactivations, many of which events are not associated with clinical signs of disease.

Route of infection

For both EHV-1 and EHV-4 the route of infection is via the upper respiratory tract by virus-infected fomites or aerosol. Transmission of virus to susceptible animals occurs either from horses with acute or reactivated EHV respiratory infections or from contact with aborted fetuses. The source of infectious virus in the first two examples is respiratory tract secretions, whilst from pregnant mares, the aborted fetus and placenta are rich in infectious virus.

Latency and reactivation

In common with other alphaherpesviruses, infection with EHV-1 and EHV-4 results in the establishment of latency, which is often lifelong and ensures persistence of infection in horse populations through periodic reactivations (Edington 1992, Edington et al 1994). After EHV-1 infection of the respiratory tract, latent infection is established in the lymphoreticular system, both in circulating lymphocytes and in lymph nodes, and in sensory ganglia such as the trigeminal ganglion. The sites of latent infection may be detected by PCR or by prolonged co-cultivation of indicator cells with cells from the tissues of interest.
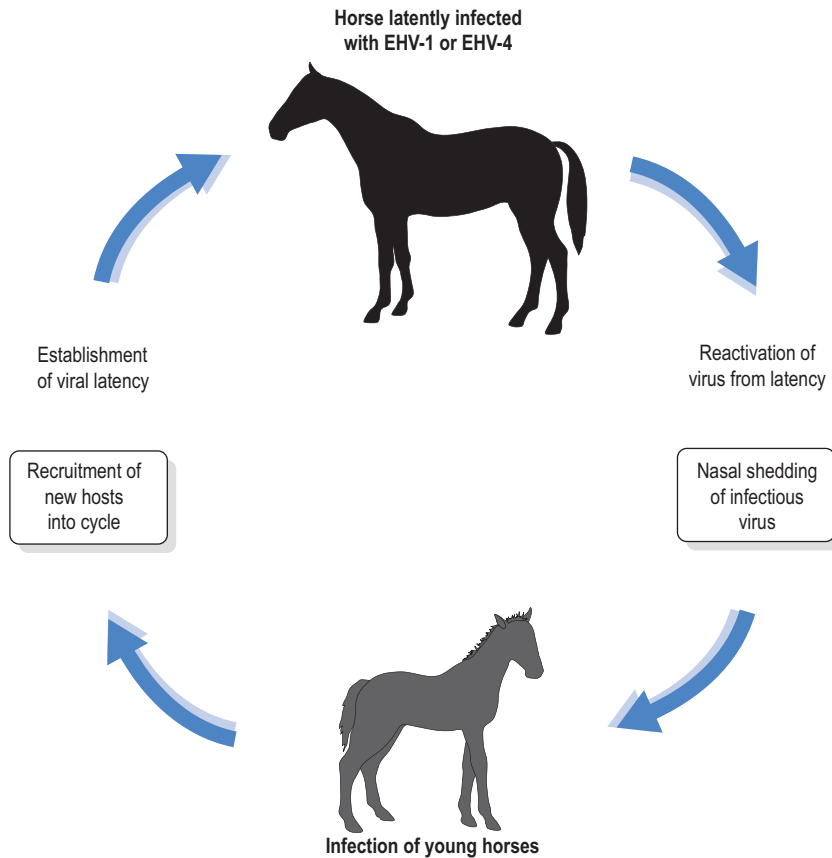
Fig. 22.10. The cycle of primary infection, latency and reactivation of EHV-1 infections in horses. Reproduced with the permission of Professor George Allen.

Using these techniques, it is generally easier to detect latent virus in lymphoreticular tissues than ganglia. Sites of EHV-4 latency also involve sensory ganglia and lymphoreticular tissues, including circulating lymphocytes (despite the rarity of EHV-4 viremia) (Borchers et al 1997).

Reactivation of EHV-1 or -4 infection may result in viremia (thereby rendering the affected horse at risk of abortion or paralysis) and/or shedding of infectious virus in respiratory tract secretions (thus infecting new, susceptible horses). Reactivation of latent EHV-1 and -4 infections in field situations has been observed following transport, handling, re-housing and weaning, and reactivation has been achieved experimentally by treatment with corticosteroids. Modern management of horses, especially long-distance travel in racing and competition horses, results in frequent reactivation of latent infections. Importantly, clinical respiratory disease is often absent following reactivation and such horses are therefore silent shedders of virus. This presents obvious management difficulties: new cases of EHV respiratory disease develop in susceptible, in-contact horses that have had no apparent contact with horses with respiratory disease. Similarly, abortion as a result of EHV-1 reactivation may occur in a pregnant

mare that has no history of recent EHV-1 respiratory disease in in-contact horses. The central role of latency and reactivation in maintaining EHV-1 in horse populations is illustrated in Fig. 22.10.

Immune responses to EHV-1 and EHV-4 infection

Both antibody-mediated (humoral) and cell-mediated immune responses are stimulated by EHV-1 or EHV-4 infection, and generally lead to recovery within 2 or 3 weeks, although immunity to re-infection is short-lived, lasting 3–6 months (Allen et al 1999). Repeated infections may, however, substantially increase the duration of immunity.

The antibody responses may be divided into systemic and mucosal types, with the former having received more detailed study in relation to diagnostic testing and assessment of immunity. Thus it has been known for several decades that, following infection with EHV-1 or EHV-4, CF and VN antibodies may be detected in serum samples. CF antibody lasts only 60–90 days, whereas VN antibody is longer lived and can be detected 12 months

after infection. There is antigenic cross-reactivity and a degree of cross-protection between EHV-1 and EHV-4, although the extent of cross-reactivity and cross-protection varies between virus isolates and between studies. In general, studies have shown that cross-protection is greater after multiple infections (with either virus) than single infections. It is also noteworthy that repeated infections can substantially increase the overall duration of immunity.

Mucosal antibody secretion represents a first line of defense against EHV-1 or EHV-4 infection of the respiratory tract, although detailed study of this arm of the immune system has been relatively neglected. Nevertheless, virus-specific antibody has been detected in the respiratory tract, and antibody with VN activity has been detected in mucosal secretions collected from horses for a few weeks after EHV-1 infection (Allen et al 1999, Breathnach et al 2001). These mucosal antibodies are primarily of the IgA isotype. Effective protection at the mucosal surface is also likely to involve cytokine responses, although there are similarly sparse data on these. Putative type I interferon secretion has been detected in nasal secretions and serum during the first 10 days after experimental EHV-1 infection, and synthesis of interferon-γ has been shown to increase in both CD4$^+$ and CD8$^+$ peripheral blood T lymphocytes collected at 10 days p.i. (Edington et al 1989).

Cell-mediated immune responses are vital in recovery from EHV-1 or -4 infection. This is because both viruses become intracellular within hours of contact with host cells, thereby evading the neutralizing effects of serum antibodies. However, attainment of a robust cellular immune response to infection is complicated by the fact that EHV-1 (and to a lesser extent EHV-4) is capable of replicating in lymphocytes and impairing their function. It has been shown that lymphocytes collected from animals infected experimentally with virulent strains of EHV-1 have a reduced ability to respond optimally to either inactivated virus or mitogen for periods varying between 2 and 10 weeks. This period of impaired lymphocyte function is succeeded by a phase of increased lymphocyte responsiveness to antigen, which then declines to baseline levels by 3 months p.i. (Hannant et al 1991). The basis of the impaired lymphocyte function has been studied *in vitro*, by measuring the proliferation of lymphocytes (either resting or mitogen-stimulated) in response to live or inactivated EHV-1. This has shown that lymphocytes that have been exposed to live EHV-1, either *in vivo* or *in vitro*, fail to proliferate in response to incubation with live virus *in vitro.* It is likely therefore that one factor contributing to the immunodepression is viral replication within the lymphocytes, which interferes with the ability of the infected cells to enter mitosis and thereby to proliferate in response to viral antigens. Thus, EHV-1, and presumably EHV-4, have evolved sophisticated mechanisms to subvert the function of key effector cells responsible for viral clearance and recovery.

Research into EHV-1 and EHV-4 immunology is now entering an exciting phase, with the development of sensitive assays for cellular responses that correlate with protection. Foremost among these is the detection of cytotoxic T lymphocyte (CTL) precursors as a measure of T cells capable of lysing virus-infected cells (Allen et al 1995, Kydd et al 2003). This cytotoxic immune response is major histocompatibility complex class I restricted and mediated by CD8$^+$ lymphocytes, and is not mediated by lymphokine-activated killer cell activity. CTL activity and CTL precursor frequency increase after EHV-1 infection and are of long duration. This may be up to 1 year in multiply infected ponies, although perhaps less in younger animals. Identification of those viral genes and proteins most important in stimulating CTL activity in horses is now an attainable goal, and is critical in rational vaccine design (Allen et al 1999).

Control of EHV-1 and EHV-4

Management of control

EHV-1 and EHV-4 can infect both bloodstock and horses in training but greater emphasis has been placed on the control of EHV-1 because of its abortigenic potential in pregnant mares. In the UK, the Horserace Betting Levy Board (HBLB) publishes a Code of Practice recommending precautions to prevent or limit infection of pregnant mares with EHV-1 (available online on www.hblb.org.uk). The Code is not mandatory but is adhered to in the thorough-bred industries in Britain, France, and Ireland. Similar management practices are used in North America (Ostlund 1993). Elsewhere, EHV-1 management is less formal.

Since EHV-1 and -4 are present as latent infections in the majority of horses, it is not feasible to prevent infection being introduced into horse populations. Management procedures may nevertheless have a major impact in limiting spread of infection, and associated clinical disease such as abortion, within populations. Infectious EHV-1 virions are present in aerosols shed from the respiratory tract and may also be present in other body fluids (tears, saliva, urine, feces, mucosal surfaces, blood, lymph) during the acute phase of disease. The aborted fetus and placenta are also highly infectious. Where abortion occurs within 2 weeks of respiratory infection or reactivation, the mare herself may shed virus from her respiratory tract. Reactivation of latent virus to result in nasopharyngeal shedding and/or abortion also complicates control measures. Therefore, horses of all ages and either sex must be considered potential sources of infection. This is particularly important when planning the husbandry of pregnant mares, and measures to limit contact between young horses that may be shedding EHV-1 infection and pregnant mares that are susceptible to EHV-1 abortion are of paramount importance, particularly in the last trimester of pregnancy.

Disease prevention is often divided into three areas, namely management, hygiene and vaccination. Adherence to the principles outlined in the HBLB Code of Practice, summarized below, remains the mainstay of successful control of EHV-1 on equine breeding premises.

Summary of measures to be taken on breeding premises following EHV-1 abortion

- Isolate mare, destroy bedding and disinfect box.
- Stop horse movement on and off the stud.
- Notify local breeders' association.
- Divide close contact pregnant mares into small groups.
- Contact owners of mares that are at the infected stud or are due to be sent there.
- Contact studs to which mares have been sent or are due to be sent.
- Pregnant mares should remain on the stud until foaling.
- Do not move any horse for 28 days from last abortion.
- Non-pregnant mares can move earlier than 28 days if they have been isolated from pregnant mares and handled by separate staff, and if blood samples at 14-day intervals do not indicate seroconversion.
- Aborting mares should be isolated from pregnant mares for 56 days.
- The following year, aborted and in-contact mares should foal in isolation, preferably at home.
- Review vaccination policy.

With regard to horses-in-training, EHV-1 and EHV-4 are responsible for only a low incidence of respiratory disease. In naive young-stock, disease is obvious but after several infections and/or vaccination the infection is subclinical, which in the athletic horse may be responsible for loss of performance. Although less infectious than equine influenza virus, both EHV-1 and EHV-4 spread between animals at grass or in nose-to-nose contact with each other. Spread around training yards also occurs and appears to be facilitated where airspaces are shared, as in American Barn housing systems.

Good animal husbandry and management in training yards can alert the trainer and veterinary surgeon to a horse with subclinical disease. Many yards now monitor rectal temperatures daily and use regular hematological examinations and cytology on tracheal washes as a means of early detection of infectious disease. This information gives the trainer the option of reducing the workload or even resting the animal while investigations are continued. Strict isolation of all acutely infected animals, perhaps detected by early diagnosis of pyrexia, can be an effective means of limiting spread of infection around a training establishment.

Clinical treatment of diagnosed cases of EHV respiratory disease should include rest from strenuous exercise and particular care with the management of stabled horses to minimize stress and dust inhalation, which may require a change of bedding. In many cases in horses-in-training, secondary bacterial infections are not particularly evident, but where they are, they should be treated aggressively with standard antibacterial therapy.

Control by vaccination

During the decade from 1941, several EHV vaccines were tested by intramuscular administration in pregnant mares. Both inactivated vaccines prepared from virus grown in equine fetal tissues and live vaccines derived from hamster-adapted virus were used in these early studies. Unfortunately, adverse side effects were common features of these vaccines (although intranasal delivery of the live vaccine to young horses did protect them from challenge infection 1 month later). Subsequently, a planned infection program was initiated in Kentucky to immunize pregnant mares against virus abortion (Doll & Bryans 1963). Vaccination of 9,480 pregnant mares during the next decade was associated with a reduction of the incidence of abortion from 15% in unvaccinated animals to 0.93%, although the effect of the vaccine is impossible to separate from the effects of management changes implemented over this period. However, safety problems continued because there was a suggestion of vaccine-induced abortions. In the 1960s, a new attenuated virus was developed from a high-titer porcine-cell-culture-adapted virus that had been first isolated from an aborted fetus and then passaged in hamsters (isolate RAC-H), resulting in a virus that had impaired capacity to replicate but nonetheless retained some immunogenicity (Mayr & Pette 1968). Derivatives of this isolate are still in commercial use in northern Europe today. Attempts were made to enhance further the efficacy of the vaccine in the 1970s, by further passage of the porcine-cell-culture-adapted virus in rabbit kidney cells and then equine dermal cells to reach a final passage of p263. The resultant vaccine became available in North America and parts of Europe, but safety problems were identified, with abortion occurring in 50% of pony mares experimentally challenged with the vaccine virus (Dutta & Shipley 1975). Largely as a result of this concern about the safety of live virus vaccines, workers in Lexington in the 1980s demonstrated serviceable immunity to EHV-1 in pregnant mares (successful foaling and reduction in virus shedding) using a formalin-inactivated, whole virus adjuvanted vaccine, which was administered intramuscularly. These results were substantiated by a large field trial of the same vaccine after it became commercially available as Pneumabort K (Bryans & Allen 1982). The accumulated frequency of EHV-1 abortions among vaccinated mares was 1.6/1000 (14/8,638) as compared with a

frequency of 6.8/1000 (140/20,732) in the remainder of the study population. However, subsequent studies in the UK were not so encouraging: Burrows and colleagues showed that intramuscular vaccination with this inactivated vaccine in yearlings and 2-year-old pregnant pony mares was associated with reduced viremia, but that there was no difference in the occurrence of abortion between vaccinated and unvaccinated controls (Burrows et al 1984a).

Towards the end of the 1990s in the UK, the use of the earlier inactivated product was generally replaced by the use of a similar, but carbomer-adjuvanted, inactivated product (Heldens et al 2001). Typically, this vaccine is used in pregnant mares in the 5th, 7th, and 9th months of their gestation. The vaccine is also licensed for protection against respiratory disease, when 6-monthly boosting of all susceptible horses is recommended. Another inactivated vaccine is licensed in the UK for protection against EHV-1 and EHV-4 respiratory disease and equine influenza, but is not licensed for protection against abortion. Neither vaccine is licensed for protection against neurological disease.

Notwithstanding safety concerns, it is predictable that live virus vaccines will be more effective than inactivated vaccines in stimulating protective immune responses associated with CTL. To balance the risk of such viruses reverting to virulence against the benefit of stimulating more robust immunity, efforts have been directed towards constructing live attenuated viruses with deletions in specific genes associated with pathogenicity. Several such deletion mutants have been constructed, and vaccine trials are underway in experimental ponies. The results are awaited with interest, although success may be confounded by the paradox that those genes associated with pathogenicity, and thereby deleted from the vaccines, may also be the most immunogenic.

The route of vaccine administration will also condition the type of immune response that is stimulated. Given that EHV-1 and -4 initially infect the respiratory tract, it is logical to predict that stimulation of immunity at the respiratory mucosal surface, by intranasal administration of vaccine, may be more successful that parenteral administration. The attraction of mucosal vaccines is that the induction of a layer of antibody with VN activity should neutralize inhaled virus particles before epithelial cell infection occurs. This may prove useful for vaccinating in-contact horses in the face of an ongoing outbreak or to protect bloodstock passing through the sale ring by limiting the spread of disease. This technique has been studied for influenza virus vaccination, using cholera toxin as an adjuvant to enhance immunogenicity, but awaits application to EHV-1, although it has been shown to be successful in bovine herpesvirus 1 infection in cattle.

DNA vaccines also offer promise as a means of introducing immunogenic viral genes into animal cells, without the risk of viral replication and consequent disease. Such vaccines consist of a bacterial plasmid with a strong viral promoter, the required gene sequence and a polyadenylation/transcriptional termination sequence. On administration of the plasmid DNA vector by intramuscular injection or bombardment of DNA-coated gold beads intradermally, the antigen is expressed *in situ*, leading to the induction of antigen-specific immunity, including vigorous cellular immune responses. The immune response generated after DNA vaccination can also be modulated by the inclusion of cytokine sequences. In the horse, one potential advantage of DNA vaccination may be induction of immunity in foals, even under cover of colostrally derived antibodies.

It is highly likely that, whatever progress is made with the development of new vaccines, the use of novel products will never replace the important role that management practices play in the prevention and control of disease associated with EHV-1 and EHV-4.

Equine Arteritis Virus

Historical context

The symptoms of the disease "pink-eye", now known as equine viral arteritis (EVA), were first reported over 100 years ago, but there was clearly some confusion with other equine viral diseases as the condition was also referred to as "equine influenza". Early workers described the clinical signs as including "weakness, watering of the eyes, injected conjunctiva, swelled legs, and diffuse swelling on the under-part of the abdomen". There was also recognition that the disease could be spread from stallions to mares at covering and that this spread continued for a long period after stallions were first affected. Abortion was sometimes observed and it was recognized that strict isolation could successfully control the disease.

The next major advance in the understanding of EVA came when the causative agent, equine arteritis virus (EAV), was first isolated from horses during an outbreak of severe respiratory disease and abortion on a standardbred stud farm in the town of Bucyrus, Ohio in 1953 (Bryans et al 1957). The observation that different disease outbreaks often presented with differing severity of signs then led to speculation that different strains of EAV could exist. Differences in virulence between isolates were recognized and this was further modified and exploited through the development of a modified virus strain in a live vaccine (Doll et al 1968, McCollum 1969). Following its initial isolation, the morphological and physicochemical properties of EAV were described by the early 1970s, by which time it had been appreciated that there were two different viruses capable of causing abortion in mares (i.e. EHV and EAV). Around the same time, detailed observations on the respiratory form of the disease were made in Kentucky during experimental infections which contributed to understanding of the pathogenesis of EVA (McCollum et al

1971). The serological assays of CF and VN were developed and described by the mid-1970s (Senne et al 1985). These techniques allowed accurate diagnosis and surveillance of EVA in many areas of the world.

Virology and immunity

EAV is the prototype member of the family Arteriviridae, a group of small enveloped, single-stranded RNA viruses. Other members of this family are lactate dehydrogenase-elevating virus, simian hemorrhagic fever virus, and porcine reproductive and respiratory syndrome virus.

EAV has an icosahedral nucleocapsid that contains a 12.7-kilobase polyadenylated infectious RNA molecule surrounded by a lipid bilayer envelope. The genome contains nine recognized open reading frames (ORFs 1a, 1b, 2a, 2b and 3–7), of which the two largest (1a and 1b) encode the viral replicase. Five structural proteins have been characterized, namely the nucleocapsid protein (N, encoded by ORF 7), two glycosylated envelope proteins (G_L and G_S, encoded by ORFs 5 and 2b, respectively) and two non-glycosylated envelope proteins (M and E), encoded by ORFs 6 and 2a, respectively (de Vries et al 1992). Recently, GP3 and GP4, encoded by ORFs 3 and 4, have been demonstrated to form part of the virion envelope (Wieringa et al 2002). Proteins N, M and G_L are the major antigens of EAV (Balasuriya et al 1995, Chirnside et al 1995, MacLachlan et al 1998). Although ORF 3 is also thought to encode an immunogenic protein (Hedges et al 1999), all hitherto known neutralizing epitopes are contained within the putative ectodomain of G_L (Balasuriya et al 1997). The enveloped virus is easily inactivated by lipid solvents and disinfectants (Castillo-Olivares et al 2001).

Animals that recover from EAV infection develop a long-lasting immunity against the disease, although not always against re-infection. It is thought that this immunity is mediated mainly by VN antibodies, since their appearance in serum, usually within a week of infection, coincides with the elimination of virus from circulation. Furthermore, passive transfer of colostral antibodies from immune mares to foals has been found to moderate or prevent EVA (McCollum 1976). However, virus-specific $CD8^+$ CTL responses are stimulated by EAV (Castillo-Olivares et al 2003a) and high levels of protection have been achieved in the absence of a strong VN antibody using a G_L deletion mutant vaccine, indicating that alternative effector mechanisms of immunity (CTL responses) are important for protection (Castillo-Olivares et al 2003b). In chronically infected stallions, EAV replication, which is restricted to cells of the accessory sex glands, persists for several months or years, despite high levels of circulating VN antibody (Timoney et al 1987).

Clinical signs and pathogenesis

EAV replicates primarily in the endothelium of small arteries and in macrophages. Secondary sites of virus replication include the epithelia of adrenals, kidney, liver, seminiferous tubules, and mesothelium. The result of infection is variable and depends on the virus strain, the age of the host and environmental factors (Timoney & McCollum 1991). Affected animals may or may not develop a syndrome that is typically characterized by one or a combination of the following clinical signs: pyrexia (up to 41°C), depression, conjunctivitis, palpebral edema, ocular and nasal discharges, edema of the periorbital region or ventral parts of the body, urticarial skin rash and lymph node swelling (Fig. 22.11). An important sequel of infection in the pregnant mare is abortion. Typically this occurs around 2 weeks after acute infection and often, but not invariably, follows illness in the mare. It is not always associated with fetal infection, although it is possible to recover virus from the tissues in some fetuses (Johnson et al 1991). If neonatal foals become infected, signs including fever, leukopenia and pneumonia can be severe and there is significant mortality. At necropsy, an interstitial pneumonia and an arteritis are frequently present (Del Piero et al 1997). In the outbreak in the UK in 1993, two foals on the index stud were born prematurely following illness in their dams; one died from pneumonia and another was weak, with its dam having little milk following a severe illness.
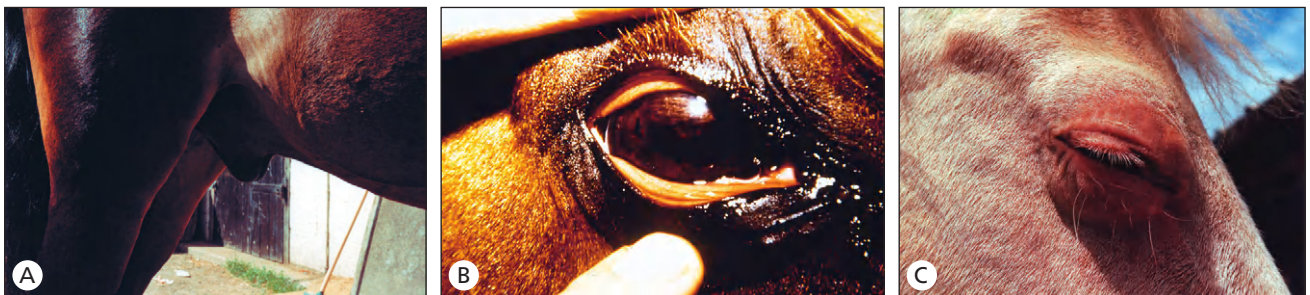
Fig. 22.11. Horses with clinical EVA demonstrating (A) edema of the sheath, (B) conjunctivitis, blepharitis, and uveitis, and (C) severe periorbital edema.

Importantly, the nature and severity of the clinical signs are not affected by the route of infection – mares infected venereally will demonstrate identical signs to those infected via the respiratory route.

Infection with EAV typically produces a viremia that persists for up to 20 days (McCollum et al 1971), although experimental studies with some strains of virus have demonstrated a cell-associated viremia for several weeks longer than this (J. Castillo-Olivares and N. Davis-Poynter, personal communication). During this period, all bodily secretions may be infectious, including ocular and nasal discharges, saliva, urine, milk, feces, and semen. The virus has been isolated from nasal discharges for up to 16 days after infection. After the acute period, the infection is eliminated from geldings, mares, and sexually immature colts (McCollum et al 1971). However, the infection may persist in the accessory glands of the adult male reproductive tract for longer periods and around 30% of infected stallions have been reported to be persistent carriers (Timoney et al 1987). Virus is shed in the semen and infects mares, whether covered naturally or inseminated with frozen or chilled semen.

The clinical signs presented, and their severity, vary widely between outbreaks and between animals within outbreaks. In many occasions, EAV causes subclinical infections. Of particular significance for the equine industry is the capacity of EAV to cause abortion in pregnant mares and to establish persistent infection in stallions (Fukunaga 1994). Infection is also transmitted by nasal secretions during the acute phase of the disease as well as through semen of chronically infected stallions. The latter animals are asymptomatic virus carriers and can introduce the disease to naive equine populations, as demonstrated in the 1993 outbreak in the UK.

Diagnosis

The appearance of disease suggestive of EVA in mares 1–2 weeks after mating should lead to suspicion of EVA, as should respiratory transmission of a disease with these clinical features. However, diagnosis of the disease can only be made following laboratory confirmation, either by detection of virus or viral RNA, or through demonstration of a four-fold or greater increase in specific antibody levels.

The current international standard and OIE-recognized serological assay still remains the virus or serum neutralization test (VN or SNT) (Timoney 2000). The CF test has been used in the past, but has no advantages over VN for routine use. There have been problems regarding inter-laboratory differences with VN tests which were addressed, at least in some European laboratories, through sharing of reagents (in particular of virus strains with given passage histories and positive control antisera) and protocols following a formal inter-laboratory comparison (Edwards et

al 1999). The VN test should be undertaken as described in the standard protocol in the OIE Manual of Standards for Diagnostic Tests and Vaccines (Timoney 2000).

More recently, problems have been reported with serum cytotoxicity when undertaking the VN test in old mares that have been repeatedly immunized with tissue-culture-grown equine herpesvirus vaccines (Newton et al 2004). Factors like this and issues such as cost and speed of assay have directed attention towards replacing the VN test with an ELISA. Excellent data on a prototype ELISA based on G_L have been published (Nugent et al 2000) and while this assay is not yet commercially available, efforts are being made to introduce it and inter-laboratory trials are underway. However, several commercially available ELISAs have poor specificity and/or sensitivity, are not supported by good published data or inter-laboratory comparisons, and their use cannot be supported in any setting.

Virus can be detected in blood samples or nasopharyngeal swabs collected from acute cases, either through its growth in tissue culture in a wide range of cell types, or through detection of its RNA through reverse transcription (RT)-PCR. Semen samples (which are frequently toxic to cells in tissue culture) are usually pretreated before inoculation by short-term sonication followed by centrifugation to sediment the spermatozoa. Monolayer cultures should be inoculated in duplicate (at least) with tenfold serial dilutions of the sonicated seminal plasma, or buffy coat collected from heparinized blood samples. The virus can be difficult to isolate or detect in tissue culture in the laboratory and not all strains are always immediately cytopathic.

Diagnosis of EVA in the fetus is problematic, as a high proportion of fetuses aborted during this infection are virologically negative, with placental separation occurring secondary to viral replication in the pregnant uterus (Coignoul et al 1984b). Confirmation of EVA infection in the aborting mare is necessary in such cases. Where transplacental spread of EAV to the fetus occurs then lesions of vasculitis may be detected in the fetal organs, and viral antigen may be demonstrated by immunohistochemistry in a similar manner to EHV-1 abortion (Del Piero 2000).

Detection of persistently infected ("shedding") stallions

Detailed studies of persistently infected stallions have been undertaken, reflecting the key role that these animals play in the persistence and transmission of EAV, in particular by Professor Peter Timoney at the Gluck Equine Research Center in Kentucky (Timoney et al 1986, 1987, Timoney & McCollum 2000). All persistently infected stallions have a titer of 1 : 4 or greater in VN tests and the VN test performed on serum is a well-validated first screening test to determine if stallions have been infected. Care should be taken to use only laboratories experienced in this assay.

In stallions that have detectable neutralizing antibody, efforts should be directed to determine whether or not they are persistently infected. Virus is thought to be shed continuously in the semen and can be found in any fraction, so if sensitive methods are used, it should be possible to determine whether or not a stallion is a shedder through examination of one semen sample. Semen should be delivered chilled to the laboratory, preferably within 12 h of collection, although frozen semen straws can also be used. Where possible, a combination of validated RT-PCR and virus isolation techniques should be employed to determine whether or not the sample is infected.

Most stallions will shed virus in the first few weeks following infection, but the proportion infected then decreases over several weeks to around 30%. Some stallions will shed virus for many years – effectively for their lifetime. The testing of semen can be repeated if it is felt that the stallion may have ceased to shed. If laboratory tests suggest that stallions have ceased shedding or if there is any question relating to these results, the shedding status of a stallion can be determined by test mating at least two naive, proven seronegative mares. The mares should be placed in strict isolation before the test mating and then tested serologically 2 weeks later. They should then be covered at least twice a day for two consecutive days (i.e. each mare should be covered at least four times), kept in strict isolation and then retested 14–28 days later to determine if they have seroconverted (see the HBLB Code of Practice for details).

Epidemiology

EAV spreads readily by both the respiratory and the venereal routes. The outbreak in Kentucky in 1984 led to a major breakthrough in the understanding of the epidemiology of EVA that provided an explanation for the postcoital infections that had been described at the turn of the century. Testosterone-dependent persistence of EAV infection localized in the accessory sexual organs was found to occur in around one-third of infected sexually mature stallions and was reproduced experimentally (Holyoak et al 1993, McCollum et al 1994). This understanding of the epidemiology of EVA offered for the first time the realistic prospect of control and even elimination of this disease from endemically infected horse populations. However, treatment of persistently shedding stallions with testosterone antagonists has not yet produced consistently useful results (Fortier et al 2002).

Spread through the venereal route is highly efficient and the fundamental role of persistently infected stallions in the spread and persistence of this infection cannot be overemphasized. It has been estimated that around 90% of susceptible mares will become infected for each mating or insemination with infected semen. Virus is preserved by treatments aimed at preserving semen quality (e.g. chilling or freezing) and shipping semen is a most effective means of spreading the infection over long distances (Timoney & McCollum 1993).

The infection is not highly contagious by the respiratory routes, certainly transmitting less rapidly and readily than diseases like influenza. Respiratory spread usually requires fairly close contact and even then may not occur, although major respiratory outbreaks have been described, including on major North American racetracks. In outbreaks on stud farms that involve both venereal and respiratory infection, the venereal route is usually the more important of the two. Care must also be taken to prevent contact between susceptible mares and aborted fetuses and other products of parturition, as these can also act as important sources of infection.

Infection on stud farms is frequently introduced by the use of infected semen. Once mares are acutely infected, then infection can spread to close contacts via the respiratory route. If pregnant mares become infected, abortion or neonatal foal death can occur and if mares due for covering are infected, then they can pass the infection on to other resident stallions. Typically, a large proportion of the clinical cases observed during an outbreak will be derived from venereally transmitted infections. A representation of a typical epidemic lifecycle on a stud farm is shown in Fig. 22.12. The route of transmission from naive mare to nursing foal, perhaps following insemination by infected semen, is unclear and could involve milk or close respiratory contact; it is, however, most efficient.

Although not particularly well characterized, epidemiological investigations of outbreaks suggest that there are marked differences between strains of virus in their ability to spread via the respiratory route (as well as differences in virulence, which may be related) (Moore et al 2002). This can lead to very different patterns of infection from that described above being observed on some stud farms. For example, we investigated, in conjunction with the local veterinarians, spread of infection on a very well-managed UK warmblood stud farm where one of the two resident stallions had been shedding virus in its semen for a number of years (as represented in Fig. 22.13). All infections had been entirely subclinical. Interestingly, other than spread from all maiden mares covered by the shedder to their suckling foals, there had been, over several years, no respiratory spread to other animals from acutely infected mares or their foals. This was evident from finding that all mares that had ever been covered by the shedder were seropositive and that all of those only covered by the other, seronegative, stallion remained seronegative. The mares had been kept as a single band over several years, emphasizing the highly variable transmissibility of EAV under different circumstances. Such results are consistent with the variation seen between isolates *in vitro* (Moore et al 2002, 2003).

Shedding stallion

Semen

Mares

Foals

Stallions

Off stud

Mares

Off stud

Fetuses and neonatal foals

Foals

Venereal spread

Respiratory spread
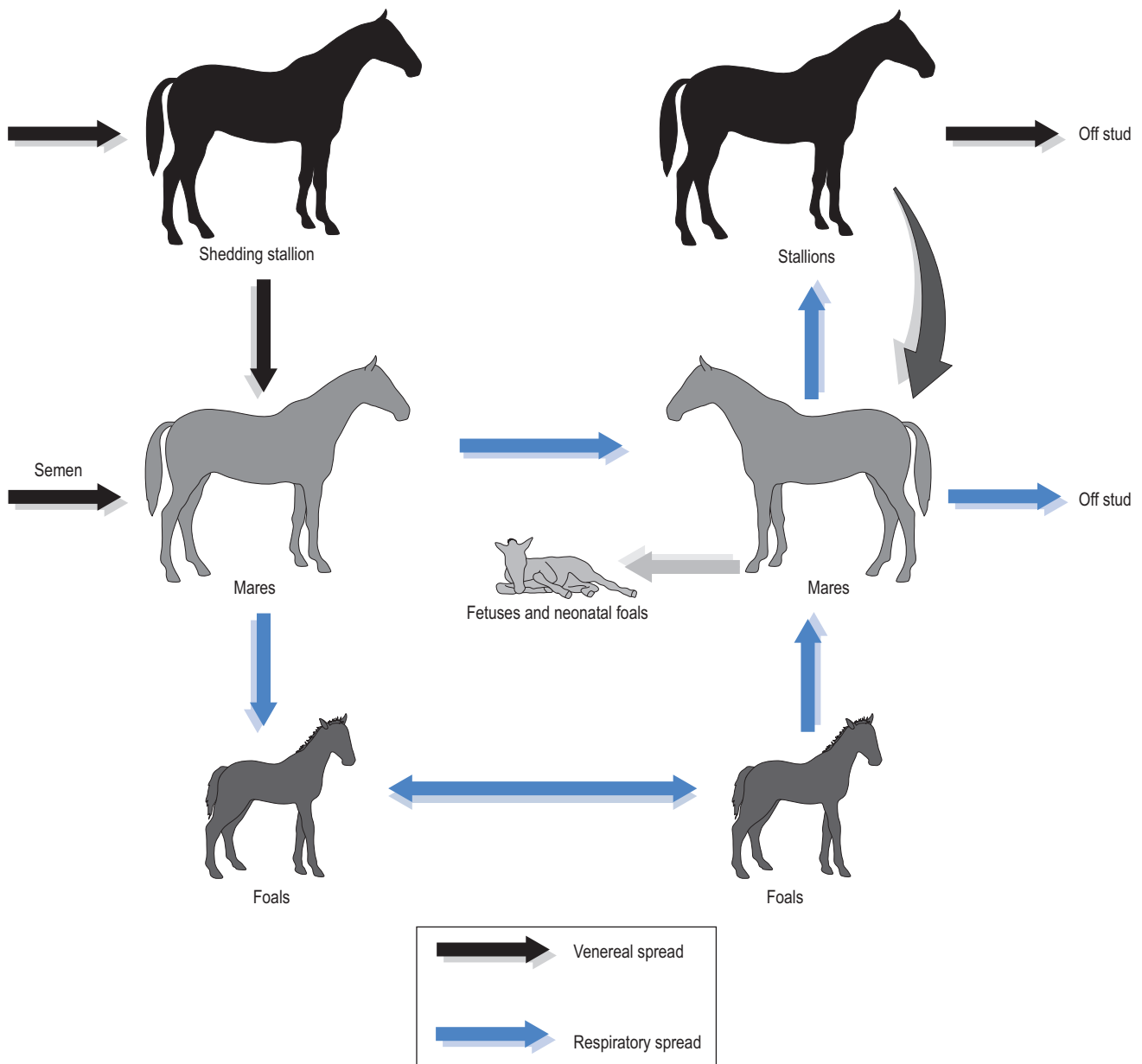
Fig. 22.12. Representation of the typical spread of EAV on a stud farm.

There are marked differences between different breeds in the prevalence of infection and also between breeds in different countries. The two extremes are the thoroughbred, in which the infection is generally rare and fairly tightly controlled, and the standardbred, in which the infection is generally common and relatively uncontrolled. The infection has also been reported to be common in warmblood breeds. That these breed differences are frequently consistent between continents emphasizes the critical role played by reproduction in the spread of infection with EAV. Until recently, the infection had not caused many problems in major European thoroughbred populations, but subclinical outbreaks have occurred in the first few years of the 21st century in both France and Ireland (see below under Control). These outbreaks emphasize the importance of ongoing disease control and surveillance in preventing this infection.

Prevention and control

Prevention and control programs for EVA need to focus on the pivotal role that the shedding stallion plays in the transmission of this infection. Protecting stallions from infection can be based on vaccination, on quarantine of all animals having contact with stallions or on a combination of the two. The avoidance of natural covering by stallions
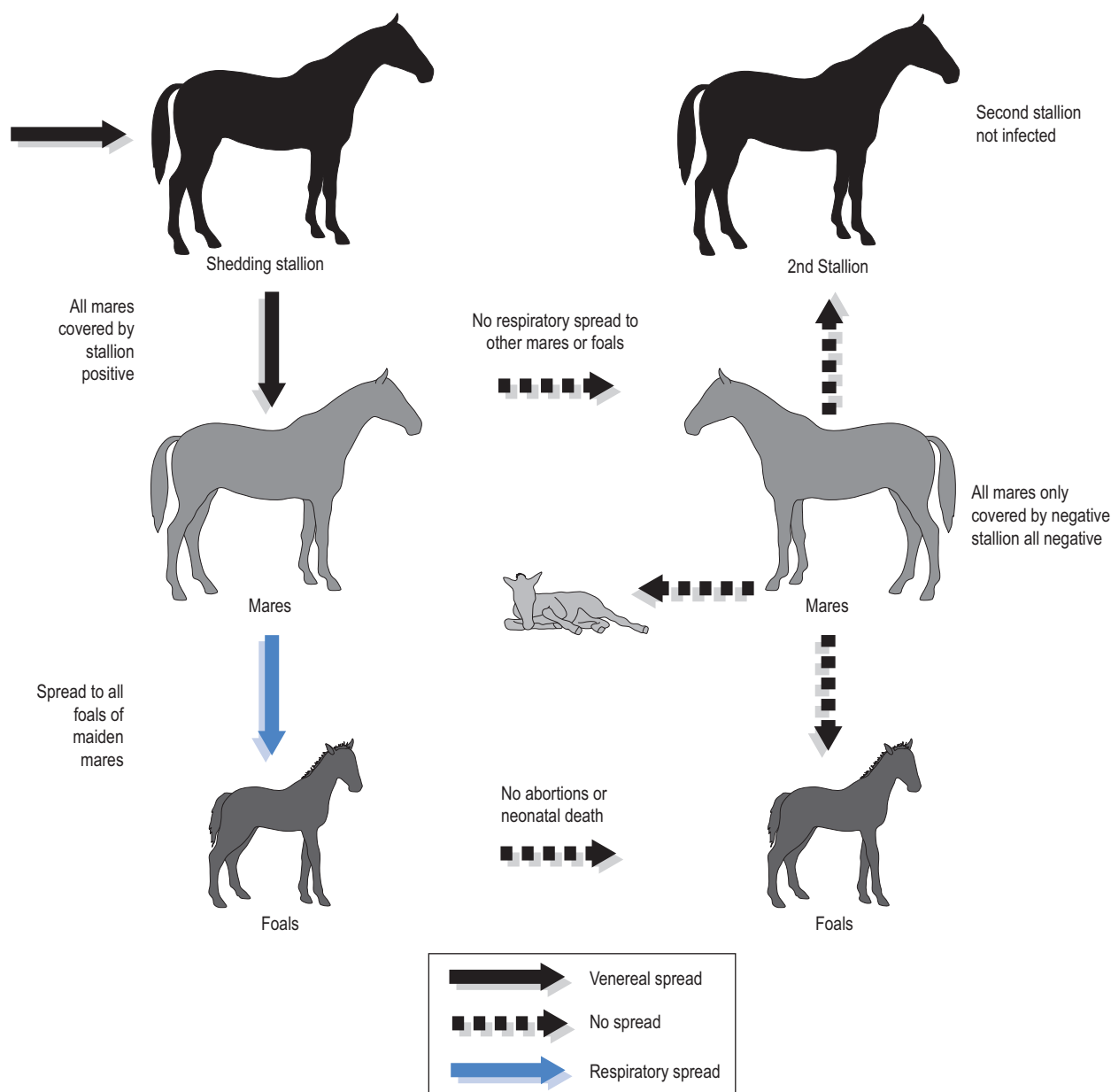
Fig. 22.13. Restricted pattern of spread of EAV on a warmblood stud farm.

through the use of artificial breeding techniques, so that stallions have no contact with incoming mares, is a highly effective means of preventing spread but is currently not possible in thoroughbreds. Considerable care should always be taken to ensure that stallions being used for artificial insemination are not infected; transmission of virus through semen is an entirely avoidable means of spreading the infection over particularly long distances.

Because of the role of stallions, extensive efforts are made by governments to ensure that infected stallions and semen are not traded between different countries. A major exception to this is the situation within the EU, in which intracommunity trade of EAV-infected semen and impor-

tation of infected stallions from outside the EU are prevented by legislation, but where there is no requirement to test stallions before movement within the EU. In addition, the disease is notifiable in Britain (only in stallions), Ireland, and other countries and statutory disease control procedures provide an important means of controlling animals and their owners when there is no compliance with voluntary control measures.

Vaccination

Tissue culture-adapted and formalin-inactivated whole virus vaccines have been used to control EAV infection. The

live vaccine was attenuated by serial passages in horse kidney (HK), rabbit kidney (RK) and equine dermal (ED) cells. Despite occasional isolation of the attenuated virus after vaccination (Fukunaga et al 1982, Timoney et al 1988), no reversion to virulence was observed after sequential passages of the vaccine virus in horses (Doll et al 1968, McCollum 1969). However, vaccination of pregnant mares is not recommended. The commercial live virus vaccine (passage history HK-131/RK-80/ED-24) and its less attenuated earlier versions have been shown to be effective for clinical protection (McCollum 1969, 1986, Timoney & McCollum 1988). These animals also demonstrated reductions in duration of virus excretion from nasal secretions and in duration of viremia.

An experimental, non-adjuvanted, formalin-inactivated whole virus vaccine was shown to stimulate high levels of VN antibody after repeated inoculations with high titer virus preparations (Fukunaga et al 1996, 1997). Clinical trials with this vaccine showed a reduction in clinical signs, nasal excretion of virus and viremia in vaccinated horses, which appear to correlate with the VN antibody titer at the time of challenge. In 1993, an adjuvanted killed virus vaccine was licensed in the UK under an Animal Test Certificate, based on some experimental efficacy data. It has subsequently been fully licensed in several European countries and has been widely used since then. No significant adverse effects have been detected with the widespread use of this product in stallions and there has been no evidence of vaccine breakdown, although it is unclear how often vaccinated animals have experienced field infections.

Both commercially available products have principally been used in stallions and a particular challenge arises with the vaccination of stallions with them. These animals develop circulating neutralizing antibody that is indistinguishable from that induced by infection. Therefore, all stallions to be vaccinated should have a sample taken to demonstrate that they are seronegative at the time of vaccination. Wherever possible, they should then be boosted at least annually and possibly, in the case of those receiving the whole virus inactivated product, every 6 months for the first few years (J. Cardwell, J.R. Newton and J. Wood, unpublished observations). Ideally, they should also be serologically monitored to ensure that they have not become infected under the cover of vaccination. Most trading countries will accept vaccinated stallions if they have been shown to be seronegative at the time of first vaccination and then regularly boosted, although some still require testing of semen samples for virus in all seropositive animals.

The development of a subunit vaccine for EAV would enable differentiation between vaccinated and infected animals. Immunodominant proteins of EAV have now been identified and bacterially expressed glutathione *S*-transferase (GST) fusion proteins of ORFs 2b–7 have been screened by ELISA with EAV-positive equine sera; G_L has been demonstrated to be the most immunoreactive polypeptide (Chirnside et al 1995). A fusion protein and a synthetic peptide, both derived from G_L, induce neutralizing antibodies when injected into rabbits and ponies, thus demonstrating the potential of the subunit approach. Further studies demonstrated that other G_L-based antigens also induced strong neutralizing antibody responses and could confer protection in ponies against experimental infection (Castillo-Olivares et al 2001). The combined use of the G_L-based subunit vaccine and a differential diagnostic test against EAV would allow the discrimination of vaccinated from naturally infected horses. Other EAV antigens, such as N and M, are frequently recognized by sera from EAV-infected horses and initial steps to develop ELISA diagnostic tests based on these antigens have been taken (MacLachlan et al 1998); so a G_L-based subunit vaccine could be generally administered to horse populations without compromising disease surveillance by serological screening. Such an approach should be very useful in those areas of low EAV prevalence, or for international trade or for the control of outbreaks of EAV by enabling the protection of susceptible populations by vaccination and, at the same time, permitting the surveillance of disease progression by serological testing.

Control

Most attention is given to the prevention of EVA on stud farms because of the huge costs that EAV can cause in this setting. Using artificial insemination to prevent contact between uninfected stallions and visiting brood mares can be a most effective means of protecting stallions, but is not currently possible in registered thoroughbreds. Protection of stallions through vaccination is the cornerstone of many stud-based control programs. Serological and clinical surveillance in brood-mare populations is also vital to protect studs that use covering from potential challenges to their stallions and from outbreaks of abortion. A detailed description of the formal disease prevention program employed in Europe is in the HBLB Code of Practice, along with a program for EHV-1 (www.hblb.org.uk).

Briefly, it is recommended that all mares should be screened serologically within 28 days of mating. Seropositive animals, if not previously identified as such, should be isolated and retested 14–28 days later to determine if the infection is still active. If the animal was managed as part of a group, then investigations in this group may at times be necessary. Animals being imported from higher risk areas should be placed in isolation on arrival and tested serologically after 14 days. Stallions should preferably be vaccinated. Where unvaccinated, they should be screened at least annually at the start of the breeding season. Where vaccinated they should be boosted at least annually and, preferably, serologically monitored for changes in titer as well.

Considerable care should be taken to ensure that stallions being brought on to premises for either natural

covering or semen collection are not infected before arrival. Animals being used for semen collection should not be mixed with others of lower health status after their arrival on the collection station. Frozen semen can be proven free from infection by showing that stallions are seronegative both before and 28 days after the last date of collection. Chilled semen is harder to certify, but stallions should be shown to be uninfected within 2 weeks of collection, having been kept in isolation during this period.

Despite the existence of these control programs, outbreaks of EAV infection can still occur. For example, extensive and entirely subclinical spread of EAV occurred in several studs in Normandy in northern France in 2000, resulting in at least two thoroughbred stallions seroconverting; one of these stallions became a long-term shedder of the virus and has subsequently had to be used under stringent restrictions. Less information has been made available on some EAV infections in the thoroughbred population in Ireland in 2003 when a problem became apparent after a prospective "shuttle" stallion was routinely tested in late summer before export and was found to be unexpectedly EAV seropositive. Further serological screening demonstrated evidence of subclinical infection on at least three stud farms, but in contrast to the French outbreak, no stallions were reported to have become shedders.

This increasing number of EVA outbreaks reported in Europe, and those that continue elsewhere, identify that the use of high value (in economic or breeding terms) stallions that have unfortunately become shedders must be considered. There is extensive experience in the USA (Timoney & McCollum 1991) and increasingly elsewhere as well, that suggests that, with care and considerable attention to biosecurity, these animals can be used safely with no general threat to the equine population. Mares should be seropositive prior to covering and should be kept in strict isolation for at least 28 days after covering.

As we move forward in this new millennium, recent EVA outbreaks serve to remind us that the international movement of horses or their semen still poses a real threat. The situation for EVA is exacerbated because different nations currently have very different attitudes to this disease.

Equine Rhinitis Viruses

Historical context

Equine rhinitis virus A and B, previously classified as equine rhinovirus-1, -2, and -3, are common in horse populations but knowledge of their epidemiology, pathogenesis and association with disease is rather limited. The viruses have recently been reclassified and other equine picornaviruses, such as "acid-stable picornavirus" (ASPV), may yet also be reclassified.

Virology and immunity

Several different picornaviruses have been isolated from horses (Plummer 1962, Studdert & Gleeson 1977, Mumford & Thomson 1978, Fukunaga et al 1983) and most have been rhinoviruses, isolated from the respiratory tract, mouth, blood, and feces.

The rhinoviruses were originally grouped into three categories, equine rhinovirus-1 (ERV-1), ERV-2 and ERV-3. ERV-1 has now been reclassified as equine rhinitis A virus (ERAV) (Varrasso et al 2001). ERV-2 has been renamed equine rhinitis B virus (ERBV) and has been reclassified as an Erbovirus, a new genus in the Picornaviridae (Hinton & Crabb 2001). ERV-3 has also recently been classified as an Erbovirus and named ERBV2 in view of its close sequence homology with ERBV (Huang et al 2001). ASPV, which shares the properties of enteroviruses and rhinoviruses, has not been reconsidered in recent years.

Infection with ERAV stimulates a long-lasting neutralizing antibody response which is thought to prevent further disease, although repeated infections in racehorses in training can occur (Mumford 1994). Infection with ERAV does not stimulate cross-reactive neutralizing antibody to either ERBV or ASPV (Mumford & Thomson 1978, Steck et al 1978, Mumford 1994). There is little information on cell-mediated immune responses to other equine picornaviruses.

Clinical signs and pathogenesis

Both natural and experimental infection of seronegative horses with ERAV has been associated with pyrexia (<40.4°C) for up to 5 days, nasal discharge and swelling of retropharyngeal lymph nodes and a moist cough (Plummer 1962, Plummer & Kerry 1962). Natural infections may frequently be subclinical, particularly in seropositive animals (Studdert & Gleeson 1978).

Although ERBV infections may sometimes cause slight pyrexia and mild respiratory signs (Steck et al 1978), infections are usually subclinical and infection of gnotobiotic foals failed to induce clinical signs of respiratory disease (Mumford & Thomson 1978). There is little or no information on the ability of ERBV2 to cause disease.

ASPV was initially isolated from a thoroughbred racehorse in training, although it was not showing signs of respiratory disease at the time (Mumford & Thomson 1978). Experimental infection of a gnotobiotic foal failed to produce signs of respiratory disease, but seroconversion did occur and virus was recovered from the animal. Serological investigations of naturally occurring outbreaks of respiratory disease have produced confusing results and there is little evidence that this virus is a cause of equine respiratory disease, although the very limited amount of work reported precludes firm conclusions from being drawn.

Diagnosis

Equine rhinitis virus infections may be diagnosed by isolation of virus in tissue culture, detection of viral RNA by PCR (Li et al 1997) or through demonstration of viral seroconversion using either CF or VN tests (Mumford & Thomson 1978).

Epidemiology

ERAV has a worldwide distribution. Infection can spread by respiratory contact and outbreaks of disease associated with ERAV have been reported (Mumford 1994, Li et al 1997). Prolonged excretion of ERAV in urine in racehorses is common, particularly in 2- and 3-year-olds (McCollum & Timoney 1992) and is probably an important source of infection.

Many foals in the UK remain seronegative to ERAV and racehorses usually experience their first ERAV infection in their second or third year, whilst in training (Powell et al 1974). A similar situation has also been reported elsewhere, including North America (Holmes et al 1978). During outbreaks, ERAV is highly contagious and transmission rates in naive populations may approach 100% (Holmes et al 1978, Studdert & Gleeson 1978). Initial infections are usually associated with clinical signs (Burrows 1979, Li et al 1997, Klaey et al 1998), but repeated infections with ERAV, detectable in racehorses using the CF test (Mumford & Thomsen 1978), are frequently not associated with signs of disease. Most foals become infected with ERBV during the first few months of life (Holmes et al 1978, Burrows 1979) and repeated infections are frequently observed. It is clear that infections with ERBV and ERBV2 are common in Australia, where they have been most often studied (Huang et al 2001). Most infections are not associated with obvious clinical signs.

Prevention

No commercially available equine rhinovirus vaccines currently exist. However, ERAV infection stimulates strong clinical immunity and immunization with inactivated virus stimulates VN antibody and protection against experimental infection (Burrows 1979).

As a result of the lack of commercially available vaccines, controlled infection programs with ERAV in racehorses have been suggested as a means of ensuring that disease does not occur shortly before important race meetings, but so far these have not been reported (Klaey et al 1998).

No information is available on stimulation of immunity to ERBV or ERBV2, but natural infection is often associated with prolonged excretion (Fukunaga et al 1983) and virus neutralizing antibody levels are not maintained at high levels, suggesting that natural immunity may be poor.

Hendra Virus

Historical context

In September 1994 there was an outbreak of acute severe respiratory disease in racehorses stabled in the Hendra suburb of Brisbane, Queensland, Australia. During the outbreak, which lasted around 20 days, 18 horses were clinically affected and 14 died or were euthanased. Two human contacts, the trainer (who later died of a pneumonic condition) and a stable hand, became ill, with the latter subsequently slowly recovering (Selvey et al 1995). Following notification to the Department of Primary Industries, a veterinary team conducted an exhaustive investigation of the outbreak. Quarantine and movement restrictions were immediately implemented, all horseracing in Queensland was halted, and all equine deaths were made notifiable (Mackenzie et al 2003). The investigation did not demonstrate infection with any known equine infectious agents, but quickly identified a previously unrecognized virus (initially termed equine morbillivirus and now referred to as Hendra virus – HeV).

A program of extensive experimental and *in vitro* studies, many conducted at the Commonwealth Scientific and Industrial Research Organization (CSIRO) and complicated by the extensive care required when working with such dangerous category 4 pathogens in large animals, have fulfilled Koch's postulates and confirmed the etiology (K Murray et al 1995, 1998, Hooper et al 2001). Detailed epidemiological studies have demonstrated that this is a rare, incidental infection in horses, being endemic in fruit bats in the region (Westbury 2000). Only a few other cases have been diagnosed in either horse or humans since that time, but far more extensive outbreaks of disease caused by the closely related Nipah virus in pigs and people have occurred over Southeast Asia.

Virology

Initially described as a morbillivirus, the viruses isolated from both horses and people in the initial outbreak were shown to be closely related on the basis of two-way cross-neutralization assays (K Murray et al 1995). Hendra and the related Nipah virus have now been classified in the Henipah genus of the Paramyxoviridae as they were not typical of the morbillivirus group. The viruses are pleomorphic and enveloped, with two or three glycosylated transmembrane proteins. The viruses have single-stranded RNA with negative polarity, of 5×10^6 to 7×10^6 b. p. (base pairs), which can encode 10–12 proteins. The function of these are not described here, and readers are referred to more detailed descriptions (Rodriguez & Horvath 2004). The virus will grow in a wide variety of different cell types. Virions are labile and are rapidly inactivated by heat, lipid solvents, and detergents. HeV shares between 80 and 90% sequence homology with the closely related Nipah virus.

Clinical signs and pathogenesis

Following a typical incubation period of 8–11 days, not extending beyond 16 days, similar clinical signs develop in both the natural disease and that induced by either inoculation of laboratory-grown virus or lung from field cases. These signs consist of a high temperature (up to 41°C) with anorexia and depression, accompanied by signs of a primary viral interstitial pneumonia including hyperpnea and dyspnea, sweating, congested mucous membranes, and copious frothy nasal discharge. Ataxia may be present, along with head pressing in terminal cases and subcutaneous edema in a few animals (K Murray et al 1995, 1998). Death often rapidly ensues, although the outbreak in Hendra was also associated with significant levels of subclinical infection.

The primary pathology in horses is in the lung, where massive subpleural edema and distension of lymphatics accompanies the interstitial pneumonia, along with thoracic and pericardial fluid effusion. Virus can be found in blood vessel walls and in pulmonary epithelium; pulmonary capillary endothelium is extensively damaged, leading to the edema, and giant cell syncytia are present in lung capillaries and arterioles. More detailed reviews of pathology in horses and cats are available (Hooper et al 2001).

Diagnosis

The infection can be diagnosed by detection of the virus in tissue culture, detection of viral RNA by RT-PCR (Guillaume et al 2004), demonstration of typical histological lesions, supported by immunoperoxidase staining or by demonstration of seroconversion. A VN assay was used in all initial investigations, but an ELISA has been used more recently, particularly for screening of healthy animals for international trade purposes.

Diagnosis should not be attempted by field veterinarians and suspicion of disease should always be notified to competent authorities because of its extremely high case fatality rate in human contacts.

Epidemiology

Since the original outbreak, very few other cases have been diagnosed in horses or humans, despite extensive retrospective and prospective investigations. In October 1995 a farmer in northern Queensland, 1000 km from the original outbreak, died in hospital following a progressive neurological illness associated with Hendra virus infection. In August 1994 the farmer had assisted with necropsies on two horses, diagnosed then as poisoning but retrospectively diagnosed with HeV infection. He had suffered mild meningoencephalitis soon after but had recovered without apparent complication before later relapsing. A further case of equine HeV infection occurred in January 1999 in Australia with the death of an adult thoroughbred mare within 24 h of becoming ill. There was no evidence of spread of infection to another mare in the same paddock in a residential area of Cairns and no other contact with other horses.

Investigations that followed the earlier outbreaks of HeV demonstrated no evidence of infection in any other people or horses, suggesting that a wildlife reservoir might exist. After investigation of many other species, a seroprevalence of >20% was demonstrated in pteroptid bats in eastern Australia (Halpin et al 2000). Three species of these large frugiverous bats, otherwise known as fruit bats or flying foxes, are found around coastal regions of Australia and they are generally assumed to be the reservoir hosts of this virus. Detailed understanding of its persistence in bats does not exist, but the isolation of virus from bat uterine fluids (Halpin et al 2000) and the demonstration of vertical transmission in bats (Williamson et al 1998) have led to suggestions that transmission of infection may occur around birth. These animals often give birth to their young on the ground and it may be that inquisitive horses come into contact with infection at this time.

Field investigations, supported by experimental studies, have demonstrated that the virus is not highly contagious between horses. Transmission of the virus between horses generally required close contact, as it did for transmission between horses and people. Cats can be infected experimentally and can transmit the infection to horses. Importantly, infection occurs in the kidneys of many infected horses and virus can be detected in urine (Williamson et al 1998). Infection did not spread from experimentally infected grey-headed fruit bats to horses in contact with them, but the bats were not breeding at the time.

These studies and observations demonstrate that, as the disease is not very contagious, it should generally be easy to control, but they emphasize the care that must be taken in managing cases and suspect cases and their fomites and bedding. A greater challenge is to improve our understanding of the transmission of HeV in fruit bats and how this might be controlled.

Prevention and control

Fruit bats are common in Australia and the high seroprevalence of HeV in them suggests that elimination from this wildlife reservoir would be problematic. While stabling of horses during the fruit bat reproductive season can be proposed in theory, it is hard to envisage how this can be achieved in practice. Nonetheless, it is important to note the rarity of transfer of the infection to horses. It is critical that clinical cases are diagnosed at the earliest possible stage so that appropriate precautions can be taken for handlers and veterinary staff (Mackenzie et al 2003). Diagnosed cases of HeV infection in horses should be destroyed because of the unacceptable human health risks of managing such animals in the field.

Control procedures implemented in Europe after the initial outbreaks included a demonstration that horses traveling from Australia, particularly from the northeast, should be demonstrated to be Hendra-seronegative within 2 weeks of travel. This requirement has now lapsed and the generally high clinical expression of infection and the short incubation period provide a reasonable safeguard that subclinically affected horses do not travel.

The control of the Hendra outbreaks and the subsequent investigations provide an excellent example of how a novel and fatal viral zoonosis can be effectively investigated collaboratively between veterinary and medical specialists and then controlled and monitored.

Parainfluenza-3

Clinical signs

Parainfluenza-3 (PI3) was first isolated from a horse in Canada in 1961 (Ditchfield et al 1963). The virus was associated with an outbreak of severe upper respiratory disease in one group of horses. Temperatures were raised (38.6–39.7°C) and these pyrexias were accompanied by anorexia, seropurulent nasal discharges, and dyspnea. Conjunctivitis was seen in 22 of 48 affected animals and bronchitis was observed in five. In most cases the disease was self-limiting within 7–9 days. Since then, there have been few reports of any association between this virus and respiratory disease in horses, despite several reports of serological evidence of infection. PI3 was isolated from horses with lung disease in China (Zhu et al 1989).

One study of respiratory disease in children found that specific degenerative changes of respiratory epithelium, termed ciliocytophthoria, were a sensitive and specific indicator of PI3 infection (Naib et al 1968). These findings have been extrapolated to the horse and ciliocytophthoria, which is not uncommon in both acute (Whitwell & Greet 1984) and chronic (Beech 1975) respiratory disease in the horse, is now regarded by many as indicative of "viral" infection (Beech 1975, Whitwell & Greet 1984), even though it appears to be a specific feature of PI3 infection in humans and it has never been associated with PI3 infection in the horse.

Diagnosis

PI3 infection may be diagnosed by isolation of virus from respiratory secretions or through demonstration of seroconversion (Ditchfield et al 1963). The initial virus isolates were obtained with the use of primary rhesus monkey kidney cell lines. The HI test is the most commonly used serological test in the horse (Ditchfield et al 1963).

Epidemiology

In the initial outbreak in Canada, infection was widespread and antibody to PI3 was found in all adults in the affected group. There is a marked contrast between several serological studies of respiratory disease in horses. Several studies have reported a PI3 seroprevalence of 0% in racehorses (Canadian standardbred racehorses and thoroughbred racehorses in Chile and Japan). In contrast, a seroprevalence of 3–33% has been reported in racehorses and riding horses in different parts of Europe. No serological studies of PI3 infection in horses in the UK have been published and given the widespread occurrence of this virus in other species, further studies of this infection in horses are warranted.

African Horse Sickness

Historical context

African horse sickness (AHS) is typically a peracute or acute infectious, but non-contagious, disease of Equids transmitted by *Culicoides* midges. The outcome is highly dependent on the equid species infected and while the mortality rate in naive horses is between 70 and 95%, it varies between 50 and 70% in mules and is very low in South African donkeys, although donkeys from the Middle East may experience a mortality rate of up to 10% (Coetzer & Erasmus 1994). Clinical signs are not generally recognized in zebras. Rates of transmission through South African donkeys and zebras, in which the infection is usually subclinical, can however be high (Lord et al 1997a, 2002).

AHS is common in most countries in sub-Saharan Africa and is the only OIE list A disease of horses. AHS causes sporadic, but socio-economically devastating, epidemics in other countries such as Morocco and Ethiopia (Coetzer & Erasmus 1994). It is an important reason why Equids have not been more widely used as draught animals in Africa and so continues to have significant socio-economic impact in the African continent. Also, many affected horses have considerable monetary value either as performance horses or in their use in other forms of recreation. Furthermore, exportation of horses from areas where AHS occurs can only be accomplished, if at all, following strict quarantine and testing procedures. The virus caused massive mortality in the 19th century in imported horses, with outbreaks in 1854/5 and 1891/2 being associated with the deaths of 70,000 and 25,000 horses respectively.

Major outbreaks of the disease outside the hyperendemic areas occurred in Egypt in 1928, 1943, 1953, 1958, and 1971, in Yemen in 1930, and across the Middle East in 1944. In 1959, an outbreak caused by serotype 9 occurred in Iran and between 1960 and 1961 this spread through the Persian Gulf area into Afghanistan, Pakistan, India and Turkey, killing in excess of 300,000 animals (Coetzer & Erasmus 1994). The disease occurred in several North African countries in 1965, spreading across the straits of Gibraltar into Spain in 1966. Further outbreaks

occurred between 1987 and 1991 in Spain, Portugal, and Morocco, the infection having been introduced into the region by a group of zebra from Namibia (Lubroth 1988, Hamblin et al 1991b). The disease also occurred in Saudi Arabia in 1989, after an absence of 30 years (Mellor et al 1990).

Virology

AHS virus (AHSV) is in the genus Orbivirus in the family Reoviridae. It shares many properties with other orbiviruses such as bluetongue virus and equine encephalosis virus and is a double-stranded RNA unenveloped virus with a segmented genome. The viruses are highly resistant to disinfection and other forms of inactivation.

There are nine serotypes of AHSV, the last of which was isolated in 1960 (Howell 1962). Serotype is determined by the surface glycoprotein VP3. Antisera raised to homologous virus may cross-neutralize different serotypes, although the extent of cross-protection in natural mammalian hosts, other than the zebra (Lord et al 1997b), has not been formally evaluated to any extent. The cross-neutralization between antisera produced against different strains of orbiviruses depends to a great extent on the species in which the antisera have been produced (Howell 1962, Howell et al 2002). In AHSV, there is cross-neutralization between serotypes 6 and 9, 5 and 8 and, to a lesser extent, between strains 1 and 2 and strains 3 and 7 (Coetzer & Erasmus 1994). These cross-reactions can complicate the interpretation of postinfection serology, especially after infection with a number of different serotypes. Clinical evidence suggests that there is a lack of intratypic variation, but this has not been evaluated formally either through molecular or serological means.

Clinical signs and pathogenesis

The incubation period following AHSV infection in susceptible hosts is typically 5–7 days. The clinical signs are markedly affected by the host species and its immune status. Experimentally, the incubation period may vary between 2 and 10 days and disease severity depends markedly on virus dose and virulence. There are four different forms of the disease: the pulmonary form ("Dunkop"), the cardiac form ("Dikkop"), the mixed form, and horsesickness fever. Although the mixed form is probably the commonest, it is rarely diagnosed because pulmonary or cardiac signs usually predominate in individual animals (Coetzer & Erasmus 1994).

After inoculation by biting *Culicoides*, initial multiplication occurs in the regional lymph nodes, followed by a disseminating viremia with subsequent infection of target organs (Mellor & Hamblin 2004). Replication of AHSV in these organs, particularly in capillary endothelium, is associated with a secondary cell-associated viremia of variable duration and with clinical signs. Much of the pathology results from increased permeability of capillary

endothelium (Mellor & Hamblin 2004). Virus strains vary in their organ tropisms (Gomez-Villamandos et al 1999).

In the pulmonary form, a fever (up to 41°C) lasting up to 2 days is followed by development of severe dyspnea, paroxysmal coughing, and a copious nasal discharge of frothy, serofibrinous fluid (Coetzer & Erasmus 1994). The disease is characterized by a massive pulmonary edema and hydrothorax, often with several liters of fluid accumulating. Death can occur within a few hours of onset of signs and less than 5% of animals with this form, which is the typical form in young susceptible animals, survive. The cardiac or edematous form is chiefly characterized clinically by subcutaneous swelling of the neck and head, particularly the supra-orbital fossa. At post-mortem, yellow gelatinous edema of subcutaneous and intermuscular connective tissues is the most obvious feature. Microscopic pathology in cardiac tissues can be hard to detect, other than for serotype 9, where foci of myocyte degeneration and necrosis occur. In more severe cases, in which mortality is more likely, all cranial soft-tissue structures are affected, along with the shoulders, neck, and chest. Petechial hemorrhages are a poor prognostic indicator. Mortality from the cardiac form, which is milder than the pulmonary form, is around 50% (Coetzer & Erasmus 1994).

Horsesickness fever is the most common form in horses with pre-existent immunity to one or more serotypes. It is the mildest form of the disease and is probably very underdiagnosed. It is characterized by a pyrexia (39–40°C) lasting 1–6 days and some edema of the supra-orbital fossa. The pyrexia may also be accompanied by transient loss of appetite, slight dyspnea, and increased heart rate.

Diagnosis

In most cases, in endemic or epidemic areas, clinical diagnosis of AHS will often be correct. Confirmation of diagnosis of AHS, as for most viral diseases, can be accomplished by either serological means detecting rises in antibody levels, or by virus detection through isolation or the detection of specific viral RNA using PCR. In AHS, serologic methods commonly used in diagnostic laboratories include a group-specific ELISA which enables detection of antibodies following infection with any of the nine viral serotypes (Hamblin et al 1990, House et al 1990) or through specific serotype VN assays (Howell 1962, House et al 1990). Virus can be isolated from tissues or blood samples from acutely affected animals using a variety of different cell culture systems (e.g. BHK or Vero) and characterized in neutralization or other assays (House et al 1990, Coetzer & Erasmus 1994); for example antigen detection ELISA (Hamblin et al 1991c) and PCR assays have been reported which are either group-specific (Zientara et al 1993, Sailleau et al 1997) or serotype-specific (Sailleau et al 2000). AHSV can also be detected in midge species by virus isolation or ELISA (Hamblin et al 1991a).

Postinfection serology has been used in epidemiological investigations in donkey (Hamblin et al 1991b, Williams et al 1993, el Hasnaoui et al 1998, Lord et al 2002) and zebra (Barnard & Paweska 1993, Lord et al 1997a) populations, although the cross-reactions between serotypes can complicate the interpretation of the results, particularly for the more cross-reactive types (Lord et al 1997a, 2002).

Epidemiology and prevention

In addition to the sporadic, but internationally reported, epidemics of AHS, cases in horses occur every year in South Africa, despite the practice of widespread vaccination with a polyvalent attenuated vaccine (Coetzer & Erasmus 1994). There was a major outbreak caused by serotype 2 in the Transkei region of the Eastern Cape Province from November 2000 to May 2001, in which over 1,000 horses died. These horses were in poor rural areas, where they were typically used for transport and work, and the outbreak had a substantial economic impact.

The distribution and spread of AHSV is determined to a great extent by the distribution of its insect vector. In most parts of Africa and southern Europe, there is a large body of evidence that indicates that the major role is played by *Culicoides imicola* (Coetzer & Erasmus 1994, Mellor & Boorman 1995), as is also the case for the closely related bluetongue virus (Baylis et al 2001, Purse et al 2005). Extensive work has had considerable success in predicting the distribution of *C. imicola*, and hence of AHS and bluetongue, from satellite data (Baylis et al 1997, 1998a, 2001, Rawlings et al 1997, Purse et al 2005). The distribution of this species is dependent to a great extent on the phase of the annual "normalized difference vegetative index" (NDVI) cycle (Baylis et al 2001). NDVI is a composite measure and is influenced by a variety of factors, including soil type, rainfall, and environmental temperatures.

Investigations of some recent outbreaks of AHS in South Africa have implicated *C. bolitinos* in transmitting some strains of AHSV (Meiswinkel & Paweska 2003) in regions where *C. imicola* was absent. There is less published material on the usefulness of satellite imagery to predict the distribution of *C. bolitinos* in South Africa, or of the more recently identified northern European bluetongue vectors *C. pulicaris* and *C. obsoletus* (Capela et al 2003, Caracappa et al 2003, Purse et al 2005).

The transmission of orbiviruses by *Culicoides* is under genetic control and is strongly influenced by environmental temperatures, because of the requirement for virogenesis in the vector after feeding on an infected mammalian host, followed by feeding again on another susceptible host (Mellor et al 1998). The temperature dependence of the virogenesis in bluetongue virus is affected by the virus serotype and the species of *Culicoides* (Paweska et al 2002). For AHS and bluetongue, both the infection rate of midges

and the rate of virogenesis are temperature dependent (Mellor et al 1998, Paweska et al 2002). AHSV serotype 4 replication is low at temperatures <15°C. While the vector's lifespan is extended at lower temperatures (Mellor et al 1998) or at lower night-time wind speeds (Baylis et al 1998b), the rate of virogenesis decreases so that at environmental temperatures of 10°C virus becomes undetectable, although there is some, apparently non-replicative, persistence of virus at these temperatures (Mellor et al 1998). If the ambient temperature increases, the virus will replicate and vectors will be able to transmit the virus. The rates of infection and titers of infectivity of different *Culicoides* spp. vary substantially and are also affected by the strain and specific isolate of virus (Venter et al 1998); such variability is likely to exist for AHSV but has not yet been characterized.

Quantitative studies of the epidemiology of AHS in different provinces of South Africa have produced estimates of the "force of infection" of AHSV serotypes as a whole, for two separate time periods (Lord et al 1996). However, there are no published estimates of serotype-specific transmission rates or of forces of infection, despite these being fundamental measures (Lord et al 1997b) and despite there being evidence that transmission may vary between serotypes (Lord et al 1998). Elegant, deterministic mathematical models of large-scale AHSV transmission, which included the role of two mammalian hosts (horse and donkey) and one vector species (*C. imicola*) in viral transmission, have been published (Lord et al 1996, 1997b).

Control is currently hampered by an insufficient understanding of the quantitative aspects of transmission that are required for scientifically based control programs. For example, given sometimes large populations of donkeys and the fact that donkey populations can experience high rates of AHSV infection (Lord et al 2002), immunization of donkeys (Lord et al 1997b, 1998, el Hasnaoui et al 1998, Hamblin et al 1998) has been suggested as critical in the success of vaccination (to eradicate) programs. Despite this, donkeys have largely been ignored in previous epidemics in non-endemic areas, probably because of their low economic value and the fact that they are clinically unaffected by AHS and the uncertainty of the precise role that they can play in the epidemiology of AHS.

Recent spread of the closely related bluetongue virus through the Mediterranean area (Mellor & Wittmann 2002, Purse et al 2005) has been associated with remarkable and rapid spread of the shared primary insect vector, transmission by novel vectors and overwintering in new northern regions. This experience suggests that AHS may also cause unforeseen problems in these regions and that efforts should be made now to understand and quantify the transmission of the virus and manage its control (Anon 1997, The Royal Society 2002).

As is the case for bluetongue virus, the only currently available vaccine against AHSV is a live attenuated vaccine. As a result of the possible reversion to virulence in

vaccinated animals and transmission by vectors and the possible reassortment with field virus, this is not the ideal vaccine for use in naive areas. An inactivated monovalent vaccine was used as an adjunct to control during the most recent outbreak in southern Europe. Currently, however (as of 2006), the license for this product has lapsed and it is no longer commercially available in Europe.

Management factors that reduce exposure to midge bites are a most effective means of preventing the disease in horses, and stabling without the use of lights at night has long been recognized to be highly effective in areas where *C. imicola* is implicated in the transmission, even if the stabling is open and not insect proof. However, the feeding behavior of northern European *Culicoides* species may differ and this means of prevention may not be as effective in non-*C. imicola* areas.

Adenovirus

There are several reports of the isolation of equine adenovirus from foals (Wilks & Studdert 1972, Studdert et al 1974, Dutta 1975, Whitlock et al 1975, Konishi et al 1977) and, less frequently, from adult horses, with pneumonia or signs of upper respiratory disease (unpublished observations). In some reports, the infection has been associated with diarrhea (Studdert & Blackney 1982) or a loosening of fecal material (Powell et al 1974). Generally, all isolates have been remarkably antigenically similar (Studdert 1978), but one study reported the isolation of a distinct isolate from cases of diarrhea (Studdert & Blackney 1982). The typical isolates are referred to as equine adenovirus type 1 and the isolate from cases of diarrhea is referred to as equine adenovirus type 2.

The infection can be diagnosed by isolation of virus, detection of viral DNA, or by demonstration of seroconversion using serum neutralization tests or HI assays.

Equine adenovirus is a very important cause of morbidity and mortality in Arabian foals suffering from severe combined immunodeficiency (SCID) (Whitlock et al 1975, Thompson et al 1976, Perryman et al 1978). In addition, the virus has been isolated from the spinal cord of a few horses with cauda equina neuritis (Edington et al 1984), but its role, if any, in this rare disease remains unclear.

Experimental infection studies have demonstrated that the virus can cause signs of respiratory disease, including mild nasal discharge, rhinitis, tracheitis, bronchopneumonia, and an interstitial pneumonia (McChesney et al 1974, Pascoe et al 1974, Gleeson et al 1978). Signs of disease were worsened in colostrum-deprived foals and also diminished in foals aged > 2 months compared to those aged 48 hours. Clinical signs had largely resolved, even in colostrum-deprived animals, within 10 days (McChesney et al 1974).

Different serological surveys have demonstrated that antibodies to equine adenovirus-1 are common in many horse populations, often being close to 100% (Studdert

1996). Antibodies to equine adenovirus-2 also appear to be common (Studdert 1996). However, despite this, structured studies in both foals (Hoffman et al 1993, Dunowska et al 2002) and young thoroughbred racehorses (Burrell et al 1996, Christley et al 2001, Newton et al 2003, Wood et al 2005) have failed to demonstrate any association between clinical respiratory disease and the presence of adenovirus or seroconversion to this agent. Occasional outbreaks of respiratory disease or pyrexia in adult animals associated with adenovirus infection do undoubtedly occur, but, in the authors' experience, are not common.

Infection with equine adenovirus is common, but the virus does not generally appear to be an important cause of equine disease, particularly in the adult. The advent of genetic tests that have markedly reduced the numbers of Arab foals being produced with SCID reduces the apparent significance of equine adenovirus further.

Conclusion

This chapter illustrates the rapid advances that have been made in equine respiratory virology in the last decades, and identifies new challenges for the future. There is a need for improved, more rapid, and internationally validated diagnostic tests that will allow consistent and early diagnosis of disease and early implementation of control. Further improvements in our understanding of viral disease epidemiology, pathogenesis, virulence determinants, and correlates of immunity are also required. Such advances would enable better models of disease to be developed and subsequently lead to the marketing of effective and safer vaccines. Improved and multidisciplinary approaches to surveillance and control of equine diseases are also required, particularly in the light of the increasing international movement of horses and not least because of the potential for novel inter-species and zoonotic viral transmission. Key to all of these advances is good communication between all those with interests in the disease, from the clinician dealing with the suspect case and working with the official to prevent the disease in the population, through epidemiologists to laboratory diagnosticians and basic researchers who should provide the necessary tools to the pharmaceutical industry to develop the necessary commercially available products.

REFERENCES

Allen GP, Bryans JT, Pandey R 1986 Molecular epizootiology, pathogenesis, and prophylaxis of equine herpesvirus-1 infections. Veterinary microbiology molecular and clinical perspectives. Progress in Veterinary Microbiology and Immunology 2: 78–144

Allen G, Yeargan M, Costa LR et al 1995 Major histocompatibility complex class I-restricted cytotoxic T-lymphocyte responses in horses infected with equine herpesvirus 1. Journal of Virology 69: 606–612

Allen GP, Kydd JH, Slater JD et al 1999 Advances in understanding of the pathogenesis, epidemiology and immunological control of equine herpesvirus abortion. In: Proceedings of the Eighth International Conference on Equine Infectious Diseases, Dubai. R&W Publications Ltd, Newmarket UK

Anon. 1997 African horse sickness. Conclusions and recommendations of a scientific meeting. Bulletin of the Office of International Epizootics 4: 406–411

Balasuriya UB, Timoney PJ, McCollum WH et al 1995 Phylogenetic analysis of open reading frame 5 of field isolates of equine arteritis virus and identification of conserved and nonconserved regions in the GL envelope glycoprotein. Virology 214: 690–697

Balasuriya UB, Patton JF, Rossitto PV et al 1997 Neutralization determinants of laboratory strains and field isolates of equine arteritis virus: identification of four neutralization sites in the amino-terminal ectodomain of the G(L) envelope glycoprotein. Virology 232: 114–128

Barnard BJ, Paweska JT 1993 Prevalence of antibodies against some equine viruses in zebra (*Zebra burchelli*) in the Kruger National Park, 1991–1992. Onderstepoort Journal of Veterinary Research 60: 175–179

Baylis M, El Hasnaoui H, Bouayoune H et al 1997 The spatial and seasonal distribution of AHS and its potential *Culicoides* vectors in Morocco. Medical and Veterinary Entomology 11: 203–211

Baylis M, Bouayoune H, Touti J et al 1998a Use of climatic data and satellite imagery to model the abundance of *Culicoides imicola*, the vector of African horse sickness virus, in Morocco. Medical and Veterinary Entomology 12: 255–266

Baylis M, Touti J, Bouayoune H et al 1998b Studies of the mortality rate of *Culicoides imicola* in Morocco. Archives of Virology (Supplement) 14: 127–136

Baylis M, Wittman EJ, Mellor PS et al 2001 Prediction of areas around the Mediterranean at risk of bluetongue by modelling the distribution of its vector using satellite imaging. Veterinary Record 149: 639–643

Beech J 1975 Cytology of tracheobronchial aspirates in horses. Veterinary Pathology 12: 157–164

Blunden AS, Smith KC, Binns MM et al 1995 Replication of equid herpesvirus 4 in endothelial cells and synovia of a field case of viral pneumonia and synovitis in a foal. Journal of Comparative Pathology 112: 133–140

Borchers K, Wolfinger U, Lawrenz B et al 1997 Equine herpesvirus 4 DNA in trigeminal ganglia of naturally infected horses detected by direct in situ PCR. Journal of General Virology 78: 1109–1114

Breathnach CC, Yeargan MR, Sheoran AS et al 2001 The mucosal humoral immune response of the horse to infective challenge and vaccination with equine herpesvirus-1 antigens. Equine Veterinary Journal 33: 651–657

Browning GF, Ficorilli N, Studdert MJ 1988 Asinine herpesvirus genomes: comparison with those of the equine herpesviruses. Archives of Virology 101: 183–190

Bryans JT, Allen GP 1982 Application of a chemically inactivated, adjuvanted vaccine to control of abortigenic infection of mares by equine herpesvirus 1. Developments in Biological Standardization 52: 493–498

Bryans JT, Crowe ME, Doll ER et al 1957 Isolation of a filterable agent causing arteritis of horses and abortion by mares; its differentiation from the equine abortion (influenza) virus. Cornell Veterinarian 47: 3–41

Burrell MH, Wood JL, Whitwell KE et al 1996 Respiratory disease in thoroughbred horses in training: the relationships between disease and viruses, bacteria and environment. Veterinary Record 139: 308–313

Burrows R 1979 Equine rhinovirus and adenovirus infections. In: Proceedings of the 24th Annual Convention of the American Association of Equine Practitioners. St. Louis, MI, pp.299–306

Burrows R, Goodridge D, Denyer M S 1984a Trials of an inactivated equid herpesvirus I vaccine: challenge with a subtype 1 virus. Veterinary Record 114: 369–374

Burrows R, Goodridge D, Wittmann G 1984b Studies of persistent and latent equid herpesvirus 1 and herpesvirus 3 infections in the Pirbright pony herd. In: Gaskell RM, Rziha HJ (editors) Latent Herpesvirus Infections in Veterinary Medicine. Series: Current Topics in Veterinary Medicine and Animal Science, vol 27. Martinus Nijhoff, Dordrecht, pp.307–319

Capela R, Purse BV, Pena I et al 2003 Spatial distribution of *Culicoides* species in Portugal in relation to the transmission of African horse sickness and bluetongue viruses. Medical and Veterinary Entomology 17: 165–177

Caracappa S, Torina A, Guercio A et al 2003 Identification of a novel bluetongue virus vector species of *Culicoides* in Sicily. Veterinary Record 153: 71–74

Castillo-Olivares J, de Vries AA, Raamsman MJ et al 2001 Evaluation of a prototype sub-unit vaccine against equine arteritis virus comprising the entire ectodomain of the virus large envelope glycoprotein (G(L)): induction of virus-neutralizing antibody and assessment of protection in ponies. Journal of General Virology 82: 2425–2435

Castillo-Olivares J, Tearle, JP, Montesso F et al 2003a. Detection of equine arteritis virus (EAV)-specific cytotoxic CD8+ T lymphocyte precursors from EAV-infected ponies. Journal of General Virology 84: 2745–2753

Castillo-Olivares J, Wieringa R, Bakonyi T et al 2003b Generation of a candidate live marker vaccine for equine arteritis virus by deletion of the major virus neutralization domain. Journal of Virology 77: 8470–8480

Chambers,TM, Holland RE, Tudor LR et al 2001 A new modified live equine influenza virus vaccine: phenotypic stability, restricted spread and efficacy against heterologous virus challenge. Equine Veterinary Journal 33: 630–636

Chirnside ED, de Vries AA, Mumford JA et al 1995 Equine arteritis virus-neutralizing antibody in the horse is induced by a determinant on the large envelope glycoprotein GL. Journal of General Virology 76: 1989–1998

Christley RM, Hodgson DR, Rose RJ et al 2001 A case–control study of respiratory disease in Thoroughbred racehorses in Sydney, Australia. Equine Veterinary Journal 33: 256–264

Coetzer JAW, Erasmus B 1994 African horse sickness. In: Coetzer JAW, Thompson GR, Tutsin RC (editors) Infectious Diseases of Livestock, with Special Reference to Southern Africa. Oxford University Press, Oxford, pp.460–475

Coignoul FL, Bertram TA Cheville NF 1984a Pathogenicity of equine herpesvirus 1 subtype 2 for foals and adult pony mares. Veterinary Microbiology 9: 533–542

Coignoul FL, Cheville NF 1984b Pathology of maternal genital tract, placenta, and fetus in equine viral arteritis. Veterinary Pathology 21: 333–340

Collinson PN, O'Rielly J, Ficorilli N et al 1994 Isolation of equine herpesvirus type 2 (equine gammaherpesvirus 2)

from foals with keratoconjunctivitis. Journal of the American Veterinary Medical Association 205: 329–331

Cook RF, Sinclair R, Mumford JA 1988 Detection of influenza nucleoprotein antigen in nasal secretions from horses infected with A/equine influenza (H3N8) viruses. Journal of Virological Methods 20: 1–12

Crabb BS, Studdert MJ 1995 Equine herpesvirus 4 (equine rhinopneumonitis virus) and 1 (equine abortion virus). Advances in Virus Research 45: 153–190

Crawford PC, Dubovi EJ, Castleman WL et al 2005 Transmission of equine influenza virus to dogs. Science 310: 482–485

Cullinane A, Weld J, Osborne M et al 2001 Field studies on equine influenza vaccination regimes in thoroughbred foals and yearlings. Veterinary Journal 161: 174–185

Daly JM, Lai ACK, Binns MM et al 1996 Antigenic and genetic evolution of equine H3N8 influenza A viruses. Journal of General Virology 77: 661–671

Daly JM, Yates RJ, Browse G et al 2003 Comparison of hamster and pony challenge models for evaluation of effect of antigenic drift on cross protection afforded by equine influenza vaccines. Equine Veterinary Journal 35: 458–462

Daly JM, Yates PJ, Newton JR et al 2004 Evidence supporting the inclusion of strains from each of the two co-circulating lineages of H3N8 equine influenza virus in vaccines. Vaccine 22: 4101–4109

Daly JM, Whitwell KE, Miller J et al 2006 Investigation of equine influenza cases exhibiting neurological disease: coincidence on association. Journal of Comparative Pathology (in press)

de la Rua-Domenech R, Reid SW, Gonzalez-Zariquiey AE et al 1999 Modelling the spread of a viral infection in equine populations managed in Thoroughbred racehorse training yards. Preventative Veterinary Medicine 47: 61–77

de Vries AA, Chirnside ED, Horzinek MC et al 1992 Structural proteins of equine arteritis virus. Journal of Virology 66: 6294–6303

Del Piero F 2000 Equine viral arteritis. Veterinary Pathology 37: 287–296

Del Piero F, Wilkins PA, Lopez JW et al 1997 Equine viral arteritis in newborn foals: clinical, pathological, serological, microbiological and immunohistochemical observations. Equine Veterinary Journal 29: 178–185

Dimock WW, Edwards PR 1936 The differential diagnosis of equine abortion with reference to a hitherto unknown form of epizootic abortion in mares. Cornell Veterinarian 26: 231–240

Ditchfield J, Zbitnew A, MacPherson LW 1963 Association of Myxovirus Para-Influenzae 3 (RE 55) with upper respiratory infection of horses. Canadian Veterinary Journal 4: 175–180

Doll ER, Bryans JT 1962 Incubation periods for abortion in equine viral rhinopneumonitis. Journal of Biochemistry (Tokyo) 140: 351–354

Doll ER, Bryans JT 1963 A planned infection program for immunizing mares against viral rhinopneumonitis. Cornell Veterinarian 53: 249–262

Doll ER, Bryans JT, Wilson JC et al 1968 Immunization against equine viral arteritis using modified live virus propagated in cell cultures of rabbit kidney. Cornell Veterinarian 48: 497–524

Dunowska M, Wilks CR, Studdert MJ et al 2002 Equine respiratory viruses in foals in New Zealand. New Zealand Veterinary Journal 50: 140–147

Dutta SK 1975 Isolation and characterization of an adenovirus and isolation of its adenovirus-associated virus in cell culture from foals with respiratory tract disease. American Journal of Veterinary Research 36: 247–250

Dutta SK, Shipley WD 1975 Immunity and the level of neutralization antibodies in foals and mares vaccinated with a modified live-virus rhinopneumonitis vaccine. American Journal of Veterinary Research 36: 445–448

Edington N 1992 Latency of equine herpesviruses. In: Proceedings of the Sixth International Conference on Equine Infectious Diseases, Cambridge. R&W Publications, Newmarket, pp.195–200

Edington N, Wright JA, Patel JR et al 1984 Equine adenovirus 1 isolated from cauda equina neuritis. Research in Veterinary Science 37: 252–254

Edington N, Bridges CG, Patel JR 1986 Endothelial cell infection and thrombosis in paralysis caused by equid herpesvirus-1: equine stroke. Archives of Virology 90: 111–124

Edington N, Bridges CG, Griffiths L 1989 Equine interferons following exposure to equid herpesvirus-1 or -4. Journal of Interferon Research 9: 389–392

Edington N, Welch HM, Griffiths L 1994 The prevalence of latent Equid herpesviruses in the tissues of 40 abattoir horses. Equine Veterinary Journal 26: 140–142

Edwards S, Castillo-Olivares J, Cullinane A et al 1999 International harmonisation of laboratory diagnostic tests for equine viral arteritis. In: Proceedings of the Eighth International Conference on Equine Infectious Diseases, Dubai. R&W Publications, Newmarket, pp.359–362

el Hasnaoui H, el Harrak M, Zientara S et al 1998 Serological and virological responses in mules and donkeys following inoculation with African horse sickness virus serotype 4. Archives of Virology (Supplement) 14: 29–36

Foote CE, Love DN, Gilkerson JR et al 2004 Detection of EHV-1 and EHV-4 DNA in unweaned Thoroughbred foals from vaccinated mares on a large stud farm. Equine Veterinary Journal 36: 341–345

Fortier G, Vidament M, DeCraene F et al 2002 The effect of GnRH antagonist on testosterone secretion, spermatogenesis and viral excretion in EVA-virus excreting stallions. Equine reproduction VIII. In: Proceedings of the Eighth International Symposium on Equine Reproduction Theriogenology 58. Elsevier, New York, pp.425–427

Fukunaga Y 1994 Equine viral arteritis: diagnostic and control measures. Journal of Equine Science 5: 101–114

Fukunaga Y, Wada R, Hirasawa K et al 1982 Effect of the modified Bucyrus strain of equine arteritis virus experimentally inoculated into horses. Bulletin of Equine Research Institute, Japan 19: 97–101

Fukunaga Y, Kumanimodo T, Kamada M et al 1983 Equine picornavirus: isolation of virus from the oral cavity of healthy horses. Bulletin of the Equine Research Institute 20: 103–109

Fukunaga Y, Wada R, Kanemaru T et al 1996 Immune potency of lyophilized, killed vaccine for equine viral arteritis and its protection against abortion in pregnant mares. Journal of Equine Veterinary Science 16: 217–221

Fukunaga Y, Wada R, Imagawa H et al 1997 Venereal infection of mares by equine arteritis virus and use of killed vaccine against the infection. Journal of Comparative Pathology 117: 201–208

Gerber H 1970 Clinical features, sequelae and epidemiology of equine influenza. In: Proceedings of the Second

International Conference on Equine Infectious Diseases. Karger AG, Basel, pp.63–80

Gibson JS, Slater JD, Awan AR et al 1992 Pathogenesis of equine herpesvirus-1 in specific pathogen-free foals: primary and secondary infections and reactivation. Archives of Virology 123: 351–366

Glass K, Wood JL, Mumford JA et al 2002 Modelling equine influenza 1: a stochastic model of within-yard epidemics. Epidemiology and Infection 128: 491–502

Gleeson LJ, Studdert MJ, Sullivan ND 1978 Pathogenicity and immunologic studies of equine adenovirus in specific-pathogen-free foals. American Journal of Veterinary Research 39: 1636–1642

Gomez-Villamandos JC, Sanchez C, Carrasco L et al 1999 Pathogenesis of African horse sickness: ultrastructural study of the capillaries in experimental infection. Journal of Comparative Pathology 121: 101–116

Gross DK, Hinchcliff KW, French PS et al 1998 Effect of moderate exercise on the severity of clinical signs associated with influenza virus infection in horses. Equine Veterinary Journal 30: 489–497

Gross DK, Morley PS, Hinchcliff KW et al 2004 Pulmonary ultrasonographic abnormalities associated with naturally occurring equine influenza virus infection in standard-bred racehorses. Journal of Veterinary Internal Medicine 18: 718–727

Guillaume V, Lefeuvre A, Faure C et al 2004 Specific detection of Nipah virus using real-time RT-PCR (TaqMan). Journal of Virological Methods 120: 229–237

Guthrie AJ, Stevens KB, Bosman PP 1999 The circumstances surrounding the outbreak and spread of equine influenza in South Africa. Review of Science and Technology 18: 179–185

Halpin K, Young PL, Field HE et al 2000 Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. Journal of General Virology 81: 1927–1932

Hamblin C, Graham SD, Anderson EC et al 1990 A competitive ELISA for the detection of group-specific antibodies to African horse sickness virus. Epidemiology and Infection 104: 303–312

Hamblin C, Mellor PS, Boned J 1991a The use of ELISA for the detection of African horse sickness viruses in *Culicoides* midges. Journal of Virological Methods 34: 221–225

Hamblin C, Mellor PS, Graham SD et al 1991b Antibodies in horses, mules and donkeys following monovalent vaccination against African horse sickness. Epidemiology and Infection 106: 365–371

Hamblin C, Mertens PP, Mellor PS et al 1991c A serogroup specific enzyme-linked immunosorbent assay for the detection and identification of African horse sickness viruses. Journal of Virological Methods 31: 285–292

Hamblin C, Salt JS, Mellor PS et al 1998 Donkeys as reservoirs of African horse sickness virus. Archives of Virology (Supplement) 14: 37–47

Hannant D, Mumford JA, Jessett DM 1988 Duration of circulating antibody and immunity following infection with equine influenza virus. Veterinary Record 122: 125–128

Hannant D, O'Neil T, Jessett DM et al 1991 Evidence for non-specific immunosuppression during the development of immune responses to equid herpesvirus-1. Equine Veterinary Journal Supplement 12: 41–45

Hannant D, Mumford JA 1996 Equine influenza. In: Studdert MJ (ed.) Virus Infections of Equines. Elsevier, Amsterdam, pp.285–293

Hedges JF, Balasuriya UB, MacLachlan NJ 1999 The open reading frame 3 of equine arteritis virus encodes an immunogenic glycosylated, integral membrane protein. Virology 264: 92–98

Heldens JG, Hannant D, Cullinane AA et al 2001 Clinical and virological evaluation of the efficacy of an inactivated EHV1 and EHV4 whole virus vaccine (Duvaxyn EHV1,4). Vaccination/challenge experiments in foals and pregnant mares. Vaccine 19: 4307–4317

Hinton TM, Crabb BS 2001 The novel picornavirus Equine rhinitis B virus contains a strong type II internal ribosomal entry site which functions similarly to that of Encephalomyocarditis virus. Journal of General Virology 82: 2257–2269

Hoffman AM, Viel L, Prescott JF et al 1993 Association of microbiologic flora with clinical, endoscopic, and pulmonary cytologic findings in foals with distal respiratory tract infection. American Journal of Veterinary Research 54: 615–1622

Holmes DF, Kemen MJ, Coggins L 1978 Equine rhinovirus infection – serological evidence of infection in selected United States horse populations. In: Proceedings of the Fourth International Conference on Equine Infectious Diseases, Lyon. Veterinary Publications, Princeton, NJ, pp.315–319

Holyoak GR, Little TV, McCollam WH et al 1993 Relationship between onset of puberty and establishment of persistent infection with equine arteritis virus in the experimentally infected colt. Journal of Comparative Pathology 109: 29–46

Hooper P, Zaki S, Daniels P et al 2001 Comparative pathology of the diseases caused by Hendra and Nipah viruses. Microbes and Infection 3: 315–322

House C, Mikiciuk PE, Berninger ML 1990 Laboratory diagnosis of African horse sickness: comparison of serological techniques and evaluation of storage methods of samples for virus isolation. Journal of Veterinary Diagnostic Investigation 2: 44–50

Howell PG 1962 The isolation and identification of further antigenic types of African horsesickness virus. Onderstepoort Journal of Veterinary Research 29: 139–149

Howell PG, Groenewald D, Visage CW et al 2002 The classification of seven serotypes of equine encephalosis virus and the prevalence of homologous antibody in horses in South Africa. Onderstepoort Journal of Veterinary Research 69: 79–93

Huang JA, Ficorilli N, Hartley CA et al 2001 Equine rhinitis B virus: a new serotype. Journal of General Virology 82: 2641–2645

Johnson B, Baldwin C, Timoney P et al 1991 Arteritis in equine fetuses aborted due to equine viral arteritis. Veterinary Pathology 28: 248–250

Klaey M, Sanchez-Higgins M, Leadon DP et al 1998 Field case study of equine rhinovirus 1 infection: clinical signs and clinicopathology. Equine Veterinary Journal 30: 267–269

Kleiboeker SB, Schommer SK, Johnson PJ et al 2002 Association of two newly recognized herpesviruses with interstitial pneumonia in donkeys (*Equus asinus*). Journal of Veterinary Diagnostic Investigation 14: 273–280

Konishi SI, Harasawa R, Mochizuki M et al 1977 Studies on equine adenovirus. I. Characteristics of an adenovirus isolated from a thoroughbred colt with pneumonia. Nippon Juigaku Zasshi 39: 117–125

Kumar S, Tamura K, Jakobsen IB, Nei M 2001 MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17: 1244–1245

Kydd JH, Smith KC, Hannant D et al 1994a Distribution of equid herpesvirus-1 (EHV-1) in respiratory tract associated lymphoid tissue: implications for cellular immunity. Equine Veterinary Journal 26: 470–473

Kydd JH, Smith KC, Hannant D et al 1994b Distribution of equid herpesvirus-1 (EHV-1) in the respiratory tract of ponies: implications for vaccination strategies. Equine Veterinary Journal 26: 466–469

Kydd JH, Wattrang E, Hannant D 2003 Pre-infection frequencies of equine herpesvirus-1 specific, cytotoxic T lymphocytes correlate with protection against abortion following experimental infection of pregnant mares. Veterinary Immunology and Immunopathology 96: 207–217

Lai AC, Chambers TM, Holland RE et al 2001 Diverged evolution of recent equine-2 influenza (H3N8) viruses in the Western Hemisphere. Archives of Virology 146: 1063–1074

Li F, Drummer HE, Ficorilli N et al 1997 Identification of noncytopathic equine rhinovirus 1 as a cause of acute febrile respiratory disease in horses. Journal of Clinical Microbiology 35: 937–943

Livesay GJ, O'Neill T, Hannant D et al 1993 The outbreak of equine influenza (H3N8) in the United Kingdom in 1989: diagnostic use of an antigen capture ELISA. Veterinary Record 133: 515–519

Lord CC, Woolhouse ME, Rawlings P et al 1996 Simulation studies of African horse sickness and *Culicoides imicola* (Diptera: Ceratopogonidae). Journal of Medical Entomology 33: 328–338

Lord CC, Woolhouse ME, Barnard BJ 1997a Transmission and distribution of virus serotypes: African horse sickness in zebra. Epidemiology and Infection 118: 43–50

Lord CC, Woolhouse ME, Mellor PS 1997b Simulation studies of vaccination strategies in African horse sickness. Vaccine 15: 519–524

Lord CC, Woolhouse ME, Barnard BJ 1998 Transmission and distribution of African horse sickness virus serotypes in South African zebra. Archives of Virology (Supplement) 14: 21–28

Lord CC, Venter GJ, Mellor PS et al 2002 Transmission patterns of African horse sickness and equine encephalosis viruses in South African donkeys. Epidemiology and Infection 128: 265–275

Lubroth J 1988. African horse sickness and the epizootic in Spain 1987. Equine Practice 10: 26–33

Lunn DP, Holmes MA, Gibson J et al 1991 Haematological changes and equine lymphocyte subpopulation kinetics during primary infection and attempted re-infection of specific pathogen free foals with EHV-1. Equine Veterinary Journal 23: 35–40

Lunn DP, Hussey S, Sebing R et al 2001 Safety, efficacy, and immunogenicity of a modified-live equine influenza virus vaccine in ponies after induction of exercise-induced immunosuppression. Journal of the American Veterinary Medical Association 218: 900–906

Mackenzie JS, Field HE, Guyatt KJ 2003 Managing emerging diseases borne by fruit bats (flying foxes), with particular reference to henipaviruses and Australian bat lyssavirus. Journal of Applied Microbiology 94 Suppl: 59S–69S

MacLachlan NJ, Balasuriya UB, Hedges JF et al 1998 Serologic response of horses to the structural proteins of equine arteritis virus. Journal of Veterinary Diagnostic Investigation 10: 229–236

Matsumura T, Sugiura T, Imagawa H et al 1992 Epizootiological aspects of type 1 and type 4 equine herpesvirus infections among horse populations. Journal of Veterinary Medical Science 54: 207–211

Mayr A, Pette J 1968 1st experiments concerning the vaccination of horses against rhino-pneumonia (viral abortion of mares) with a live vaccine from cell cultures. Bulletin of the Office of International Epizootics 70: 133–140

McChesney AE, England JJ, Whiteman CE et al 1974 Experimental transmission of equine adenovirus in Arabian and non-Arabian foals. American Journal of Veterinary Research 35: 1015–1023

McCollum WH 1969 Development of a modified virus strain and vaccine for equine viral arteritis. Journal of the American Veterinary Medical Association 155: 318–322

McCollum WH 1976 Studies of passive immunity in foals to equine viral arteritis. Veterinary Microbiology 1: 45–54

McCollum WH 1986 Responses of horses vaccinated with avirulent modified-live equine arteritis virus propagated in the E. Derm (NBL-6) cell line to nasal inoculation with virulent virus. American Journal of Veterinary Research 47: 1931–1934

McCollum WH, Timoney PJ 1992 Studies on the seroprevalence and frequency of equine rhinovirus-I and -II infection in normal horse urine. In: Proceedings of the Sixth International Conference on Equine Infectious Diseases, Cambridge. R&W Publications, Newmarket, pp.83–87

McCollum WH, Prickett ME, Bryans JT 1971 Temporal distribution of equine arteritis virus in respiratory mucosa, tissues and body fluids of horses infected by inhalation. Research in Veterinary Science 12: 459–464

McCollum WH, Little TV, Timoney PJ et al 1994 Resistance of castrated male horses to attempted establishment of the carrier state with equine arteritis virus. Journal of Comparative Pathology 111: 383–388

Meiswinkel R, Paweska JT 2003 Evidence for a new field *Culicoides* vector of African horse sickness in South Africa. Preventative Veterinary Medicine 60: 243–253

Mellor PS, Boorman J 1995 The transmission and geographical spread of African horse sickness and bluetongue viruses. Annals of Tropical Medicine and Parasitology 89: 1–15

Mellor PS, Wittmann EJ 2002 Bluetongue virus in the Mediterranean Basin 1998–2001. Veterinary Journal 164: 20–37

Mellor PS, Hamblin C 2004 African horse sickness. Veterinary Research 35: 445–466

Mellor PS, Hamblin C, Graham SD 1990 African horse sickness in Saudi Arabia. Veterinary Record 127: 41–42

Mellor PS, Rawlings P, Baylis M et al 1998 Effect of temperature on African horse sickness virus infection in *Culicoides*. Archives of Virology (Supplement) 14: 155–163

Moore BD, Balasuriya UB, Hedges JF et al 2002 Growth characteristics of a highly virulent, a moderately virulent, and an avirulent strain of equine arteritis virus in primary equine endothelial cells are predictive of their virulence to horses. Virology 298: 39–44

Moore BD, Balasuriya UB, Watson JL et al 2003 Virulent and avirulent strains of equine arteritis virus induce different quantities of TNF-alpha and other proinflammatory cytokines in alveolar and blood-derived equine macrophages. Virology 314: 662–670

Morley PS, Hanson LK, Bogdan JR et al 1995 The relationship between single radial hemolysis, hemagglutination inhibition, and virus neutralization assays used to detect antibodies specific for equine influenza viruses. Veterinary Microbiology 45: 81–92

Morley PS, Townsend HG, Bogdan JR et al 1999 Efficacy of a commercial vaccine for preventing disease caused by influenza virus infection in horses. Journal of the American Veterinary Medical Association 215: 61–66

Mumford JA 1994 Equine rhinovirus infections. In: Coetzer JAW, Thomson GR, Tustin RC, Kriek NPJ (editors) Infectious Diseases of Livestock with Special Reference to South Africa. Oxford University Press, Oxford, vol. II, pp.820–822

Mumford JA 1999 The equine influenza surveillance program. Advances in Veterinary Medicine 41: 379–387

Mumford JA, Thomson GR 1978 Studies on picornaviruses isolated from the respiratory tract of horses. In: Proceedings of the Fourth International Conference on Equine Infectious Diseases, Lyon. Veterinary Publications, Princeton, NJ, pp.419–429

Mumford JA, Wood J 1992 Establishing an acceptability threshold for equine influenza vaccines. Developments in Biological Standardization 79: 137–146

Mumford JA, Wood JM, Folkers C et al 1988 Protection against experimental infection with influenza virus A/equine/Miami/63 (H3N8) provided by inactivated whole virus vaccines containing homologous virus. Epidemiology and Infection 100: 501–510

Mumford JA, Jessett DM, Rollinson EA et al 1994a. Duration of protective efficacy of equine influenza immunosti-mulating complex/tetanus vaccines. Veterinary Record 134: 158–162

Mumford JA, Wilson H, Hannant D et al 1994b Antigenicity and immunogenicity of equine influenza vaccines containing a Carbomer adjuvant. Epidemiology and Infection 112: 421–437

Mumford JA, Wood J, Jessett DM et al 1994c Efficacy of H3N8 influenza vaccines prepared from virus cultured in eggs or mammalian cells. In: Equine infectious diseases VII: Proceedings of the Seventh International Conference, Tokyo. R&W Publications, Newmarket, pp.342–343

Murray K, Selleck P, Hooper P et al 1995 A morbillivirus that caused fatal disease in horses and humans. Science 268: 94–97

Murray K, Dunn K, Murray G 1998 Hendra virus (equine morbillivirus): the outbreaks, the disease and lessons for preparedness. In: Proceedings of the Eighth International Conference on Equine Infectious Diseases, Dubai. R&W Publications, Newmarket, pp.3–10

Murray MJ, Eichorn ES, Dubovi EJ et al 1996 Equine herpes-virus type 2: prevalence and seroepidemiology in foals. Equine Veterinary Journal 28: 432–436

Murray MJ, del Piero F, Jeffrey SC et al 1998 Neonatal equine herpesvirus type 1 infection on a thoroughbred breeding farm. Journal of Veterinary Internal Medicine 12: 36–41

Naib ZM, Stewart JA, Dowdle WR et al 1968 Cytological features of viral respiratory tract infections. Acta Cytologica 12: 162–171

Newton JR, Verheyen, K, Wood JL et al 1999 Equine influenza in the United Kingdom in 1998. Veterinary Record 145: 449–452

Newton JR, Lakhani KH, Wood JL et al 2000a Risk factors for equine influenza serum antibody titres in young thoroughbred racehorses given an inactivated vaccine. Preventive Veterinary Medicine 46: 129–141

Newton JR, Townsend HG, Wood JL et al 2000b Immunity to equine influenza: relationship of vaccine-induced antibody in young Thoroughbred racehorses to protection against field infection with influenza A/equine-2 viruses (H3N8). Equine Veterinary Journal 32: 65–74

Newton JR, Wood JL, Chanter N 2003 A case control study of factors and infections associated with clinically apparent respiratory disease in UK Thoroughbred racehorses. Preventative Veterinary Medicine 60: 107–132

Newton JR, Geraghty RJ, Castillo-Olivares J et al 2004 Evidence that use of an inactivated equine herpes-virus vaccine induces serum cytotoxicity affecting the equine arteritis virus neutralisation test. Vaccine 22: 4117–4123

Nordengrahn A, Rusvai M, Merza M et al 1996 Equine herpesvirus type 2 (EHV-2) as a predisposing factor for *Rhodococcus equi* pneumonia in foals: prevention of the bifactorial disease with EHV-2 immunostimulating complexes. Veterinary Microbiology 51: 55–68

Nugent J, Sinclair R, deVries AA et al 2000 Development and evaluation of ELISA procedures to detect antibodies against the major envelope protein (G(L)) of equine arteritis virus. Journal of Virological Methods 90: 167–183

Ostlund EN 1993 The equine herpesviruses. Veterinary Clinics of North American; Equine Practice 9: 283–294

Park AW, Wood JL, Newton JR et al 2003 Optimising vaccination strategies in equine influenza. Vaccine 21: 2862–2870

Park AW, Wood JL, Daly JM et al 2004 The effects of strain heterology on the epidemiology of equine influenza in a vaccinated population. Proceedings of the Royal Society of London. Series B, Biological Sciences 271: 1547–1555

Pascoe RR, Harden TJ, Spradbrow PB 1974 Letter: Experimental infection of a horse with an equine adenovirus. Australian Veterinary Journal 50: 278–279

Paweska JT, Venter GJ, Mellor PS 2002 Vector competence of South African *Culicoides* species for bluetongue virus serotype 1 (BTV-1) with special reference to the effect of temperature on the rate of virus replication in *C. imicola* and *C. bolitinos*. Medical and Veterinary Entomology 16: 10–21

Perryman LE, McGuire TC, Crawford TB 1978 Maintenance of foals with combined immunodeficiency: causes and control of secondary infections. American Journal of Veterinary Research 39: 1043–1047

Plummer G 1962 An equine respiratory virus with entero-virus properties. Nature 195: 519–520

Plummer G, Kerry JB 1962 Studies on an equine respiratory virus. Veterinary Record 74: 967–970

Powell DG, Burrows, R, Goodridge D 1974 Respiratory viral infections among thoroughbred horses in training during 1972. Equine Veterinary Journal 6: 19–24

Prickett ME 1970 The pathology of disease caused by equine herpesvirus-1. Proceedings of the Second International Conference on Equine Infectious Diseases. Karger, Basel

Purse BV, Mellor PS, Rogers DJ et al 2005 Climate change and the recent emergence of bluetongue in Europe. National Review of Microbiology 3: 71–181

Rawlings P, Pro MJ, Pena I et al 1997 Spatial and seasonal distribution of *Culicoides imicola* in Iberia in relation to the transmission of African horse sickness virus. Medical and Veterinary Entomology 11: 49–57

Rees WA, Harkins JD, Woods WE et al 1997 Amantadine and equine influenza: pharmacology, pharmacokinetics and neurological effects in the horse. Equine Veterinary Journal 29: 104–110

Rees WA, Harkins JD, Lu M et al 1999 Pharmacokinetics and therapeutic efficacy of rimantadine in horses experimentally infected with influenza virus A2. American Journal of Veterinary Research 60:888–894

Rodriguez JJ, Horvath CM 2004 Host evasion by emerging paramyxoviruses: Hendra virus and Nipah virus v proteins inhibit interferon signalling. Viral Immunology 17: 210–219

Roizmann B, Desrosiers RC, Fleckenstein B et al 1992 The family Herpesviridae: an update. The Herpesvirus Study Group of the International Committee on Taxonomy of Viruses. Archives of Virology 123: 425–449

Sailleau C, Moulay S, Cruciere C et al 1997 Detection of African horse sickness virus in the blood of experimentally infected horses: comparison of virus isolation and a PCR assay. Research in Veterinary Science 62: 229–232

Sailleau C, Hamblin C, Paweska JT et al 2000 Identification and differentiation of the nine African horse sickness virus serotypes by RT-PCR amplification of the serotype-specific genome segment 2. Journal of General Virology 81: 831–837

Scott JC, Dutta SK, Myrup AC 1983 *In vivo* harboring of equine herpesvirus-1 in leukocyte populations and subpopulations and their quantitation from experimentally infected ponies. American Journal of Veterinary Research 44: 1344–1348

Selvey LA, Wells RM, McCormack JG et al 1995 Infection of humans and horses by a newly described morbillivirus. Medical Journal of Australia 162: 642–645

Senne DA, Pearson JE, Carbrey EA 1985 Equine viral arteritis: a standard procedure for the virus neutralization test and comparison of results of a proficiency test performed at five laboratories. In: Proceedings of the United States Animal Health Association 89: 29–34

Sharma PC, Cullinane AA, Onions DE et al 1992 Diagnosis of equid herpesviruses -1 and -4 by polymerase chain reaction. Equine Veterinary Journal 24: 20–25

Shortridge KF, Chan WH, Guan Y 1995 Epidemiology of the equine influenza outbreak in China, 1993–94. Veterinary Record 136: 160–161

Slater JD, Borchers K, Thackray AM et al 1994 The trigeminal ganglion is a location for equine herpesvirus 1 latency and reactivation in the horse. Journal of General Virology 75: 2007–2016

Smith KC, Blunden AS, Whitwell KE et al 2003 A survey of equine abortion, stillbirth and neonatal death in the UK from 1988 to 1997. Equine Veterinary Journal 35: 496–501

Steck F, Hofer B, Schaeren B et al 1978 Equine rhinoviruses: new serotypes. In: Proceedings of the Fourth International Conference on Equine Infectious Diseases, Lyon. Veterinary Publications, Princeton, NJ, pp.321–328

Studdert MJ 1978 Antigenic homogeneity of equine adenoviruses. Australian Veterinary Journal 54: 263–264

Studdert MJ 1996 Equine adenovirus infections. In: Studdert MJ (ed.) Virus Infections of Vertebrates, vol. 6, Virus Infections of Equines. Elsevier Science, Amsterdam, pp.67–80

Studdert MJ, Gleeson LJ 1977 Isolation of equine rhinovirus type 1. Australian Veterinary Journal 53: 452

Studdert MJ, Gleeson LJ 1978 Isolation and characterisation of an equine rhinovirus. Zentralblatt für Veterinärmedizin B 25: 225–237

Studdert MJ, Blackney MH 1982 Isolation of an adenovirus antigenically distinct from equine adenovirus type 1 from diarrheic foal feces. American Journal of Veterinary Research 43: 543–544

Studdert MJ, Wilks CR, Coggins L 1974 Antigenic comparisons and serologic survey of equine adenoviruses. American Journal of Veterinary Research 35: 693–699

Telford EAR, Watson MS, McBride K et al 1992 The DNA sequence of equine herpesvirus-1. Virology 189: 304–316

Telford EAR, Watson MS, Perry, J et al 1998 The DNA sequence of equine herpesvirus-4. Journal of General Virology 79: 1197–1203

The Royal Society 2002 Infectious Diseases of Livestock. Royal Society, London

Thompson DB, Spradborw PB, Studdert M 1976 Isolation of an adenovirus from an Arab foal with a combined immunodeficiency disease. Australian Veterinary Journal 52: 435–437

Thomson GR, Mumford JA, Campbell J et al 1976 Serological detection of equid herpesvirus 1 infections of the respiratory tract. Equine Veterinary Journal 8: 58–65

Timoney PJ 2000 Equine viral arteritis. In: Manual of Standards for Diagnostic Tests and Vaccines. Lists A and B Diseases of Mammals, Birds and Bees. Office International des Epizooties, Paris, pp.582–594

Timoney PJ, McCollum WH 1988 Equine viral arteritis – epidemiology and control. Journal of Equine Veterinary Science 8: 54–59

Timoney PJ, McCollum WH 1991 Equine viral arteritis: current clinical and economic significance. In: Proceedings of the thirty-sixth annual convention of the American Association of Equine Practitioners, Lexington, KY

Timoney PJ, McCollom WH 1993 Equine viral arteritis in perspective in relation to international trade. Journal of Equine Veterinary Science 13: 50–52

Timoney PJ, McCollum WH 2000 Equine viral arteritis: further characterisation of the carrier state in stallions. Journal of Reproduction and Fertility Supplement 56: 3–11

Timoney PJ, McCollum WH, Murphy TW et al 1987 The carrier state in equine arteritis virus infection in the stallion with specific emphasis on the venereal mode of virus transmission. Journal of Reproduction and Fertility Supplement 35: 95–102z

Timoney PJ, McCollum WH, Roberts AW 1988 Current strategies for the control of equine viral arteritis. Proceedings of the United States Animal Health Association 92: 211–218

Timoney PJ, McCollum WH, Roberts AW et al 1986 Demonstration of the carrier state in naturally acquired equine arteritis virus infection in the stallion. Research in Veterinary Science 41: 279–280

Townsend HG, Penner SJ, Watts TC et al 2001 Efficacy of a cold-adapted, intranasal, equine influenza vaccine: challenge trials. Equine Veterinary Journal 33: 637–643

Varrasso A, Drummer HE, Huang JA et al 2001 Sequence conservation and antigenic variation of the structural proteins of equine rhinitis A virus. Journal of Virology 75: 10550–10556

Venter GJ, Paweska JT, Van Dijk AA et al 1998 Vector competence of *Culicoides bolitinos* and *C. imicola* for South African bluetongue virus serotypes 1, 3 and 4. Medical and Veterinary Entomology 12: 378–385

Westbury HA 2000 Hendra virus disease in horses. Review of Science and Technology 19: 151–159

Whitwell, KE and Greet, TR 1984. Collection and evaluation of tracheobronchial washes in the horse. Equine Veterinary Journal 16: 499–508

Whitlock RH, Dellers RW, Shively JN 1975 Adenoviral pneumonia in a foal. Cornell Veterinarian 65: 393–401

Wieringa R, de Vries AA, Raamsman MJ et al 2002 Characterization of two new structural glycoproteins, GP(3) and GP(4), of equine arteritis virus. Journal of Virology 76: 10829–10840

Wilks CR, Studdert MJ 1972 Isolation of an equine adeno-virus. Australian Veterinary Journal 48: 580–581

Williams R, Du Plessis DH, Van Wyngaardt W 1993 Group-reactive ELISAs for detecting antibodies to African horsesickness and equine encephalosis viruses in horse, donkey, and zebra sera. Journal of Veterinary Diagnostic Investigation 5: 3–7

Williamson MM, Hooper PT, Selleck PW et al 1998 Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses and cats. Australian Veterinary Journal 76: 813–818

Wood JL, Newton JR, Chanter N et al 2005 Association between respiratory disease and bacterial and viral infections in British racehorses. Journal of Clinical Microbiology 43: 120–126

Wood JM, Mumford J, Folkers C et al 1983a Studies with inactivated equine influenza vaccine. 1. Serological responses of ponies to graded doses of vaccine. Journal of Hygiene 90: 371–384

Wood JM, Schild GC, Folkers C et al 1983b The standardization of inactivated equine influenza vaccines by single-radial immunodiffusion. Journal of Biological Standardization 11: 133–136

Wood JM, Mumford J, Schild GC et al 1986 Single-radial-immunodiffusion potency tests of inactivated influenza vaccines for use in man and animals. Developments in Biological Standardization 64: 169–176

Wood JM, Mumford JA, Dunleavy U et al 1988 Single radial immunodiffusion potency tests for inactivated equine influenza vaccines. In: Proceedings of the 5th International Conference on Equine Infectious Diseases. University Press of Kentucky, Lexington, KY, pp.74–79

Yates P, Mumford JA 2000 Equine influenza vaccine efficacy: the significance of antigenic variation. Veterinary Microbiology 74: 173–177

Zhu XR, Wang CR, Shen BY et al 1989 Microbiological examination of horses with chronic obstructive pulmonary disease in Hexi district, Gansu province. Acta Veterinaria et Zootechnica Sinica 20: 238–241

Zientara S, Sailleau C, Moulay S et al 1993 Diagnosis and molecular epidemiology of the African horse sickness virus by the polymerase chain reaction and restriction patterns. Veterinary Research 24: 385–395

Bacterial Infections of the Equine Respiratory Tract

Josh Slater

Equine Streptococci

Streptococci are the most important respiratory bacterial pathogens of the horse and are the major etiological agents, either as single infections or mixed infections with other pathogens, of all four of the major equine bacterial respiratory syndromes:

- strangles
- bronchopneumonia in foals up to 6–8 months old
- inflammatory airway disease
- pneumonia/pleuropneumonia in mature horses.

There are four principal equine streptococci:

- *Streptococcus equi* subsp. *equi*
- *Streptococcus equi* subsp. *zooepidemicus*
- *Streptococcus dysgalactiae* subsp. *equisimilis*
- *Streptococcus pneumoniae.*

General features of the equine streptococci are listed in Box 23.1.

A typical equine streptococcus is shown schematically in Fig. 23.1. The scanning electron micrograph shows chains of *S. equi* colonizing equine ciliated respiratory epithelium (×2,000). The streptococcal cell wall consists of peptidoglycan and teichoic acid. A variety of surface proteins (including M-like proteins and lipoproteins) are anchored to the cell wall by their C terminus, their N terminus, or by other chemical interactions. These proteins are fibrillar structures that project into the environment around the bacterium and are responsible for many of the interactions between the bacterium and the horse's epithelium and immune system. Although the functions of all the surface proteins have not been determined, they are known to be responsible for certain key events in pathogenesis including adhesion to respiratory epithelium, evasion of phagocytosis and immunogenicity. The bacterial cell is surrounded by a loose network of polysaccharides known as a capsule, through which the surface proteins project. With the exception of *S. pneumoniae*, the capsule of equine streptococci consists of hyaluronic acid. Since hyaluronic acid is a polysaccharide found in mammalian tissues, it is not recognized as foreign by the horse, i.e. it is non-immunogenic and opsonizing antibodies are not raised against the capsule. The capsule also protects the bacterium from the horse's inflammatory defenses, including prevention of opsonization by complement (C3b), and thus confers resistance to phagocytosis. Streptococci secrete a variety of proteins, referred to as exotoxins, that are cytolytic to host cells including respiratory epithelium and leukocytes.

Differentiating equine streptococci

Most equine streptococcal clinical isolates are β-hemolytic and belong to Lancefield group C (Fig. 23.2), the exception being *S. pneumoniae* which is α-hemolytic and does not have a Lancefield group antigen. The Group C streptococci are closely related to each other (Lancefield 1933, Quinn et al 1994, Chanter et al 1997) but can be differentiated by their biochemical properties. Between-subspecies and

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Box 23.1. General features of the equine streptococci

- Gram-positive cocci approximately 1 μm in diameter
- Occur as pairs or chains depending on the culture environment: usually as pairs (diplococci) in the animal but as elongated chains *in vitro*; diplococci are more virulent
- Facultative anaerobes
- Catalase and oxidase negative
- Non-motile and non-spore-forming (therefore limited environmental persistence and reservoir of infection)
- β-hemolysis (complete) on blood agar plates (except *S. pneumoniae* which is α-hemolytic)
- Lancefield Group C (except *S. pneumoniae* which has no Lancefield group antigen)
- Opportunist pathogens of the respiratory tract
- Cause both mucosal respiratory infections and invasive systemic disease
- Host range varies from strictly Equids (*S. equi* subsp. *equi*) to a variety of animal species and humans (*S. equi* subsp. *zooepidemicus*; *S. pneumoniae*)
- Variety of virulence and defense mechanisms including the antiphagocytic capsule and surface (M) protein
- Variably transient immunity following natural infection; some species have huge antigenic diversity between strains with little cross-protection
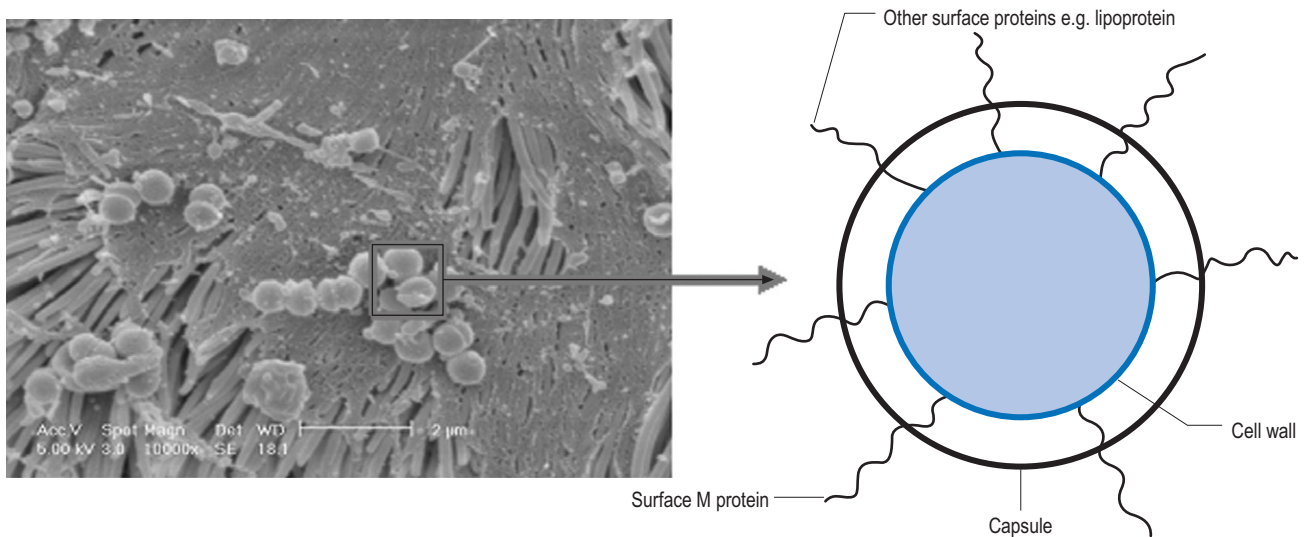
Fig. 23.1. Principal features of an equine streptococcus.

within-subspecies differentiation is also possible by genetic "fingerprinting" at chromosome level (restriction fragment length polymorphisms and pulsed field gel electrophoresis) or nucleotide level using polymerase chain reactions (PCR) for amplification of single gene (16s and 23s rRNA and M protein) or multiple gene sequences (multilocus sequence typing).

Hemolysis

Hemolysis occurs around streptococcal colonies growing on blood agar plates because of production of the cytolytic toxin streptolysin. Streptolysin causes transmembrane pore formation in red blood cells resulting in osmotic lysis. Three patterns of hemolysis occur.

- α-Hemolysis: growth on blood plates causes incomplete destruction of blood cells. This produces dark green discoloration around bacterial colonies, reflecting the presence of biliverdin and other hemoglobin breakdown products.
- β-Hemolysis: growth on blood plates causes complete destruction of blood cells, resulting in transparency of the region surrounding bacterial colonies.
- γ-Hemolysis: no observable destruction of blood cells surrounding bacterial colonies.
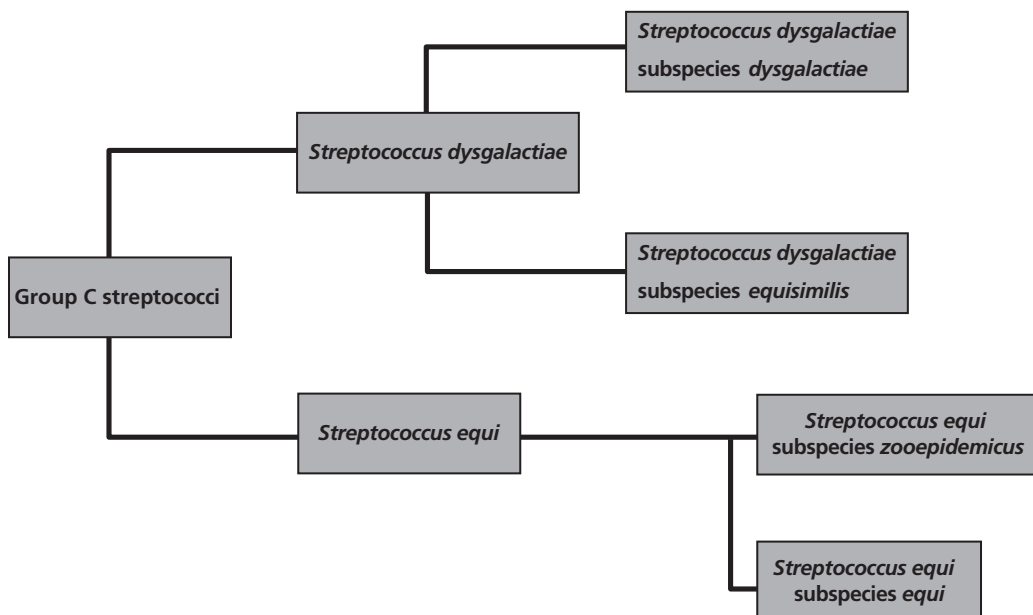
Fig. 23.2. Lancefield group C.

Table 23.1. Sugar fermentation patterns of the equine group C streptococci

Species	Trehalose	Sorbitol	Lactose	Ribose
Streptococcus equi subspecies *equi*	–	–	–	–
Streptococcus equi subspecies *zooepidemicus*	–	+	+	+
Streptococcus dysgalactiae subspecies *equisimilis*	+	–	±	+

Fig. 23.3. *Streptococcus equi*: Gram-stained smears show chains of Gram-positive cocci. Colony smears show elongated chains as in this case, while smears from nasal discharge or abscesses show shorter chains and often diplococci (magnification ×100).

Pathogenic equine streptococci are hemolytic and most are β-hemolytic. Non-hemolytic streptococci may be isolated from the equine upper respiratory tract (URT) but are regarded as commensals without pathogenic potential.

Lancefield groups

Lancefield grouping is a serological method for classifying streptococci into one of 20 groups (designated by a letter) based on the presence of polysaccharide and teichoic acid antigens in the bacterial cell wall (Lancefield 1933). The technique is now performed using commercial latex agglutination test kits, which allow rapid detection of clinically important streptococcal groups. Some streptococci, for example *S. pneumoniae*, have not been assigned to a group because their antigen extracts fail to react with group antisera. With the exception of *S. pneumoniae* all the equine streptococci belong to Lancefield group C.

Biochemical properties

The equine streptococci are all oxidase and catalase negative. The group C streptococci are differentiated on the basis of their ability to ferment the sugars salicin, sucrose, trehalose, sorbitol, lactose, and ribose. They all ferment salicin and sucrose; *S. equi* subsp. *equi* does not ferment any other sugars but *S. equi* subsp. *zooepidemicus* and *S. equi* subsp. *equisimilis* ferment at least two other sugars (Table 23.1).

Streptococcus equi subsp. *equi*

General features

Streptococcus equi subsp. *equi* (*S. equi*) is the cause of strangles. It is a Gram-positive, chain-forming (Fig. 23.3), Lancefield group C β-hemolytic streptococcus (Fig. 23.4). *Streptococcus equi* is prevalent worldwide and is a remark-
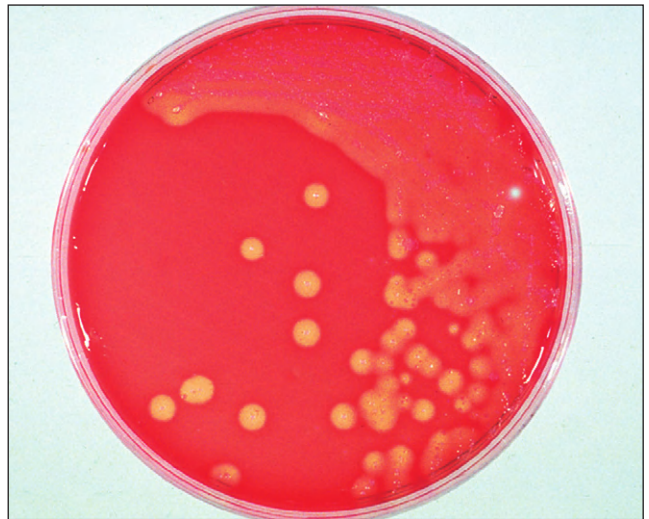
Fig. 23.4. *Streptococcus equi* colonies are generally mucoid and β-hemolytic on blood agar plates.

ably homogeneous, apparently clonal, organism which appears to have evolved comparatively recently from the closely related *S. equi* subsp. *zooepidemicus* (Chanter et al 1997). Different isolates from diverse geographical locations demonstrate antigenic homogeneity on gel diffusion precipitin analysis, immunoblot of M-protein (SeM) and serum neutralization experiments. Genetic homogeneity of *S. equi* has also been demonstrated through chromosomal DNA "fingerprinting" and sequence comparisons of the 16s and 23s rRNA intergenic spacer regions of different strains. However, detailed genetic analysis has shown that the previously held belief that *S. equi* is a single serotype with no genetic variation is incorrect (Al-Ghamdi et al 2000, Takai et al 2000). At least seven different genetic subtypes with particular

geographical distributions have been identified, allowing molecular epidemiology and the tracing of subtype transmission during and between outbreaks to be performed (Al-Ghamdi et al 2000, Takai et al 2000).

Pathogenesis and virulence factors

The pathogenesis of *S. equi* infections has not been completely elucidated at a cellular level and, although some bacterial virulence mechanisms have been identified, the detailed bacterial mechanisms employed in the processes of adhesion to respiratory epithelium, invasion into the lamina propria, entry into lymphatics, and evasion of phagocytosis are incompletely understood.

Acquisition of *S. equi* infection appears to be mainly via infected droplets directly from other horses or indirectly via fomites rather than by aerosol transmission. Acquisition may be via nasal or oral routes. Following entry, bacteria adhere to, and colonize, the epithelium in the URT, possibly preferentially in the nasopharynx and pharyngeal tonsil region although this has not been demonstrated *in vivo*. Invasion through the epithelium into the lamina propria and lymphatics appears to occur rapidly because bacteria can be isolated from drainage lymph nodes within hours of infection (Timoney 1988a). *S. equi* efficiently evades phagocytosis despite efficient chemotaxis of neutrophils to the sites of infection. The continued survival of bacteria within lymph nodes drives further recruitment of neutrophils and abscess formation. In most horses bacteria are confined to the lymph nodes draining the head (submandibular, parotid, retropharyngeal, and cervical lymph nodes). In a minority of cases *S. equi* is released in efferent lymph and gains entry to the blood circulation via the thoracic duct, with systemic dissemination of bacteria resulting in metastatic abscessation. Bacterial antigens (either whole bacteria or bacterial surface proteins) in the circulation can trigger purpura hemorrhagica, an immune-complex-mediated vasculitis, the pathogenesis of which is not completely understood. Although classically associated with *S. equi* infection, it can be triggered by a variety of bacterial antigens, and some horses may have no history of exposure to infectious agents (Pusterla et al 2003). Immune complexes contain immunoglobulin A (IgA) complexed to streptococcal M protein. Affected horses generally have high IgA titers and low IgG titers, with IgG titers only rising in the convalescent phase. The reason for the apparent association with IgA and the late rise in IgG in affected horses is not clear.

Several virulence factors have been identified and characterized in *S. equi* and the genome sequencing projects for *S. equi* and *S. zooepidemicus* (www.sanger.ac.uk) have enabled rapid progress to be made in identifying other virulence mechanisms (Box 23.2; Harrington et al 2002).

Adhesion is the first and key step in pathogenesis: without bacterial adhesion to the epithelium of the URT,

colonization and the subsequent steps in pathogenesis cannot progress. Adhesion almost certainly involves multiple components on the bacterial surface. Our understanding of the molecular events and regulation of *S. equi* adhesion is incomplete. Although several *S. equi* adhesins have been identified and characterized, there are many surface proteins to which functions have not been ascribed and it is likely, based on sequence homology, that many of these are involved with host cell adhesion. Like most bacteria, *S. equi* possesses adhesins that bind to host extracellular matrix molecules (these adhesins are referred to as microbial surface components recognizing adhesive matrix molecules – MSCRAMMs). These include the fibrinogen-binding proteins SFS and FNE (Lindmark & Guss 1999, Lindmark et al 2001). It is not clear whether the hyaluronic acid capsule functions as an adhesin or whether, by virtue of its negative charge and physical masking of surface proteins, it acts to prevent adhesion. Studies with *S. pyogenes* have suggested that the capsule acts as a ligand for CD44 receptors on human cell lines, but experiments with non-capsulated isolates of *S. equi* have either found no difference in adhesion or an increase in adhesion compared to encapsulated isolates. Evasion of host defenses (opsonophagocytosis) is central to pathogenesis, enabling bacterial persistence despite neutrophil chemotaxis, and drives abscess formation. *Streptococcus equi* possesses three antiphagocytic mechanisms: capsule, M protein, and exotoxins. The hyaluronic acid capsule is a polymer of *N*-acetylglucosamine and glucuronic acid. It interferes with opsonization (Anzai et al 1999) by preventing the formation of C3b convertase and hence the deposition of C3b on the bacterial surface. The M protein SeM, in common with all streptococcal M proteins, binds fibrinogen and is antiphagocytic by inhibiting opsonization (Galan & Timoney 1987, Timoney et al 1997) by blocking C3b deposition on the bacterial surface (fibrinogen is bound by the N terminus thus masking the C3b binding site) and by binding the Fc portion of IgG, preventing Fc binding to

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Box 23.2. Known virulence mechanisms of *S. equi*

- Bacterial cell wall
  – Peptidoglycan
- Hyaluronic acid capsule
- Surface proteins
  – Extracellular matrix binding proteins
  – Antibody binding proteins
  – M protein
- Exotoxins
  – Hyaluronidase
  – Streptolysin
  – Streptokinase
  – Pyrogenic exotoxins

Fc receptors on neutrophils (Boschwitz & Timoney 1994). SeM is a 58-kDa coiled fibrillar protein and, unlike most streptococcal M proteins, it is highly conserved showing no antigenic and little sequence variation. Although isolates with truncated M proteins have been isolated from the guttural pouches of carrier horses, these isolates appear to retain immunogenicity and virulence despite their M protein truncations (Chanter et al 2000). Survival in the host also requires a variety of nutrient acquisition mechanisms. *Streptococcus equi* possesses several ATP binding cassette (ABC) transporter systems and associated surface lipoproteins, which appear to be important in pathogenesis. It produces a number of exotoxins that interfere with horse leukocyte function. These include four pyrogenic exotoxins (SePE-H, -I, -K, -L) and an antichemotactic/cytotoxic leukotoxin (Muhktar & Timoney 1988). Invasion into the lamina propria is assisted by degradative enzymes including hyaluronidase and the hemolytic cytotoxin streptolysin-S (Flanagan et al 1998), although this step in pathogenesis is poorly understood.

Transmission

Streptococcus equi infections occur in equids of all ages, although severe clinical disease is more prevalent in young animals or in those not previously exposed (Todd 1910, Sweeney et al 1987, 1989, Yelle 1987, Hamlen et al 1994). In establishments where there is a large population and frequent introduction of new individuals, the risk of *S. equi* outbreaks increases (Box 23.3). In particular, overcrowding and coexisting disease may enhance susceptibility (Todd 1910, Yelle 1987, Jorm 1990, Hoffman et al 1991, Dalgleish et al 1993). Whilst the incidence of strangles varies both geographically and temporally, it has been estimated that *S. equi* infection accounts for approximately 30% of all reported equine infections worldwide (Todd 1910, Dalgleish et al 1993, Harrington et al 2002) and is the most commonly reported equine bacterial infection in

the UK. Morbidity is high and may approach 100% in susceptible populations (Yelle 1987, Dalgleish et al 1993, Hamlen et al 1994). Mortality is generally considered to be only 2–3% but rates of up to 20% have been recorded in individual outbreaks (Sweeney et al 1987, Spoormakers et al 2003). The estimates of disease prevalence are likely to be inaccurate because of the lack of compulsory reporting in the UK, for example, and the stigma attached to *S. equi* infections among horse owners means that detailed and comprehensive epidemiological data are not available.

Streptococcus equi is mainly transmitted via infected droplets either by direct horse-to-horse contact (George et al 1983) or indirectly by fomites including personnel (hands, clothing, and footwear) and equipment (tack, feed utensils, and water buckets/troughs). Aerosol transmission over extended distances as occurs for the respiratory viruses, such as influenza virus, does not appear to be an important feature of *S. equi* transmission. Horses with clinical *S. equi* disease are a major source of contagion and shed bacteria (determined by both culture and PCR) continuously or, more commonly, intermittently in nasal discharge and lymph node pus. The duration and magnitude of shedding is unrelated to the severity of clinical signs. Although detailed epidemiological data from field outbreaks are not available, it is likely that bacterial shedding is initially continuous and becomes intermittent with increasing time post infection (p.i.). Bacterial shedding also occurs during convalescence (George et al 1983, Timoney 1988b, Grant et al 1993, Newton et al 2000) for up to 3–4 weeks after the cessation of clinical signs. In a small proportion of recovered horses, a carrier state is established (defined as shedding of bacteria by an apparently healthy horse for more than 1 month after cessation of clinical signs) with intermittent shedding of bacteria in nasal secretions. Bacterial carriage may continue for several months or even years (Newton et al 1997). Carrier horses are probably the major reservoir of *S. equi* infection and almost certainly account for strangles outbreaks on establishments following the introduction of new animals (George et al 1983, Newton et al 1997) or the reappearance of clinical disease months to years after previous outbreaks on particular premises. The existence and importance of an environmental reservoir of infection has been the subject of debate.

Streptococcus equi is not a hardy organism and survives for only short periods (less than 10 days) if desiccated or exposed to ultraviolet light. Survival in the environment will depend on ambient temperature, humidity, rainfall, and ultraviolet light. Bacteria survive for longer periods (up to 2 months) in water or in pus smeared onto wood or tack (Jorm 1991). However, these experiments were performed in controlled conditions of high humidity and low temperature and almost certainly overestimate survival times in the environment. Irrespective of the duration of environmental survival, the contagiousness of *S. equi* decreases exponentially with time and transmission from the environment is

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Box 23.3. Risk groups for *S. equi* outbreaks

- Low risk – Closed populations that do not travel
  - Horses kept on private premises which do not mix with other horses; no or infrequent travel to events, competitions, shows
- Medium risk – Closed populations that travel
  - Horses kept in closed yards but which travel to events, competitions, shows and race meetings
- High risk – Open populations
  - Livery yards, studs, dealer yards
  - Frequent mixing of new arrivals and age groups; stabling allows contact between horses; communal feed and water troughs

Fig. 23.5. *Streptococcus equi* infection ("strangles") causes a moderate to profuse bilateral mucopurulent nasal discharge.

thus only likely with freshly (less than 1 week) discharged bacteria. Nevertheless, care should be taken with the stable and feed environment especially communal water troughs. *Streptococcus equi* is readily inactivated by commonly used disinfectants including bleach, quaternary ammonium compounds, chlorhexidine, and Virkon, thus greatly facilitating environmental control.

Clinical signs

Severe "classical" strangles

The incubation period is variable (1–14 days), even following experimental challenge, and is probably dependent on the strain of bacterium, the size of the infecting inoculum,

and the previous immune experience of the horse. Pyrexia (up to 42°C) is the earliest clinical sign and persists for 2–3 weeks p.i. Affected horses are depressed and anorexic for 1–2 weeks, possibly longer if complications develop. Nasal discharge becomes increasingly purulent (Fig. 23.5) and persists for 2–4 weeks. Bacterial shedding continues for a considerable period after resolution of pyrexia and overt clinical signs of disease. The duration of bacterial shedding, and hence contagiousness, is typically in the range of 3 to 8 weeks after infection but can be even longer in some horses.

Lymph node enlargement is palpable from 2–3 days after infection and clinically apparent abscesses usually develop 2–3 weeks later (Yelle 1987, Wilson 1988, Timoney 1993). Histological evidence of abscessation is present from 1–2 days p.i. Abscesses usually develop in the submandibular and retropharyngeal lymph nodes (Fig. 23.6). Their superficial position makes submandibular abscesses comparatively easy to diagnose and manage (Fig. 23.7). Retropharyngeal abscesses are more difficult to diagnose because of their deeper position. Retropharyngeal abscesses may burst and drain externally (over the lateral laryngeal region) or internally (dorsally into the guttural pouches). Large, unruptured retropharyngeal abscesses can cause moderate or marked airway compression with ventral deviation of the trachea (Fig. 23.8) and occlusion of the nasopharynx, resulting in inspiratory dyspnea and possibly stertorous inspiratory noise (Sweeney et al 1987). Guttural pouch empyema may develop secondary to rupture and drainage of retropharyngeal abscesses into the guttural pouch (see Fig. 28.2). However, retropharyngeal abscessation is not a prerequisite because bacteria gain access to the pouches directly from the nasopharynx via the pharyngeal ostia of the auditory tubes. Guttural pouch empyema causes intermittent, mostly unilateral, purulent

Fig. 23.6. *Streptococcus equi* infection. This Welsh Mountain Pony has a resolving retropharyngeal lymph node abscess. This photograph was taken approximately 4 weeks after the onset of clinical signs.

Fig. 23.7. *Streptococcus equi* infection. Abscesses initially develop in the lymph nodes draining the head. This horse has a draining submandibular lymph node abscess. Abscesses can also develop in the parotid and retropharyngeal lymph nodes. Reproduced with the permission of Prof. D. Paul Lunn, CSU.

Fig. 23.8. *Streptococcus equi* infection. Large, non-ruptured retropharyngeal lymph nodes may compress the nasopharynx and trachea causing dyspnea. This radiograph shows ventral deviation of the trachea by a large retropharyngeal lymph node abscess. Reproduced with the permission of Prof. D. Paul Lunn, CSU.

nasal discharge. There is usually no obvious guttural pouch swelling externally. The pouch may be painful on percussion or palpation. Chondroids (balls of inspissated pus that contain viable bacteria) may develop in chronic cases of guttural pouch empyema. Persistent (months to years) guttural pouch infection may develop in a small proportion (<10%) of recovered horses, with a carrier remaining in an estimated 50% of outbreaks (Newton et al 1997, 2000). These animals are often asymptomatically infected and shed bacteria intermittently, often in small numbers, in the respiratory tract secretions. Infection may be established in the sinuses, causing clinical signs of sinusitis (unilateral nasal discharge with or without pain and dullness on sinus percussion). The sinuses may also be sites for chronic carriage as well as, or instead of, the guttural pouches.

In most cases bacteria do not disseminate beyond the head but in a small proportion of horses the bacteria disseminate widely via the blood and lymphatics causing metastatic abscessation ("bastard strangles") in the abdomen (abdominal lymph nodes, viscera, and peritoneum), thorax (thoracic lymph nodes, lungs, pleura, and mediastinum), central nervous system, eye, skeletal and cardiac muscle, tendon and joint sheaths (Sweeney et al 1987). Metastatic abscessation causes clinical signs relating to the region where the abscesses develop together with more generalized and non-specific signs including weight loss, intermittent pyrexia, and anorexia. It is generally a chronic, progressive syndrome with obvious clinical signs developing weeks to months after infection and frequently resulting in death (Sweeney et al 1987). Clinical signs are often insidiously progressive and initially vague, including intermittent pyrexia, depression, and weight loss. Later, organ- or region-specific clinical signs may appear, including colic,
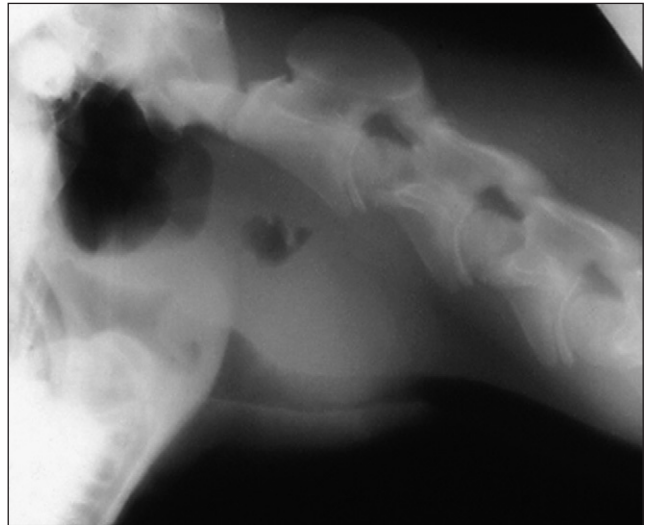
diarrhea, coughing, dyspnea, and seizures (Spoormakers et al 2003). The *S. equi* antigens in the circulation can trigger purpura hemorrhagica, an immune complex-mediated, aseptic, leukocytoclastic vasculitis, causing petechial hemorrhages and well-demarcated subcutaneous edema (Fig. 23.9). There may be oozing of serum from the skin or even skin necrosis and sloughing. Vasculitis and edema occur in organ systems throughout the body including the viscera, musculature, and kidneys, causing a variety of other signs to occur including depression, pyrexia, tachycardia, tachypnea, reluctance to move, colic, epistaxis, and weight loss (Galan & Timoney 1985a,b).

Other strangles-related clinical signs include agalactia, although this is believed to be secondary to pyrexia and anorexia and not as a direct consequence of *S. equi* invading the udder and causing disease.

"Atypical" strangles

In most outbreaks in the UK a minority of horses develop classical strangles. The majority of horses exhibit milder clinical signs and experience a transient, self-limiting infection. In some horses infection is subclinical. This milder form of *S. equi* infection is referred to as "atypical" strangles (Woolcock 1975, Prescott et al 1982, Grant et al 1993). It resembles a URT viral infectious disease with pyrexia, depression, lymph node enlargement, and nasal discharge and is frequently not recognized as *S. equi* infection if appropriate samples are not collected for microbiology. The more serious sequelae of "classical" strangles (lymph node abscesses, guttural pouch empyema, metastatic abscessation, and purpura hemorrhagica) do

Fig. 23.9. *Streptococcus equi* infection. Purpura hemorrhagica is an immune-mediated vasculitis with mucosal and visceral hemorrhages and subcutaneous edema. This horse has large plaques of proximal limb and ventral trunk edema. Reproduced with the permission of Prof. D. Paul Lunn, CSU.

not develop in the milder "atypical" form of strangles. It is not clear why there are two forms of the disease, but bacterial strain differences, horse genetic differences, and previous immune exposure of the horse, are probably all contributory factors. Bacteria isolated from "atypical" cases retain their virulence and are capable of causing more severe disease in other horses.

Diagnosis

Outbreaks of URT infectious disease with high morbidity, pyrexia, depression, purulent nasal discharge, coughing, and lymphadenopathy progressing to abscessation, especially in yards with movement and mixing of horses, is strongly suggestive of classical strangles (Timoney 1993). The milder "atypical" disease is difficult to diagnose with certainty on clinical grounds alone because it resembles other causes of URT infectious disease, including equine

influenza virus, equine herpesvirus, equine viral arteritis, and equine rhinitic virus. Hematology will usually reveal a leukocytosis with neutrophilia from 7 to 10 days p.i., but this is not diagnostic for *S. equi* infections. Horses with purpura hemorrhagica show anemia, neutrophilia, hyperproteinemia, hyperfibrinogenemia, hyperglobulinemia, and raised muscle enzymes (creatine kinase and aspartate transaminase).

Serology to detect antibody against SeM is commercially available but lacks specificity because of cross-reaction with other streptococcal M proteins, especially the *S. zooepidemicus* M protein. Confirmation of *S. equi* infection requires culture of viable bacteria or detection of bacterial DNA by PCR, using a nested PCR and primers amplifying a region of SeM (Timoney & Artiushin 1997, Newton et al 2000). Bacterial culture can be performed on nasal, nasopharyngeal, and abscess swabs; nasal washes; guttural pouch lavages; and abscess aspirates. Sample type, quality and handling after collection are important considerations because bacterial shedding is often intermittent and the number of viable bacteria is variable. Samples should be kept cool and processed as soon as practicable. Swabs should be stored in charcoal and can be posted to diagnostic laboratories. Nasal and guttural pouch lavages are performed with approx 50 ml phosphate-buffered saline via a 10-cm length of rubber tubing inserted into the nasal cavity (nasal lavage) or transendoscopically (guttural pouch lavage). The sample can be pelleted before culture to increase sensitivity. Samples need to be processed the same day if possible, making them less suitable for postal delivery. Lavages provide a superior sample to swabs because a greater surface area of epithelium is sampled. False negatives (i.e. negative results in horses infected with *S. equi*) occur with both culture and PCR. The sensitivity of culture of single samples is low (between 20 and 40%). The sensitivity of PCR is higher (between 50 and 80%). Combining these tests increases the sensitivity further.

Guttural pouch empyema causes chronic, principally unilateral, nasal discharge. There is usually no pouch distension or pain apparent on palpation. Endoscopy confirms the diagnosis. Lateral radiographs can be used to demonstrate a fluid line in the pouch but are most useful for identifying retropharyngeal lymph node enlargement and abscessation. Ultrasonography is also useful for assessing retropharyngeal lymph node enlargement and abscessation. *Streptococcus equi* carriage occurs most frequently in the guttural pouches but also occurs in other sites, including the paranasal sinuses. Guttural pouch carriage is frequently asymptomatic and occurs without frank empyema. On endoscopy there may be small quantities of pus in the pouches, chondroids, discharging lymph nodes or areas of epithelial inflammation. In some cases the pouches appear grossly normal (Fintl et al 2000). Pouch carriage is confirmed by endoscopic guttural pouch lavage using 30–50 ml saline.

Sinus carriage may also be asymptomatic, although there may be clinical signs of sinusitis. Diagnosis is by sinus radiography, sinoscopy, and lavage. Metastatic abscessation can be difficult to diagnose because abscesses can form at almost any anatomical site, often in regions that are difficult to examine physically or to image. Affected animals show intermittent pyrexia, depression, illthrift, and weight loss with variable leukocytosis and neutrophilia. There may be signs relating to the abscessed organ(s) or more general signs including colic (for abdominal abscesses), thoracic pain, dyspnea and tachypnea (for thoracic abscesses), and seizures or other cerebral signs (for central nervous system abscesses). Ultrasonography, radiography, abdominocentesis, and thoracocentesis can be useful diagnostic aids and magnetic resonance imaging has been used to diagnose central nervous system abscesses.

Horses with mild "atypical" disease are easily misidentified as not being infected with *S. equi* unless a careful clinical assessment is made. It should be remembered that in some outbreaks the majority of *S. equi*-positive (by culture or PCR) horses are apparently healthy or exhibit subtle clinical signs only. Very importantly, convalescent horses can continue to be PCR positive for *S. equi* for extended periods (up to 4 weeks) after viable bacteria can no longer be isolated. In other words, positive results on PCR in the early convalescent phase should not be interpreted as evidence for continued infection or the presence of a carrier state.

Although the majority of URT infectious disease with lymph node abscessation are the result of *S. equi* infection, other Lancefield group C streptococci, especially *S. zooepidemicus*, can also be involved either in combination with *S. equi* or as sole infections. Detection of carriers is not easy or inexpensive and requires a committed owner and clinician (Newton et al 1997, 2000). The ability to identify carriers serologically would provide a valuable, effective and simple management aid. However, serology based on SeM does not distinguish carriers from normal convalescent horses. Diagnosis, as for clinical cases, requires culture of viable bacteria or demonstration of bacterial DNA from nasopharyngeal swabs or guttural pouch lavages. Carriers shed bacteria intermittently and in low numbers, making reliable detection difficult. Nasal swabs do not provide a sample of sufficient diagnostic quality. Large, gauze nasopharyngeal swabs provide a much better sample because they absorb a large volume of secretion from the region close to the pharyngeal ostia of the guttural pouches. The sensitivity of nasopharyngeal swabs increases with serial sampling and if bacterial culture is combined with PCR (Newton et al 2000). The sensitivity of a single nasopharyngeal swab is low (approximately 30%) but is improved if three nasopharyngeal swabs are collected at weekly intervals. Further nasopharyngeal swabs are likely to increase sensitivity. PCR on a single nasopharyngeal swab improves sensitivity to 50%. Combining culture and PCR on a single nasopharyngeal swab has a sensitivity of 53% rising to 80% for three swabs at weekly intervals. The sensitivity of guttural pouch lavages is higher with a predicted sensitivity of 80–90% for carrier detection when both culture and PCR are performed. The implication of these data is that whilst serial nasopharyngeal swab samples are the most practical means of identifying carriers in a large herd, single guttural pouch lavages provide a useful alternative in small groups of animals.

Management

The three aims of managing an outbreak of strangles are (1) prevent spread of infection to new premises; (2) limit spread of infection within the infected premises; and (3) ensuring that carriers are identified and treated at the end of the outbreak. Currently, the UK does not have a compulsory reporting, tracing, testing or isolation/movement restriction policy for *S. equi*. However, movement of horses on and off the infected premises should be suspended to reduce the risk of infection spread elsewhere. Personnel should be briefed about the risk of indirect transmission and precautions should be taken with hand washing, clothing, footwear, and tack. Management of clinical cases requires strict isolation and barrier nursing. On suspicion of *S. equi* infection, the yard should be divided into clean (unaffected) and dirty (affected) areas and horses should be assigned to one group or the other based on clinical signs (initially pyrexia). Daily checks should be made and as the outbreak progresses some horses from the unaffected group that were incubating disease will require movement into the affected group. Ideally different staff should attend each group but where this is not possible staff should attend the "clean" area first. Dedicated boots, overalls, and latex gloves should be worn when attending the dirty area. Bedding should be disinfected before disposal from the dirty area. Environmental contamination by *S. equi* can be readily contained by any of the commonly used disinfectants including bleaches, phenolic compounds, quaternary ammonium compounds disinfectants, and Virkon. Feeding utensils should not be shared and particular attention should be paid to communal water troughs. These should be emptied and disinfected. Woodwork (fencing and stable partitions) should also be disinfected to reduce any possible environmental bacterial reservoir. The apparent lack of aerosol transmission of *S. equi* means that physical separation of the two groups by a solid barrier to prevent direct horse-to-horse contact should be sufficient to prevent spread between groups.

Affected horses should be kept in clean, dry, well-ventilated, and dust-free stables and fed damp, palatable feed. Antibiotic treatment of clinical cases is controversial with some clinicians advocating that antibiotics should never be used for fear of prolonging the clinical disease, reducing immunity or increasing the risk of metastatic
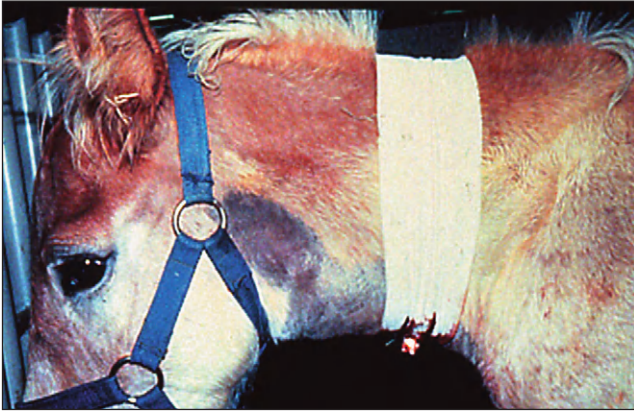
Fig. 23.10. Retropharyngeal lymph node abscesses that fail to rupture and drain can cause significant airway compression and dyspnea. This horse required a tracheotomy to alleviate its dyspnea. Reproduced with the permission of Prof. D. Paul Lunn, CSU.

abscessation. There are however no experimental or field data to support these beliefs (Sweeney et al 2005). Antibiotics may be used in early cases with pyrexia and depression, especially in foals, to improve welfare and reduce the period of bacterial shedding. "In-contact" horses can also be treated with antibiotics provided they can be moved to a clean area. Antibiotics should not be used in horses with developing abscesses: these should be managed by hot fomentation to encourage drainage. Large non-ruptured retropharyngeal lymph node abscesses may require needle drainage under ultrasound guidance if the airway is compromised and the horse develops inspiratory dyspnea. If drainage cannot be effected a temporary tracheotomy may be required until the abscess resolves (Fig. 23.10). The antibiotic of choice is penicillin because

this produces the highest bacterial cure rate and resistance appears to be uncommon. Other antibiotics including cephalosporins, fluoroquinolones, and tetracyclines can be used. Potentiated sulfonamides should be avoided because, although *S. equi* is usually sensitive *in vitro*, this antibiotic combination is ineffective in abscesses because of the high concentrations of folic acid present in pus.

At least 1 month after the end of the outbreak the yard should be screened for carriers using either serial nasopharyngeal swabs or guttural pouch lavages. Carriers can be successfully treated by guttural pouch lavage and antibiotic treatment (Verheyen et al 2000). Chondroids should be removed endoscopically using a polyp basket (or surgically if they cannot be retrieved via the nasopharyngeal ostium). The affected pouch should be lavaged daily with approximately 2 liters of saline via an indwelling Foley catheter (Fig. 23.11). Benzylpenicillin should be instilled into the pouch after each lavage and lavaging should continue until the pouch is negative on culture (PCR should not be used to test whether treatment has been successful because this does not distinguish dead from live bacteria). Horses with purpura hemorrhagica should be treated with corticosteroids [dexamethasone 0.1 mg/kg twice a day intravenously (q 12 h IV)]. Penicillin [22,000 IU/kg q 12 h intramuscularly (IM) or four times a day (q.i.d.) IV] should also be used if active *S. equi* infection is suspected. Supportive therapy, including IV fluids, is very important.

Prevention

The American College of Veterinary Internal Medicine (ACVIM; Sweeney et al 2005) has published a consensus statement containing guidelines for the treatment, control, and prevention of *S. equi*. In the UK, the Horserace Betting

Fig. 23.11. Guttural pouch empyema following *S. equi* infection. This horse's right guttural pouch is being lavaged via an indwelling Foley catheter.

Levy Board (HBLB) publishes a Code of Practice on strangles detailing the management measures required to control infection (www.hblb.org.uk) (Box 23.4).

The management measures detailed in the ACVIM Consensus Statement and the Strangles Code of Practice published by the HBLB (www.hblb.org.uk) are the cornerstone of prevention and are designed to maintain biosecurity:

- stop movements on and off the premises if disease occurs
- quarantine and test new arrivals
- investigate and isolate new clinical cases promptly; use rectal temperatures to identify new cases
- screen convalescent horses to identify and treat carriers.

New arrivals should be kept quarantined until confirmed free from *S. equi* infection by culture and PCR of three nasopharyngeal swabs collected at weekly intervals. Suspected new clinical cases should be isolated, investigated (by bacteriology), and treated promptly. Yards with endemic infection should screen all horses to identify carriers by nasopharyngeal swabs and/or guttural pouch lavage. Carriers should be isolated and treated until confirmed bacteriologically negative by culture and returned to the herd. For horses whose management presents a low–medium risk of *S. equi* infection (i.e. "closed" yards or horses from "closed" yards that travel to competitions/ events/race meetings) the Code of Practice can usually be successfully implemented and provides effective control of disease, reducing the need to vaccinate. However, in many "open" yards (e.g. studs, dealer and livery yards) the Code of Practice is difficult to implement. It is in these situations that vaccination forms an important part of *S. equi* control measures.

There has, however, been limited success in developing safe and efficacious *S. equi* vaccines (Hoffman et al 1991, Smith 1994, Jacobs et al 2000). In the USA and Australia commercial vaccines consisting of inactivated whole bacteria (Equivac-S®, CSL Limited, Victoria, Australia), M-protein extracts (StrepVax II™, Boehringer Ingelheim, St. Joseph, MO, USA), enzyme extracts of *S. equi* (Strepguard™, Intervet, Millsboro, DE, USA), all administered IM, and most recently a live, attenuated, intranasal vaccine (Pinnacle™ I.N., Fort Dodge, IA, USA) have been developed (Walker & Timoney 2002). The efficacy and/or safety or all of these vaccines has been questioned (Timoney 1988a, 1999) and

none of these products has been licensed for use in Europe. The failure of killed and subunit IM vaccines to provide effective immunity is probably because the route of administration, antigen preparation, and hence type of immune response stimulated are inappropriate (Jacobs et al 2000) because mucosal immunity (osponizing mucosal IgA and IgG) is principally required to provide protective immunity (Galan & Timoney 1985a,b, Sheoran et al 1997). Whilst live attenuated intranasal vaccines have the potential to present a near full complement of bacterial antigens to the horse's immune system in a physiologically correct context, it has proved difficult to achieve a balance between attenuation and maintenance of immunogenicity. There have been concerns, for example, about the occurrence of side effects and even the induction of clinical disease following vaccination with the Pinnacle vaccine strain. In 2005 the first European-licensed commercial strangles vaccine was launched (Equilis Strep-E, Intervet, Milton Keynes, UK). This is a live attenuated (genetically modified) vaccine and is delivered intramucosally into the lip. The genetic modification is deletion of a metabolic pathway gene (*aroA*) required for aromatic amino acid synthesis, which is required for growth in a mammalian background. This vaccine has a comparatively short duration of protection and requires revaccination at 3-month intervals to maintain immunity in an endemically infected area, although this interval can be extended to 6-monthly in a non-endemically infected area provided immediate revaccination is carried out between 3 and 6 months if disease appears in that area. The delay between vaccination and appearance of protective immunity means that vaccination of naive animals in the face of an outbreak is unlikely to be effective. The effect of vaccination on carrier animals is unknown but is unlikely to influence the carrier state.

Prognosis

The prognosis for *S. equi* infections is variable, although mortality is generally low and most horses make a complete recovery. The prognosis for mild disease ("atypical strangles") is good although it is not known whether carriers arise following this form of the disease. For the more severe disease ("classical strangles") the prognosis depends on whether complications develop. The majority of horses recover once abscesses have resolved but up to 10% of cases develop complications that delay recovery or may be fatal. Data on the duration of protective immunity after recovery from natural infection is scant but British Army records suggest that long-lasting (approximately 5 years duration) immunity develops in most (> 75%) recovered horses (Todd 1910). It should be remembered that this observation relates to large, endemically infected herds and the duration of immunity may be shorter in the modern management settings.

Bacterial Infectious Diseases of the Lower Respiratory Tract

The respiratory tract is not sterile but is populated by a dynamic population of Gram-positive and Gram-negative bacteria including *Streptococcus* spp., members of the Pasteurellaceae and *Mycoplasma* spp. that are capable of responding rapidly to changes in the airway environment and to host defenses. In healthy horses bacterial colonization is heaviest in the nares and nasopharynx and decreases along the trachea so that the airway beyond the carina is normally sterile.

Bacterial infections of the lower respiratory tract (LRT) occur in horses and ponies of all ages but are of particular importance in foals, young racehorses in training, and mature race and sport horses. The clinical disease caused by bacterial LRT infections is different in each age group (Table 23.2). Bacterial involvement in neonatal septicemia and neonatal bacterial pneumonias is dealt with in Chapters 24 and 45. In older foals (up to 8–9 months) bacterial LRT infections are common and generally cause bronchopneumonia. In young racehorses in training bacterial LRT infections are also common and cause chronic, low-grade tracheobronchitis (inflammatory airway disease). In mature race and sport horses bacterial LRT infection is not common and causes sporadic, potentially fatal, pneumonia and pleuropneumonia.

Disease summaries

Bronchopneumonia in foals

Overview

Pneumonia is common in foals up to 8–9 months old, and is generally caused by airway-resident bacteria that act as opportunistic pathogens when host airway defenses are compromised. Most affected foals have mixed infections involving one or more of the following: *Streptococcus zooepidemicus*, *S. pneumoniae*, *Actinobacillus equuli*, *Pasteurella caballi* and *Mycoplasma* sp. Other bacteria can also be involved including *Bordetella bronchiseptica*, *Escherichia coli* and *Klebsiella* sp. A variety of events damage airway defenses including respiratory virus infections, *Parascaris equorum* and *Dictyocaulus arnfieldi* migration and stresses that include other intercurrent illnesses, transport, weaning, and general anesthesia.

Clinical signs

Foals show typical, progressive clinical signs of LRT infectious disease: variable pyrexia, depression, coughing, and bilateral purulent or blood-tinged purulent nasal discharge. Nasal discharge may not be copious because mucociliary clearance is generally impaired and a scant nasal discharge should not be taken as an indication that there is little discharge in the trachea and bronchial tree. As the disease progresses, foals develop tachypnea, dyspnea, and eventually respiratory distress. Affected foals must be handled sympathetically because they have reduced respiratory capacity. Auscultation is worthwhile. There is often pooling of discharge within the trachea at the thoracic inlet, which produces coarse crackles in the trachea. Auscultation of the lung field may identify crackles and wheezes, especially in the cranioventral lung. Adventitious sounds may not always be audible and a re-breathing test can be performed to accentuate these. This procedure should not be performed in foals with severe disease or marked dyspnea. In advanced disease some areas of the lung may have reduced or absent breath sounds on auscultation and decreased resonance on percussion indicating pulmonary abscess/consolidation or pleural effusion.

Pathology

Pathological changes occur mostly in cranioventral regions of the lung: the affected lung is consolidated and red. There is copious mucopurulent or purulent discharge within the bronchi, bronchioles, and alveoli. Consolidation may be diffuse or occur in focal patches. Cut sections of lung will generally ooze mucopus or pus from the airway. There may be fibrin tags on the visceral pleura. With chronic disease the discharge often becomes more mucopurulent. Localized pulmonary abscesses and focal adhesions may develop between the visceral and parietal pleurae. Bacterial infection may extend through consolidated regions of lung to reach the pleurae and produce pleural effusion.

Diagnosis

The clinical findings of pyrexia, depression, coughing, dyspnea, tachypnea (especially when handled, stressed or running) with variable nasal discharge, and abnormal lung auscultation are highly suggestive of pneumonia. Microbiological samples are required to identify which bacterial species are present and their antibiotic sensitivity.

Table 23.2. Clinical disease syndromes caused by bacterial LRT infections by age group

Age group	Disease caused	Morbidity	Mortality	Prognosis
Foals	Bronchopneumonia	Can be high	Can be high	Variable – moderate to poor
Thoroughbred racehorses in training	Inflammatory airway disease	High	Low	Good
Adults	Pneumonia and pleuropneumonia	Low	Can be high	Moderate

Bronchopneumonia usually involves mixed infections of mostly aerobic Gram-positive (mainly streptococci) and Gram-negative bacteria (Pasteurellaceae, possibly with *Mycoplasma* sp.). The features of the different bacteria involved are discussed in the following sections. Samples for bacteriological investigation should be collected with care. For foals with moderate to severe disease that are struggling to maintain ventilation, endoscopy and tracheal or bronchoalveolar lavage may be too invasive and may reduce ventilation to dangerously low levels. Transtracheal (percutaneous) lavage is less invasive.

Management

Affected foals should be isolated (along with the mare) to limit contagion to others in the group. They should be handled with care and kept in a clean, dry, well-drained, and well-ventilated dust-free stable environment. Since bronchopneumonia in foals is usually the result of mixed infections a broad-spectrum antibiotic regimen should be used. Streptococci are almost always involved (*S. zooepidemicus* and also *S. pneumoniae*) and regimens should contain penicillin (22,000 IU/kg q 12 h IM or q.i.d. IV) together with an aminoglycoside [usually gentamicin 6.6 mg/kg once a day (s.i.d.) IM or IV] to provide cover against Gram-negative bacteria. With the exception of *Bacteroides fragilis*, which is inherently penicillin resistant, anaerobic bacteria are usually sensitive to penicillin. Metronidazole can be used to provide additional spectrum against penicillin-resistant anaerobes. Alternative antibiotics include oxytetracycline (5 mg/kg IV s.i.d. or q 12 h), ceftiofur (5 mg/kg q 12 h IM or IV) or enrofloxacin (5 mg/kg s.i.d. IV). Oxytetracycline and enrofloxacin are effective against *Mycoplasma* sp. Trimethoprim–sulfonamides can also be used but their efficacy is reduced in pus, which restricts their value for treatment of foals with large volumes of exudate. Prevention centers on limiting stocking density, maintaining good air hygiene, controlling pulmonary parasites, and reducing transport and other management stresses. There are no vaccines against the bronchopneumonia-causing bacteria of foals.

Inflammatory airway disease
Overview

Inflammatory airway disease (IAD) is a common condition affecting young racehorses in training. It may also affect older performance horses of all breeds. IAD is characterized by neutrophilic airway inflammation and accumulation of mucopurulent exudate within the trachea and bronchial tree. As awareness of this disease has increased so have diagnosis rates. The prevalence of IAD appears to be between 10 and 20% in most groups of horses in training but may be as high as 50%. IAD may also be common in housed groups of young ponies and horses of other breeds, including sport horses, subjected to training or other management stresses, although there are few data about

disease in these types of animals. The disease has a strong association with commensal airway bacteria that act as opportunistic pathogens, but up to 40% of cases do not have a clear association with bacteria and other factors, e.g. hypersensitivity to airborne allergens or hyperreactivity to airborne irritants, may be involved in these cases. A variety of bacteria are associated with IAD including *S. zooepidemicus* and *S. pneumoniae* along with *A. equuli*, *P. caballi* and *Mycoplasma* sp.

Clinical signs

Clinical signs are highly variable. In many cases infection is subclinical and detected only on endoscopic examination. Clinical signs, when present, are usually subtle and restricted to poor performance, intermittent coughing, and mucopurulent nasal discharge. Importantly, horses with IAD do not have dyspnea, depression, pyrexia or other indications of systemic illness. Disease occurs both sporadically and as outbreaks, suggesting that there can be contagious transmission from affected horses to other susceptible horses in the group. This presumably is a function of the large bacterial loads present in the airway of affected horses with transmission via infected respiratory droplets and discharges. Affected horses are often unremarkable on clinical examination but tracheal crackles may be audible at the thoracic inlet. There are usually no abnormalities on auscultation of the lung fields. Endoscopy reveals a variable quantity of mucopus within the trachea and possibly extending beyond the carina into the left and right bronchial trees. Copious volumes of mucopus may be present at the thoracic inlet (Fig. 23.12).
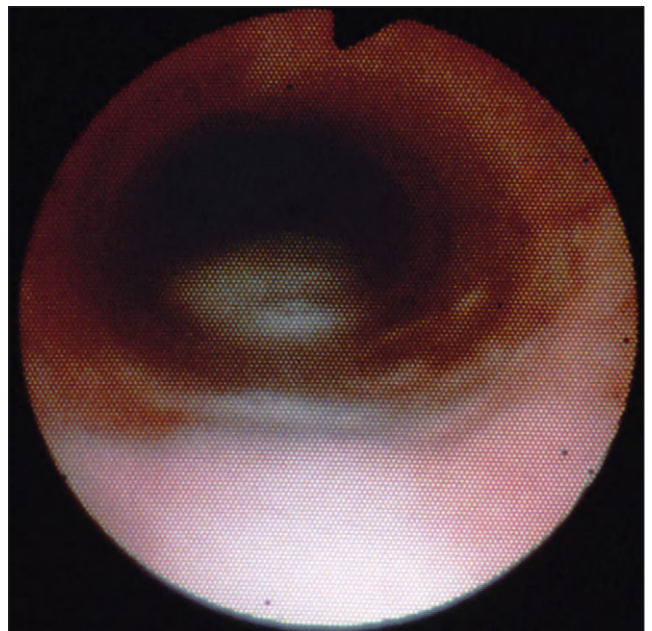
Fig. 23.12. Endoscopic image of a horse with inflammatory airway disease showing a high volume of tracheal mucopus which, on cytological examination, contained increased numbers of neutrophils.

Box 23.5. Quantitative bacteriology

- A means of enumerating the number of bacteria present in tracheal lavage samples
- Allows the clinical significance of bacterial isolates to be determined
- Viable counts of $>10^3$ cfu/ml are likely to be significant

Method
- Mix tracheal lavage sample thoroughly
- Perform serial tenfold dilutions of the sample
- Spread 100 μl of each dilution onto suitable culture plates (usually sheep blood agar)
- Incubate, and count colonies to provide a viable count per ml of tracheal lavage

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Diagnosis

Clinical signs and endoscopic findings are suggestive of IAD. Confirmation of a possible bacterial etiology requires microbiological culture of tracheal lavage samples followed by antibiotic sensitivity testing of isolates. The features of the different bacteria involved are discussed in the following sections. It is essential that quantitative bacteriology (Box 23.5) be performed when assessing microbiological samples from suspected cases of IAD. This is because whenever mucociliary clearance is impaired and mucopurulent discharge accumulates in the airway, resident bacteria are able to grow to increased titers. Consequently, simply demonstrating that bacteria are present in a tracheal lavage sample is insufficient evidence of bacterial infection and IAD: it is the number of bacteria present that is important. Bacterial counts of less than 10^3 colony-forming units (cfu) per ml of lavage should not be regarded as significant. Titers greater than 10^3 cfu/ml, and especially greater than 10^5 cfu/ml, are convincing evidence of bacterial involvement. Tracheal lavage and broncho-alveolar lavage cytology reveal airway neutrophilia possibly with increases in mast cells. Phagocytosed and free bacteria may be visible.

Management

IAD should be treated as a contagious disease and horses should be divided into affected and non-affected groups. Training should be suspended and horses should be stabled in a dry, well-drained, and well-ventilated dust-free environment. Straw should not be used for bedding and hay, if used, should be soaked and fed wet. Horses should be kept at pasture whenever possible. Where bacteria are involved in IAD they are mostly present as mixed infections and should therefore be treated using a broad-spectrum antibiotic regimen. Streptococci (*S. zooepidemicus* and also *S. pneumoniae*) are the most prevalent bacteria involved but

Gram-negative bacteria (*Actinobacillus* sp., *P. caballi*) and *Mycoplasma* sp. are also commonly involved. If treatment commences before the results of culture and sensitivity are known, penicillin (22,000 IU/kg q 12 h IM or q.i.d. IV) together with gentamicin (6.6 mg/kg s.i.d. IM or IV) is a suitable broad-spectrum regimen. The regimen may require modification once microbiology results are known; for example infection with *S. zooepidemicus* alone does not require gentamicin treatment. With the exception of *Bacteroides fragilis*, which is inherently penicillin resistant, anaerobic bacteria are usually sensitive to penicillin. Metronidazole can be used to provide additional spectrum against penicillin-resistant anaerobes. Alternative antibiotics include oxytetracycline (5 mg/kg IV s.i.d. or q 12 h), ceftiofur (5 mg/kg q 12 h IM or IV) or enrofloxacin (5 mg/kg s.i.d. IV). Oxytetracycline and enrofloxacin are effective against *Mycoplasma* sp. Trimethoprim–sulfonamides can also be used but have reduced efficacy in pus. Where an allergic etiology is suspected horses should be treated with corticosteroids by inhalation [beclomethasone 1–3 μg/kg q 12 h or three times a day (t.i.d.); fluticasone 2–4 μg/kg q 12 h or t.i.d.] or systemically (dexamethasone 0.1 mg/kg s.i.d. IM or IV). Sodium cromoglycate can be used in horses with increased mast cells in tracheal or bronchoalveolar lavage fluids. Bronchodilators (clenbuterol 0.8 μg/kg IV q 12 h or by inhalation; ipratropium bromide 1 μg/kg by inhalation t.i.d.; salmeterol 1 μg/kg by inhalation t.i.d.) can be used. Horses refractory to antibiotic treatment are sometimes treated with immunomodulators. Prevention centers on limiting stocking density, maintaining good air hygiene, controlling pulmonary parasites, and reducing transport and other management stresses.

Pneumonia and pleuropneumonia
Overview

Pneumonia and pleuropneumonia in adult horses are caused by resident commensal bacteria that cause disease opportunistically (see Chapter 46). Disease occurs following events that suppress mucosal immunity and damage airway defenses, allowing resident microbes to multiply and induce disease. These events include a variety of stresses such as long-distance transport, overcrowding in barns with poor air hygiene, hospitalization (especially being cross-tied for lengthy periods), and general anesthesia. In adult horses, in contrast to foals, primary virus infections are an uncommon predisposition to pneumonia or pleuropneumonia. The disease often presents as a peracute fibrinous pleuropneumonia but may also progress to chronic pneumonia with regions of consolidated lung and pulmonary abscess. The acute form that follows transport is referred to as "equine shipping fever" and is analogous to the similarly named disease in farm animals. Equine shipping fever is common in horses that are kept for racing and competition, especially sport horses and some

thoroughbred and standardbred racehorses, because they are frequently subjected to long-distance travel. A wide variety of aerobic and anaerobic Gram-positive and Gram-negative bacteria are associated with pneumonia/pleuro-pneumonia. Aerobes include *S. zooepidemicus*, *S. pneumoniae*, *A. equuli*, *P. caballi* and *Mycoplasma* sp. Anaerobes include *Clostridium* sp., *Fusobacterium* sp. and *Bacteroides* sp. The majority of cases are caused by *S. zooepidemicus* alone or in combination with other bacteria.

Pathogenesis

The regulation of resident microbial populations by host defenses is incompletely understood but involves physical defenses such as the mucociliary escalator, non-specific immunity such as complement and phagocytes as well as specific immunity mediated by B and T lymphocytes. Transport, especially in poor air hygiene and with the head tied in an upright position, reduces mucociliary clearance and a variety of stresses reduce the efficiency of phagocytosis by neutrophils and macrophages. Bacteria are responsive to the local airway environment and possess a variety of sophisticated sensing systems that up-regulate virulence mechanisms when host defenses change (discussed for each bacterium in the following sections of this chapter). It is mainly the cranioventral regions of the lung that are affected. Pathological changes initially occur in the airway with bronchopneumonia and mucopurulent exudate in the bronchi, bronchioles, and alveoli. Affected regions rapidly become consolidated, giving them a red appearance, and within these regions pulmonary infarction and necrosis can develop, resulting in a serosanguineous suppurative exudate. In horses that develop pleuropneumonia, bacteria invade the consolidated lung and migrate to the surface of the lung causing fibrinous pleurisy with extensive fibrin deposition on the visceral pleural surface and a variable volume pleural serosanguineous purulent effusion. Adhesions may develop between the visceral and parietal pleural surfaces and cause pocketing of pleural fluid. More chronic changes develop in some horses including pulmonary abscesses, bronchiectasis, and fibrosis.

Clinical signs

Onset of clinical signs is generally acute and occurs within hours of the stressful event that compromised the airway defenses. Clinical signs are more acute, severe, and rapidly progressive with pleuropneumonia than pneumonia. The severity of the clinical signs is proportional to the amount of lung affected, the degree of pulmonary necrosis, and the volume of pleural effusion. Affected horses show pyrexia, depression, some coughing, and variable mucopurulent nasal discharge that may be blood-tinged. Nasal discharge varies because of poor mucociliary clearance. Thus the scant nasal discharges often belie the large volumes of exudate that accumulate in the airway. There is variable

dyspnea and tachypnea: horses with localized regions of pneumonia may not show obvious dyspnea at rest whereas horses with large volumes of pleural effusion show marked respiratory distress. Coarse crackles can often be heard in the trachea at the thoracic inlet as a result of pooling of exudate in this region. In horses without pleural effusion crackles and wheezes can usually be heard in the affected lung regions. A rebreathing test can be performed with caution in horses without severe clinical signs to accentuate abnormal sounds. Horses with pleural effusion show more obvious clinical signs: there may be signs of pleural pain (pain when walking and standing with elbows abducted), breathing sounds are absent in the ventral lung field, and the ventral thorax is dull on percussion because of the pleural effusion. Cardiac sounds are muffled and, occasionally, pleural friction rubs can be heard. Rebreathing tests should not be performed on horses with pleural effusion. Other clinical signs relating to sepsis and endotoxemia may develop including circulatory collapse, abdominal pain, and laminitis.

Diagnosis

The history and clinical examination findings are suggestive. Thoracic ultrasound is extremely useful and identifies the volume, location, and nature of the pleural exudate. It will identify pleural adhesions and pocketing of pleural fluid. Pulmonary abscesses, consolidation, atelectasis, and fibrosis can be identified if the affected region of the lung is superficial or if there are adhesions between the parietal and visceral pleura. Thoracic radiography is useful for assessment of abscessation and consolidation/collapse within deeper regions of the lung that are not accessible by ultrasound. Identification of the bacteria involved requires culture of airway and pleural fluid samples. Horses with severe disease may not tolerate endoscopic examination and less invasive collection of samples, for example transtracheal (percutaneous) lavage, may have to suffice. It is important to culture pleural fluid because this is, initially at least, an anaerobic environment and the population(s) of bacteria present may be different from those in the airway. However, the most prevalent bacteria involved are *Streptococcus* sp. and these are facultative anaerobes allowing them to colonize and grow both in the airway and the pleural space. Pleural fluid can be collected by thoracocentesis (Fig. 23.13) which should, ideally, be ultrasound guided to ensure accurate sampling of fluid pockets and avoid lung puncture. Thoracocentesis generally yields a purulent exudate with a high nucleated cell count (mainly neutrophils), raised protein content and free and intracellular bacteria (Fig. 23.14). A Gram stain is useful but Pasteurellaceae and *Mycoplasma* sp. are difficult to visualize microscopically because of their small size and poor stain uptake. Quantitative bacteriology is useful to provide some indication of the likely clinical significance of bacterial isolates but is less important than

Fig. 23.13. Drainage of pleural fluid from a case of pleuropneumonia.

in IAD. The isolation of anaerobes appears to be associated with a poorer prognosis than infections associated only with aerobes.

Management

Pneumonia and pleuropneumonia are usually sporadic diseases in individual horses with only a small risk of contagious transmission. However, there are anecdotal reports of transmission within hospital yards and it appears sensible to treat affected horses as contagious and take appropriate barrier precautions, especially avoiding direct horse-to-horse contact and taking care with fomites. Pleuropneumonia in particular requires aggressive management and careful monitoring. Affected horses require detailed and frequent assessment and conscientious nursing. They should be handled sympathetically and stabled in a dry, well-drained, and well-ventilated dust-free environment. They should not be bedded on straw, and hay, if used, should be soaked and fed wet. Pleural drainage is vital (Fig. 23.13) and supportive IV fluid therapy for pleural fluid loss and endotoxemia are essential. Non-steroidal anti-inflammatory drugs improve welfare and assist in control of the endotoxin-mediated systemic inflammatory response. Mixed bacterial infections, which generally include the streptococci, are often involved and the antibiotic regimen should therefore provide broad-spectrum activity. A sensible initial regimen is penicillin (22,000 IU/kg q 12 h IM or q.i.d. IV) together with gentamicin (6.6 mg/kg s.i.d. IM or IV) and metronidazole (20 mg/kg q.i.d. orally). This may need to be modified when the results of bacterial culture and sensitivity are known. Ceftiofur (5 mg/kg q 12 h IM or IV), oxytetracycline (5 mg/kg s.i.d. or q 12 h IV) or enrofloxacin (5 mg/kg s.i.d. IV) can also be used; the latter two are active against *Mycoplasma* sp. A variety of acute and more chronic complications may develop. Acute complications include pneumothorax as a result of communication between necrotic lung and the pleural space. More chronic complications include pulmonary abscess, mediastinal abscess, pleural adhesions, pulmonary atelectasis, and fibrosis. Great improvements have been made in long-distance horse transport and better understanding of air hygiene requirements, maximum journey times, and management has greatly reduced the prevalence of equine shipping fever. Disease in hospitals is difficult to prevent except that horses should be closely monitored for pneumonia/pleuropneumonia after surgical procedures. There are no vaccines against the bacteria that cause pneumonia/pleuropneumonia in adult horses.

Fig. 23.14. Pleural fluid from pleuropneumonia cases is purulent and frequently sanguineous. This pleural fluid yielded a pure culture of *S. equi* subsp. *zooepidemicus*.

•Healthy mucociliary system
•Normal mucosal immunity
 efficient opsonization,
 phagocyte chemotaxis and
 phagocytosis
•Good air hygiene
•No management stress
•Resistant horse genotype
•Down-regulation of bacterial
 virulence factors

•Decreased mucociliary clearance
•Compromised mucosal immunity
 decreased opsonization,
 phagocyte chemotaxis or
 phagocytosis
•Poor air hygiene
•Management stress
•Susceptible horse genotype
•Up-regulation of bacterial
 virulence factors

Harmless (non-
disease-causing)
resident

Harmful (disease-
causing)
pathogen

Fig. 23.15. Change in behavior of opportunist pathogens.

Bacteria as opportunist LRT pathogens

Almost all cases of bacterial LRT disease are caused by opportunist pathogens that are part of the normal resident airway flora of many horses. The traditional view of bacterial LRT disease as strictly secondary to primary viral infection or pulmonary parasitic migration is almost certainly incorrect, although this does apply in some cases, particularly foals. It is now apparent that bacterial LRT disease in all age groups, especially horses in training and adults, often occurs without a preceding viral infection, in response to changes in dynamics of the resident airway bacterial population (Newton et al 2003, Wood et al 2005). The triggers that change the behavior of opportunist bacterial pathogens from non-disease-causing residents to disease-causing pathogens are poorly understood and probably act in combination. It is highly likely that management stresses (overcrowding, poor air hygiene, transport, general anesthesia, hospitalization) act by compromising airway defenses and changing the balance of airway flora (Anzai et al 2002). There is emerging evidence that the genotype of the horse may also play a role in LRT disease susceptibility. The advent of bacterial genome sequencing and studies of functional genomics has started to unravel the complexities of the regulation of bacterial growth, regulation/expression of virulence factors and host–pathogen interactions. Understanding is far from complete but it is now clear that the bacteria involved in equine LRT disease are sophisticated pathogens whose behavior is under complex regulatory control (Fig. 23.15).

Most bacterial LRT infections in horses involve mixed populations of bacteria and mycoplasmas (Wood et al

1993, 1997, 2005, Christley et al 2001, Ward et at 1998). A similar spectrum of Gram-positive and Gram-negative bacteria are isolated from foals with bronchopneumonia, horses in training with IAD, and adult horses with pneumonia/pleuropneumonia, even though the clinical presentations of these diseases are different. *Streptococcus zooepidemicus* is the most prevalent pathogen isolated from all age groups. In foals *S. pneumoniae*, *Pasteurella* sp. and *Actinobacillus* sp. are also commonly involved although a variety of other bacteria including *Bordetella bronchiseptica*, *Klebsiella* sp. and *E. coli* can also be isolated. IAD is most strongly associated with *S. zooepidemicus*, *S. pneumoniae*, *Actinobacillus* sp. and *Mycoplasma equirhinis* infections, but a large number of other Gram-positive and Gram-negative bacteria may be isolated from affected horses. Mixed Gram-positive/negative and aerobic/anaerobic infections are common in pneumonia and pleuropneumonia in mature horses. *Streptococcus zooepidemicus*, *S. pneumoniae*, *Staphylococcus aureus*, *Actinobacillus* sp., *Pasteurella* sp., *Enterobacter* sp. and *E. coli* are frequently isolated aerobes whilst *Bacteroides* sp., *Fusobacterium* sp., *Clostridium* sp., and *Peptostreptococcus* sp. are common anaerobes. *Mycoplasma* spp. are also implicated as causes of bacterial LRT disease, especially IAD, usually in association with one or more of the bacteria listed above but also as single infections (Wood et al 1997). *Mycoplasma equirhinis* is most strongly associated with IAD (Wood et al 2005), but other species, including *M. felis*, may also be involved (Wood et al 1997).

Streptococcus equi subsp. *zooepidemicus*

Key factors concerning *S. equi* subsp. *zooepidemicus* are listed in Box 23.6.

Box 23.6. Key facts for *Streptococcus equi* subsp. *zooepidemicus*

- Gram-positive Lancefield group C β-hemolytic cocci approximately 1 μm in diameter
  - Occur as pairs but mostly as chains, depending on culture environment
  - Facultative anaerobes
  - Catalase and oxidase negative
  - Non-motile and non-spore forming (therefore limited environmental persistence and reservoir of infection)
- Closely related to *S. equi* subsp. *equi* (>98% genome homology with almost identical protein profiles) but has very different biological behavior, i.e.:
  - Not host (equine) restricted, infects wide range of mammals
  - Mucosal resident and opportunistic pathogen of the respiratory tract
  - Causes mainly mucosal rather than invasive disease
  - Opportunistic pathogen of other sites including skin, cornea, joints, bone, udder, and genitourinary tract
- Culture on horse blood agar plates; selective plates can be used (horse blood agar supplemented with nalidixic acid and colistin sulfate)

Disease profile

Opportunist pathogen causing:
- Bronchopneumonia in foals
- IAD in horses in training
- Pneumonia and pleuropneumonia (including equine shipping fever) in adult horses

Virulence mechanisms
- Bacterial cell wall
- Hyaluronic acid capsule
- Surface proteins
- Exotoxins

Antibiotic resistance

Generally sensitive to penicillin and related antibiotics; resistance to penicillins is seldom a clinical problem. Also sensitive to a variety of other antibiotics including tetracyclines, trimethoprim–sulfonamide combinations and peptide antibiotics

Prevention
- Transient immunity following natural infection
- No vaccines available

Box 23.7. *Streptococcus zooepidemicus* virulence factors

- Bacterial cell wall
  - Peptidoglycan
- Hyaluronic acid capsule
  - Isolates usually capsulated *in vivo* but non-capsulated *in vitro*
- Surface proteins
  - Extracellular matrix binding proteins
  - Antibody binding proteins
  - M protein
- Exotoxins
  - Hyaluronidase
  - Streptolysin
  - Streptokinase
  - Pyrogenic exotoxins

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tivitis and keratitis), joints and bone, reproductive tract (metritis), and lymph nodes (abscesses) (Hoffman et al 1993). It is also a common cause of septicemia in foals. It is the most prevalent bacterial pathogen in equine LRT disease and opportunistically causes bronchopneumonia in foals, IAD in horses in training, pneumonia/pleuropneumonia in adults, and equine shipping fever (Racklyeft & Love 2000, Christley et al 2001, Newton et al 2003, Wood et al 2005).

Virulence factors

Steptococcus zooepidemicus is almost identical at a genome level to its clonal derivative *S. equi* with an estimated 98% DNA homology between the two subspecies. Despite this similarity, the disease profiles of the two bacteria are markedly different. *Streptococcus equi* is highly host adapted, is a primary pathogen of the upper respiratory tract only, is not part of the normal resident URT flora, and causes invasive disease. In contrast, *S. zooepidemicus* infects a wide range of hosts and causes disease in most organ systems. It is a URT resident and causes mainly mucosal, rather than invasive, disease. The small genome differences between these two bacteria are therefore likely to encode the virulence factors responsible for their different pathogenicities and may provide fundamental clues of the molecular basis of streptococcal biology.

Streptococcus zooepidemicus has been comparatively poorly studied but appears to possess a very similar panel of virulence factors to *S. equi* (Box 23.7).

The peptidoglycan cell wall is a pyrogen and is a key molecule responsible for bacterial recognition by the horse's immune system.

The hyaluronic acid capsule is an important antiphagocytic mechanism along with the M protein. However,

Clinical disease

Streptococcus equi subsp. *zooepidemicus* (*S. zooepidemicus*) disease is not confined to the respiratory tract. Overall it is the most frequently isolated equine bacterial pathogen and causes disease in most organ systems including skin (cellulitis and abscesses), conjunctiva and cornea (conjunc-

unlike *S. equi* where hyaluronic capsule is constitutively produced by most isolates, capsule production is variable in *S. zooepidemicus*. It appears to be regulated in response to the environment so that isolates taken directly from the horse's respiratory tract are generally capsulated but lose their capsule on culture in the laboratory. Colonies of *S. zooepidemicus* have a flat matt appearance on culture plates and are therefore usually different from the rounded, shiny, gloss colonies of *S. equi*.

Streptococcus zooepidemicus has a similar range of adhesins to *S. equi* including a number of MSCRAMMs, e.g. fibronectin binding proteins (Lindmark et al 1996).

In contrast to the near homogeneous *S. equi* M protein SeM, the *S. zooepidemicus* M protein SzP shows considerable variation at both DNA and protein levels (central and N-terminal regions) between isolates (Walker & Timoney 1998). Since the hypervariable SzP is the major immunogen recognized by the horse's immune system, there is wide variation in antigenicity between isolates resulting in little cross-protection. SzP is antiphagocytic and prevents C3b and IgG opsonization by binding fibrinogen, thus masking the C3b binding site, and also by binding the Fc portion of IgG, making it unavailable to Fc receptors on phagocytes (Timoney et al 1997).

Streptococcus zooepidemicus produces many of the same exotoxins as *S. equi* including the hemolytic cytotoxin streptolysin-S and hyaluronidase. It does not produce homologs of the *S. equi* pyrogenic exotoxins SePE-I and PsPE-H or the mitogenic leukotoxin. A combination of exotoxins is likely to be responsible for the severe necrotizing pneumonia that this bacterium produces in foals and adult horses with shipping fever (Oikawa et al 1994).

Pathogenesis

Streptococcus zooepidemicus colonizes the nasopharynx (and other mucosal sites) in normal horses and is part of the respiratory tract's resident flora. The transition from commensal to disease-causing states is associated with colonization more distally along the trachea from the nasopharynx (Anzai et al 2002). As with the other strepto-cocci, colonization is dependent on binding to epithelial cells by adhesins, including MSCRAMMs. *Streptococcus zooepidemicus* is an opportunistic pathogen causing disease when conditions within the respiratory tract favor bacterial growth. The molecular events regulating the switch in behavior from mucosal resident to opportunist pathogen are poorly understood but are likely to be multifactorial, highly regulated, and involve changes in both host and bacterium (Fig. 23.15).

The factors that trigger *S. zooepidemicus*-associated bronchopneumonia in foals include primary respiratory viral infections, poor maternal transfer of immunity, overcrowding, debilitation, transport, and anesthesia. Pneumonia/pleuropneumonia and equine shipping fever in adults are triggered by stresses that compromise airway defenses including long-distance transport and general anesthesia. The triggers for induction of IAD are unknown but probably include training stress, crowded environments, and poor air hygiene (Robinson 1997, 2003, Christley et al 2001, Newton et al 2003, Wood et al 2005). There is emerging evidence that horse genotype plays a role in disease susceptibility, possibly related to transferrin haplotype. *Streptococcus zooepidemicus* causes a rapidly progressive, severe, purulent, necrotizing hemorrhagic pneumonia often accompanied by pleuritis (Oikawa et al 1994). In the horse, but not *in vitro*, the bacteria efficiently resist phagocytosis by virtue of their hyaluronic acid capsule and are able to persist within the lung despite phagocyte (mainly neutrophil but also macrophage) chemotaxis to the site of infection. The extensive cellular damage (epithelial desquamation, necrosis), hemorrhage, interstitial changes, and pulmonary consolidation are likely to be caused both by exotoxins (especially proteolytic enzymes) and also immunopathology mediated by antigen–antibody complexes binding to pulmonary endothelium (Divers et al 1992, Yoshikawa et al 2003). The reasons for the variation in disease severity caused by *S. zooepidemicus* in different age groups of horse are not clear. Similarly there is no information about the possible molecular basis for the differences in pathogenicity between *S. zooepidemicus* isolates. It does appear, however that variation in SzP is not responsible for differences in pathogenicity (Walker & Runyan 2003). Bronchopneumonia in foals and pneumonia/pleuropneumonia/equine shipping fever in adults are sporadic diseases with low morbidity and high mortality. In contrast, IAD occurs as outbreaks with high morbidity and very low mortality. It is possible that the difference is, in part, because horses in training experience less severe compromise to their respiratory tract defenses than sick foals or adults subject to transport or anesthetic stress.

Diagnosis

Diagnosis is by culture of nasal/nasopharyngeal swabs or tracheal/bronchoalveolar lavage samples on blood agar at 37°C in 5% CO_2. Tracheal/bronchoalveolar lavage samples should be collected with great care in horses with dyspnea and should not be collected from horses with severe dyspnea. Colonies are β-hemolytic, Lancefield group C (determined by latex agglutination) and usually non-capsulated. Confirmation of identity is by sugar fermentation tests (*S. zooepidemicus* ferments sorbitol, lactose, and ribose but not trehalose).

Management

Most isolates are susceptible to the penicillins and these should be regarded as front-line antibiotics because they produce a rapid bactericidal effect and achieve the highest

bacteriological cure rates. Other antibiotics including tetracyclines and macrolides can also be used. Although sensitive to trimethoprim–sulfonamide combinations *in vitro*, the efficacy of these antibiotics is greatly reduced in the presence of pus because of the high concentrations of folic acid present in pus.

Streptococcus pneumoniae

Key factors concerning *S. pneumoniae* are listed in Box 23.8.

Clinical disease

Streptococcus pneumoniae is a major human pathogen that is a common cause of pneumonia, otitis media, bacteremia, and meningitis. It is a sporadic cause of bronchopneumonia in foals (Meyer et al 1992) and also pneumonia/pleuropneumonia in adults (Chaffin & Carter 1993, Seltzer & Byars 1996, Carr et al 1997). *Streptococcus pneumoniae* has been identified as one of the principal bacteria, along with *S. zooepidemicus* and *Actinobacillus* sp., associated with IAD in horses in training (Chapman et al 2000, Wood et al 2005).

Equine *S. pneumoniae* isolates

There are over 80 *S. pneumoniae* capsular serotypes that infect humans but all equine isolates characterized to date belong to capsular serotype 3 (Whatmore et al 1999). Molecular characterization of equine isolates by restriction fragment length polymorphism of virulence factor and housekeeping encoding genes suggests that equine isolates represent a tight clonal group and are closely related to, but distinct from, human isolates. There is no evidence that equine *S. pneumoniae* isolates are zoonotic because farm workers are generally infected with non-equine strains.

There are several important differences between human and equine *S. pneumoniae* isolates. Equine isolates lack two of the key virulence factors present in human isolates: pneumolysin and autolysin.

Pneumolysin, encoded by *ply*, is a hemolytic cytotoxin responsible for disrupting host cell membranes by binding cell-membrane-associated cholesterol and creating pores in host cell membranes, resulting in cell lysis. Unlike the hemolysins produced by *S. equi* and *S. zooepidemicus*, pneumolysin is not secreted from the bacterial cell but is a cytoplasmic protein released when bacteria lyse.

Autolysin, a bile acid (dedeoxycholic acid) activated enzyme encoded by *lytA*, cleaves the peptidoglycan bacterial cell wall, lyses the bacteria and releases pneumolysin from the cell. The genes *ply* and *lytA* are adjacent within the *S. pneumoniae* chromosome. However, equine isolates lack both pneumolysin and autolysin activity as the result of a 7-kilobase chromosomal deletion of the 3′ region of *lytA* and the 5′ region of *ply*, along with the interspersed chromosomal region. Equine

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> **Box 23.8.** Key facts for *Streptococcus pneumoniae*
>
> - Primarily a human pathogen – equine isolates are a distinct clonal group
> - Gram-positive cocci, usually occurring as diplococci, with no assigned Lancefield group
> - Capsule polysaccharide defines serotype
>   - All equine isolates are serotype 3
> - Naturally transformable – take up DNA from other bacteria including:
>   - Acquisition of new virulence and antibiotic-resistance genes
> - Equine isolates have a variety of culture, biochemical, and genetic differences from human isolates:
>   - Equine isolates form elongated chains whereas human isolates form diplococci (a pair of cocci)
>   - Equine isolates are bile insoluble, human isolates are bile soluble
>   - Equine isolates are non-hemolytic, human isolates are α-hemolytic
>   - Equine isolates do not undergo autolysis, human isolates do
>
> **Disease profile**
> - Common cause of pneumonia, otitis media, and meningitis in humans
> - Causes bronchopneumonia in foals and associated with IAD in horses in training
>
> **Virulence factors**
> - Adhesins
> - Toxins, but equine isolates lack pneumolysin and autolysin
> - Capsule
> - Cell wall
>
> **Antibiotic resistance**
> - Emerging penicillin resistance in humans, little information on equine isolates
>
> **Prevention**
> - No equine vaccines available

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isolates do possess other virulence factors found in human isolates including neuraminidases A and B (encoded by *nanA*, *nanB*), surface protein A (encoded by *pspA*) and hyaluronidase (encoded by *hyl*). However, equine isolates possess unique alleles for these virulence genes providing further evidence that isolates from horses form a subpopulation distinct from human isolates.

Pathogenesis

In a proportion of humans and horses, *S. pneumoniae* colonizes the nasopharynx and forms part of the resident flora. Colonization is dependent on binding to

epithelial cells by specific surface proteins (adhesins and MSCRAMMs). *Steptococcus pneumoniae* possess a variety of surface adhesins including pneumococcal surface protein A (PspA), pneumococcal surface adhesin, *S. pneumoniae* protein A, and choline-binding protein A. At least one of these (PspA) is present in equine isolates. It is an opportunist pathogen of the respiratory tract. The reasons for the switch from a carriage to disease-causing phenotype and the regulatory mechanisms controlling this are not fully understood but involve changes to host airway defenses, mucosal immunity, and up-regulation of bacterial virulence factors.

Streptococcus pneumoniae produces a variety of toxins, the best studied of which is pneumolysin. Human, but not equine, isolates produce pneumolysin which, although regarded as important for virulence in humans and some animal models because of cytotoxic activity, is presumably not important in horses. The polysaccharide capsule is an important virulence factor. It is antiphagocytic and acts by blocking complement (C3b) and antibody opsonization of the bacterial cell, thus preventing neutrophil binding to the bacteria and allowing bacterial persistence in the face of neutrophil chemotaxis to the site of infection. The capsule is a complex and varied polymer of sugars, amino acids, and choline; variations in its composition allow different capsular serotypes to be identified. Variation in capsular composition also accounts for variations in virulence; whilst acapsular isolates are non-virulent, the virulence of capsulated isolates varies considerably, with serotype 3 being one of the most pathogenic serotypes in humans. The cell wall components lipoteichoic acid and peptidoglycan also contribute to pathogenesis by triggering inflammation.

Diagnosis

Diagnosis is by culture of nasal/nasopharyngeal swabs or tracheal lavage samples on blood agar at 37°C in 5% CO_2. Equine colonies are usually non-hemolytic, optochin sensitive and are not bile soluble. Further characterization is by slide agglutination test to detect pneumococcal capsule antigen and serotyping using antibody directed against the polysaccharide capsule.

Management

Most strains are susceptible to the penicillins and these should be regarded as front-line antibiotics because they produce a rapid bactericidal effect and achieve the highest bacteriological cure rates. However, penicillin resistance has recently appeared in human isolates as a result of β-lactamase production and mutations in penicillin-binding proteins. Resistance to these antibiotics is uncommon although penicillin-resistant isolates have occasionally been reported. There are no equine *S. pneumoniae* vaccines.

The apparent limited duration of natural immunity, the large inter-isolate antigenic variation as a result of SzP variability and limited extent of cross-protection between isolates do not auger well for the development of vaccines against this bacterium. It seems unlikely that vaccination against *S. equi* will provide cross-protection against opportunistic *S. pneumoniae* infections.

Streptococcus dysgalactiae subsp. *equisimilis*

LRT infections with *S. dysgalactiae* are less common than those associated with *S. zooepidemicus*. Like *S. zooepidemicus* it is an opportunist pathogen and is part of the resident airway flora in many horses. It also belongs to Lancefield group C and is β-hemolytic but it is genetically distinct from both *S. equi* and *S. zooepidemicus*. In the diagnostic laboratory it is distinguished from *S. equi* and *S. zooepidemicus* by sugar fermentation. There is little detailed information on virulence factors and pathogenesis although the bacterium possesses a similar array of virulence factors as *S. equi* and *S. zooepidemicus* including hyaluronic acid capsule, M protein, a variety of adhesins, and exotoxins including hyaluronidase and fibrinolysin. The pathogenesis of *S. dysgalactiae* disease is assumed to be similar to that for *S. zooepidemicus*.

The Pasteurellaceae (*Actinobacillus* and *Pasteurella* sp.)

The key facts for the Pasteurellaceae are listed in Box 23.9.

Clinical disease

The *Pasteurellaceae* (Pasteurella family) comprise *Pasteurella*, *Actinobacillus*, and *Haemophilus* sp. They are ubiquitous commensals of the URT in all animals and are opportunist pathogens causing LRT disease when host defenses are impaired allowing bacterial growth. In horses *Actinobacillus equuli* and *Pasteurella caballi* are commensals of the URT, especially the nasopharynx and oral cavity, and are important causes of LRT disease (Ward et al 1998). *Haemophilus* sp. are rarely implicated in equine LRT disease. In addition to LRT disease in horses of all ages (Wood et al 1993, Rycroft & Garside 2000), *A. equuli* is a common cause of neonatal septicemia (Raisis et al 1996) and has been associated with a wide range of other infections including peritonitis, arthritis, endocarditis, enteritis, mastitis, and abortion in horses of all ages (Hillyer et al 1990, Peremans et al 1991, Golland et al 1994). *Pasteurella caballi* has been isolated mainly from cases of LRT disease in foals, IAD and pneumonia/pleuropneumonia in adult horses (Wood et al 1993).

Box 23.9. Key facts for Pasteurellaceae

General features
- The Pasteurellaceae family consists of the genera *Pasteurella*, *Actinobacillus*, and *Haemophilus*
- *Actinobacillus* sp. and *Pasteurella* sp. cause LRT disease in horses
- Aerobic, medium (*Actinobacillus* sp.) or small (*Pasteurella* sp.), Gram-negative rods that frequently stain as coccobacilli (especially *Pasteurella* sp.)

Disease profile
- Primarily respiratory pathogens; *Actinobacillus* sp. also cause septicemia in foals and have been associated with a wide range of infections in older horses
- Ubiquitous commensals of the URT and cause respiratory disease opportunistically when host defenses are impaired (e.g. by stress, transport, training or, occasionally, primary respiratory virus infections)
- Along with *S. zooepidemicus* and *S. pneumoniae*, infections by the Pasteurellaceae are responsible for the majority of bacterial LRT disease in foals, horses in training and mature horses

Culture characteristics
- Cultured on sheep blood agar
- Catalase and oxidase positive
- *Actinobacillus* sp. can be hemolytic or non-hemolytic
- *Pasteurella caballi* is non-hemolytic

Virulence determinants
- Capsule
- Cell wall
  - Lipopolysaccharide
- Secreted proteins
  - Hemolysins
  - Leukotoxins

Antibiotic resistance
- Usually sensitive to aminoglycosides and tetracyclines

Prevention
- There are no equine vaccines

General features

Historically the common species of *Actinobacillus* isolated from horses have been classified as *A. equuli*, variants of *A. equuli*, *A. suis* and Bisgaard taxon 11 (Blackall et al 1997). The application of DNA sequence comparisons has permitted the adoption of a more rational classification system. The common equine isolates are now classified as either *A. equuli* subsp. *equuli* or *A. equuli* subsp. *haemolyticus* (Christensen et al 2002). The *A. equuli* subsp. *equuli* is non-hemolytic and causes mainly neonatal foal septicemia

and joint ill while *A. equuli* subsp. *haemolyticus* is hemolytic and is associated mainly with LRT disease and infections of other organ systems in older horses. *Actinobacillus* species other than *A. equuli* can also be isolated from cases of equine LRT disease including *Actinobacillus* genomospecies 1 and 2 (*A. lignieresii*). However, it is common practice for diagnostic laboratories to simply report the isolation of *Actinobacillus* sp. and not to identify to species level.

The most commonly isolated *Pasteurella* sp. is *P. caballi* and this has been associated with LRT disease in horses of all ages. *Actinobacillus* and *Pasteurella* sp. are commensal bacteria of the URT, nasopharynx, and oral cavity and are opportunistic pathogens: biochemical and genetic analysis reveals no differences between *A. equuli* isolates from healthy and diseased horses (Sternberg & Brandstrom 1999). They are medium (*A. equuli*) or small (*P. caballi*) Gram-negative rods that often stain as coccobacilli because of preferential uptake of stain at the bacterial poles. They grow on sheep blood agar and produce moderate-sized gray–white colonies. *Actinobacillus equuli* is either non-hemolytic (*A. equuli* subsp. *equuli*) or hemolytic (*A. equuli* subsp. *haemolyticus*). *Pasteurella caballi* is non-hemolytic.

Virulence factors

Little is known about virulence factors for *A. equuli* and *P. caballi*. For other species within the Pasteurellaceae, the bacterial capsule confers resistance to phagocytosis. Lipopolysaccharide, located within the outer membrane of the cell wall, is believed to contribute to virulence and to be responsible for the severe clinical signs associated with septicemia. Although there is no detailed information about the role of capsule and lipopolysaccharide in *A. equuli* and *P. caballi* virulence, it is reasonable to suppose that these play a role in pathogenesis. It is also likely that these bacteria produce a variety of exotoxins that damage host defenses, including epithelial cytotoxins, tissue degradative (proteolytic) enzymes, and leukotoxins, and, although these have not been characterized, *A. equuli* cultures do produce a toxin that damages equine neutrophils (Sternberg & Brandstrom 1999). There has been considerable interest in the hemolytic activity of *A. equuli* because of the different disease-causing profiles of the non-hemolytic (mainly associated with neonatal septicemias) and hemolytic (associated with respiratory and other diseases in older horses) subspecies and the observation that serum from convalescent horses contains antibody directed against hemolysin (Rycroft & Garside 2000). *Actinobacillus equuli* produces a hemolysin belonging to the RTX family of pore-forming toxins (Berthoud et al 2002). The RTX toxins have a series of calcium-binding, glycine-rich peptide repeat units (hence "**R**epeats in **T**o**X**in") and form pores in host cell membranes destroying the cell or impairing its function. RTX toxins act as virulence factors and are directed against the host's immune cells. High

concentrations kill effector cells in the immune system (neutrophils, macrophages, and lymphocytes) and low concentrations impair phagocytosis and oxidative burst killing by phagocytes. The *A. equuli* hemolysin AqxA is encoded by the *aqxCABD* operon (Berthoud et al 2002) and exhibits selective toxicity to equine leukocytes (Kuhnert et al 2003). Although not characterized, it is likely that *P. caballi* also possesses RTX toxins that may resemble the LktA leukotoxin of other pasteurellas. RTX toxins are thus candidates as major virulence factors in *A. equuli* and *P. caballi* but it is likely that virulence is multilayered and conferred by a wide variety of determinants which have yet to be characterized.

Pathogenesis

The factors that allow switching of behavior of *A. equuli* and *P. caballi* from respiratory tract commensals to opportunistic pathogens are not understood but, as is the case with the other opportunistic respiratory tract bacterial pathogens, they likely involve complex interactions between the horse's respiratory tract defenses, mucosal immunity and up-regulation of bacterial virulence factors in response to changes in the airway environment. Primary virus infection, injury to respiratory defenses by poor environmental air hygiene, transport, and general anesthesia stress all contribute to create conditions that favor bacterial growth. It is likely that a variety of (uncharacterized) exotoxins cause epithelial injury exposing the extracellular matrix and providing additional surfaces for bacteria to colonize and nutrients for growth. Bacteria resist phagocytosis and thus persist at the site of infection by means of capsule and a variety of exotoxins including hemolysin and leukotoxin. The role of lipopolysaccharide has not been determined but it is likely to drive a widespread inflammatory cascade and is probably partly responsible for the severe systemic signs associated with *A. equuli* septicemia. The reasons for the more severe manifestations of *A. equuli* infection in neonatal foals (septicemia) compared to older foals and adults (bronchopneumonia, IAD, and pneumonia/pleuropneumonia) are not clear.

Diagnosis

Actinobacillus sp. and *P. caballi* are difficult to identify on Gram-stained nasal/nasopharyngeal swabs and tracheal/bronchoalveolar lavage samples because of their small size and weak uptake of stain. Colonies of *A. equuli* grow on sheep blood agar and produce gray–white sticky colonies (approximately 2 mm in diameter) which are either hemolytic (subsp. *haemolyticus*) or non-hemolytic (subsp. *equuli*). Identification to species level is by demonstration of Gram-negative rods or coccobacilli that are catalase, oxidase, and urease positive and ferment lactose, maltose, mannitol, melibiose, sucrose, and trehalose but not arabinose.

Pasteurella caballi also grows on sheep blood agar and produces similar-sized gray–white, non-hemolytic colonies that are oxidase positive, catalase and urease negative and that ferment glucose, lactose, maltose, and mannitol.

Management

Most isolates are sensitive to aminoglycosides and these, particularly gentamicin, are generally used as first-choice antibiotics. Resistance to aminoglycosides is not a major clinical problem. A variety of other antibacterials, for example oxytetracycline, ampicillin, trimethoprim–sulfonamide, cephalosporins, and fluoroquinolones, may be effective and can be considered as alternatives. *Pasteurella caballi* is often sensitive to penicillin, an unusual feature for Gram-negative bacteria. There are no equine vaccines for these organisms.

Mycoplasma spp.

The key facts for *Mycoplasma* spp. are listed in Box 23.10.

Clinical disease

The mycoplasmas are commensal inhabitants of the URT and cause disease opportunistically when host defenses are impaired. They may cause disease as single agents (Hoffman et al 1992, Wood et al 1997), but are more frequently present as mixed infections with the *Streptococci* sp. and *Actinobacillus* sp. (Wood et al 2005). There are at least seven species of mycoplasmas that colonize the equine URT (Allam & Lemcke 1975) and cause opportunistic LRT disease. Several of these species, such as *Mycoplasma felis*, infect other animals as well as horses, while *M. equirhinis* appears to infect horses only.

General features

Mycoplasmas are very small bacteria and have many unusual features (Razin et al 1998). They lack the rigid peptidoglycan cell wall present in other bacteria and possess only a flexible cytoplasmic membrane with some resemblance to the outer membrane of Gram-negative bacteria. This is a bilayer but, unlike the symmetrical phospholipid bilayer of the Gram-negative bacteria, it is asymmetrical with an outer layer of lipoproteins and an inner layer of phospholipids. The flexible cytoplasmic membrane gives mycoplasmas their distinctive elongated, bipolar, asymmetric shape. One pole is used to attach to host cells and the other pole is responsible for the gliding movement of these bacteria. They are extracellular bacteria, but grow in close contact with host cells and appear to require host cells for nutrient acquisition, especially the amino acid arginine, which is used as an energy substrate.

Box 23.10. Key facts for *Mycoplasma* spp.

General features

- The mycoplasmas are very small bacteria with the smallest known bacterial genomes
- They stain poorly, especially with Gram stain; they stain best with Giemsa
- Consist of two genera: *Acholeplasma* and *Mycoplasma*, with at least seven *Mycoplasma* species causing equine LRT disease
- Species are separated by biochemical profiles and serological typing

Disease profile

- Cause opportunist LRT disease in foals, horses in training and mature horses, usually in combination with other (Gram-positive and Gram-negative) bacteria
- Occasionally present as single agents in cases of LRT disease
- The principal equine respiratory pathogens are *Mycoplasma equirhinis* and *Mycoplasma felis*
- *Mycoplasma equirhinis* appears to be associated with equine respiratory disease only
- The mycoplasmas also cause opportunistic disease in other sites including the eye and genital tract

Culture characteristics

- Highly fastidious and require nutrient-rich commercial growth media
- Species within the genus *Acholeplasma* require sterol for growth
- Species within the genus *Mycoplasma* do not require sterol for growth
- Both *M. equirhinis* and *M. felis* use glucose and hydrolyze arginine

Virulence determinants

- Adhesins
- Toxins
- Evasion of phagocytosis

Antibiotic resistance

- Sensitive to tetracyclines, macrolides, and aminoglycosides
- Resistance is not a clinical problem

Prevention

- There are no equine vaccines

Virulence factors

Mycoplasmas have the smallest of all known bacterial genomes, less than 1 Mbp in size, and thus encode fewer than 1000 genes (Razin et al 1998). Not surprisingly they are comparatively simple organisms and lack many of the metabolic and regulatory genes possessed by other bacteria. They lack the genes involved in numer-

ous biosynthetic pathways including the tricarboxylic acid pathway, oxidative phosphorylation, and lipid and amino synthesis. As a consequence, mycoplasmas are highly fastidious organisms with specific nutrient requirements for growth and rely on the animal host to provide many of the nutrients required for their growth. Their strict nutrient requirements mean they are found in close association with host cells, typically on the mucous membrane surface in the respiratory tract. They are generally not invasive bacteria although some species can invade tissues.

Adhesion to respiratory epithelial cells is regulated by an organelle (the "attachment organelle") at one pole of the bacterium together with surface adhesin proteins ("cytadhesins") embedded within the outer layer of the cell membrane. A number of these adhesins appear be essential for the architecture of the attachment organelle and to act as accessory proteins, possibly by providing a scaffold for the localization and/or maturation of other adhesin proteins (Waldo et al 2005). Several of these mycoplasmal adhesin proteins have been characterized including adhesin MHP1, ciliary adhesin, adhesin P97, and adhesin-like protein P146 from *M. hyopneumoniae*; adhesin P1 from *M. pneumoniae* (Krause & Balish 2004); LppS from *M. conjunctivae* (Belloy et al 2003); and binding and activation of plasminogen from bovine Group 7 mycoplasmas (Bower et al 2003). Some surface proteins are known to confer virulence, such as MIA (mycoplasma immunodominant protein; Tu et al 2005). Specific surface adhesin proteins have not been described for *M. equirhinis* but this mycoplasma is likely to possess a similar array of adhesins.

Although mycoplasma infection does cause cytotoxicity, there is little information about possible exotoxins, although it seems highly likely that they do produce cytotoxins. The outer layer of lipoproteins may act as recognition molecules for the horse's immune system and drive the inflammatory response. It also seems likely that they produce mitogenic leukotoxins, e.g. *M. arthriditis* T-cell mitogen superantigen (Cole & Atkin 1991), although few of these have been characterized. Hydrogen peroxide production by *Mycoplasma* sp. is directly cytotoxic to host cells and is important in virulence (Khan et al 2005).

Like many bacteria, mycoplasmas are able to avoid phagocytosis and persist at the site of infection despite phagocyte chemotaxis. The mechanisms allowing phagocytosis evasion have not been elucidated but are likely to be different from those of other bacteria because the mycoplasmas do not possess a capsule.

Pathogenesis

Mycoplasmas are present as part of the normal airway flora in many horses and disease generally occurs opportunistically. In farm animals, mycoplasmas can be transmitted contagiously by aerosol (Cardona et al 2005), possibly over

large distances (Goodwin 1985). Contagious transmission is also possible in horses and is responsible for group outbreaks of disease (Wood et al 1997). Mycoplasmas attach to ciliated host epithelial cells using the adhesion organelle and associated adhesion proteins located at the adhesion pole of the bacterial cell. Like the Bordetellae, attachment to ciliated cells causes ciliostasis and epithelial cell depolarization. Induction of ciliostasis allows further bacterial attachment and colonization of the airway. Mycoplasmas induce cytotoxicity, by release of hydrogen peroxide and a range of exotoxins, and induce an intense inflammatory response, possibly in response to the outer layer of lipoproteins, which initiates most of the airway and pulmonary pathology. The host immune response is effectively avoided by uncharacterized phagocytosis evasion mechanisms and leukotoxins.

Diagnosis

Direct microscopy of nasal/nasopharyngeal swabs and tracheal/bronchoalveolar lavage samples is usually not diagnostic because of the weak and inconsistent stain uptake by mycoplasmas and their pleomorphism. In culture, mycoplasmas produce "fried-egg" microcolonies (< 0.6 mm) on agar plates. They are digitonin sensitive and urease negative. Identification of mycoplasmas to the species level is achieved by further biochemical characteristics. Fluorescent antibody and enzyme-linked immunosorbent assays can be used in tissue sections to detect mycoplasma antigens. Serology can be used to detect species-specific antibodies against *Mycoplasma* in convalescent horse serum.

Management

Mycoplasmas are resistant to the penicillins but are sensitive to a variety of antibiotics, the most suitable for equine use being the tetracyclines and fluoroquinolones. Antibiotic resistance is not a major clinical problem, possibly because the small genome size of the mycoplasmas limits their capacity for gene acquisition. There are no equine mycoplasma vaccines.

REFERENCES

Al-Ghamdi GM, Kapur V, Ames TR et al 2000 Use of repetitive sequence-based polymerase chain reaction for molecular epidemiologic: analysis of *Streptococcus equi* subspecies *equi*. American Journal of Veterinary Research 61: 699–705

Allam NM, Lemcke RM 1975 Mycoplasmas isolated from the respiratory tract of horses. Journal of Hygiene 74: 385–407

Anzai T, Timoney JF, Kuwamoto Y et al 1999 *In vivo* pathogenicity and resistance to phagocytosis of *Streptococcus equi* strains with different levels of capsule expression. Veterinary Microbiology 67: 277–286

Anzai T, Timoney JE, Kuwamoto Y et al 2002 Polymerase chain reaction-restriction fragment length polymorphism analysis of the SzP gene of *Streptococcus zooepidemicus* isolated from the respiratory tract of horses. American Journal of Veterinary Research 63: 1298–1301

Belloy L, Vilei EM, Giacometti M et al 2003 Characterization of LppS, an adhesin of *Mycoplasma conjunctivae*. Microbiology 149: 185–193

Berthoud H, Frey J, Kuhnert P 2002 Characterization of Aqx and its operon: the hemolytic RTX determinant of *Actinobacillus equuli*. Veterinary Microbiology 87: 159–174

Blackall PJ, Bisgaard M, McKenzie RA 1997 Characterisation of Australian isolates of *Actinobacillus capsulatus*, *A. equuli*, *P. caballi* and Bisgaard taxa 9 and 11. Australian Veterinary Journal 75: 52–55

Boschwitz JS, Timoney JF 1994 Inhibition of C3 deposition on *Streptococcus equi* subsp. *equi* by M protein: a mechanism for survival in equine blood. Infection and Immunity 62: 3515–3520

Bower K, Djordjevic SP, Andronicos NM et al 2003 Cell surface antigens of *Mycoplasma* species bovine group 7 bind to and activate plasminogen. Infection and Immunity 71: 4823–4827

Cardona AC, Pijoan C, Dee SA 2005 Assessing mycoplasma hyopneumoniae aerosol movement at several distances. Veterinary Record 156: 91–92

Carr EA, Carlson GP, Wilson WD et al 1997 Acute hemorrhagic pulmonary infarction and necrotizing pneumonia in horses: 21 cases (1967–1993). Journal of the American Veterinary Medical Association 210: 1774–1778

Chaffin MK, Carter GK 1993 Equine bacterial pleuropneumonia. I: Epidemiology, pathophysiology and bacterial isolates. Compendium on Continuing Education for the Practicing Veterinarian 15: 1642–1650

Chanter N, Collin N, Holmes N et al 1997 Characterization of the Lancefield group C *Streptococcus* 16S-23S RNA gene intergenic spacer and its potential for identification and sub-specific typing. Epidemiology and Infection 118: 125–135

Chanter N, Talbot NC, Newton JR et al 2000 *Streptococcus equi* with truncated M-proteins isolated from outwardly healthy horses. Microbiology 146: 1361–1369

Chapman PS, Green C, Main JP et al 2000 Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. Veterinary Record 146: 91–95

Christensen H, Bisgaard M, Olsen JE 2002 Reclassification of equine isolates previously reported as *Actinobacillus equuli*, variants of *A. equuli*, *Actinobacillus suis* or Bisgaard taxon 11 and proposal of *A. equuli* subsp. *equuli* subsp. nov. and *A. equuli* subsp. *haemolyticus* subsp. nov. International Journal of Systematic and Evolutionary Microbiology 52: 1569–1576

Christley RM, Hodgson DR, Rose RJ et al 2001 A case-control study of respiratory disease in Thoroughbred racehorses in Sydney, Australia. Equine Veterinary Journal 33: 256–264

Cole BC, Atkin CL 1991 The *Mycoplasma arthritidis* T-cell mitogen, MAM: a model superantigen. Immunology Today 12: 271–276

Dalgleish R, Love S, Pirie HM et al 1993 An outbreak of strangles in young ponies. Veterinary Record 132: 528–531

Divers TJ, Timoney JF, Lewis RM et al 1992 Equine glomerulonephritis and renal failure associated with complexes of group-C streptococcal antigen and IgG antibody. Veterinary Immunology and Immunopathology 32: 93–102

Fintl C, Dixon PM, Brazil TJ et al 2000 Endoscopic and bacteriological findings in a chronic outbreak of strangles. Veterinary Record 147: 480–484

Flanagan J, Collin N, Timoney J et al 1998 Characterization of the haemolytic activity of *Streptococcus equi*. Microbial Pathogenesis 24: 211–221

Galan JE, Timoney JF 1985a Immune complexes in purpura hemorrhagica of the horse contain IgA and M antigen of *Streptococcus equi*. Journal of Immunology 135: 3134–3137

Galan JE, Timoney JF 1985b Mucosal nasopharyngeal immune responses of horses to protein antigens of *Streptococcus equi*. Infection and Immunity 47: 623–628

Galan JE, Timoney JF 1987 Molecular analysis of the M protein of *Streptococcus equi* and cloning and expression of the M protein gene in *Escherichia coli*. Infection and Immunity 55: 3181–3187

George JL, Reif JS, Shideler RK et al 1983 Identification of carriers of *Streptococcus equi* in a naturally infected herd. Journal of the American Veterinary Medical Association 183: 80–84

Golland LC, Hodgson DR, Hodgson JL et al 1994 Peritonitis associated with *Actinobacillus equuli* in horses: 15 cases (1982–1992). Journal of the American Veterinary Medical Association 205: 340–343

Goodwin RF 1985 Apparent reinfection of enzootic-pneumonia-free pig herds: search for possible causes. Veterinary Record 116: 690–694

Grant ST, Efstratiou A, Chanter N 1993 Laboratory diagnosis of strangles and the isolation of atypical *Streptococcus equi*. Veterinary Record 133: 215–216

Hamlen HJ, Timoney JF, Bell RJ 1994 Epidemiologic and immunologic characteristics of *Streptococcus equi* infection in foals. Journal of the American Veterinary Medical Association 204: 768–775

Harrington DJ, Sutcliffe IC, Chanter N 2002 The molecular basis of *Streptococcus equi* infection and disease. Microbes and Infection 4: 501–510

Hillyer MH, Mair TS, Holmes JR 1990 Bacterial endocarditis in a Shire mare. Equine Veterinary Education 2: 5–7

Hoffman AM, Staempfli HR, Prescott JF et al 1991 Field evaluation of a commercial M-protein vaccine against *Streptococcus equi* infection in foals. American Journal of Veterinary Research 52: 589–592

Hoffman AM, Baird JD, Kloeze HJ et al 1992 *Mycoplasma felis* pleuritis in two show-jumper horses. Cornell Veterinarian 82: 155–162

Hoffman AM, Viel L, Prescott JF et al 1993 Association of microbiologic flora with clinical, endoscopic, and pulmonary cytologic findings in foals with distal respiratory tract infection. American Journal of Veterinary Research 54: 1615–1622

Jacobs AA, Goovaerts D, Nuijten PJ et al 2000 Investigations towards an efficacious and safe strangles vaccine: submucosal vaccination with a live attenuated *Streptococcus equi*. Veterinary Record 147: 563–567

Jorm LR 1990 Strangles in horse studs: incidence, risk factors and effect of vaccination. Australian Veterinary Journal 67: 436–439

Jorm LR 1991 Laboratory studies on the survival of *Streptococcus equi* subspecies *equi* on surfaces. In: Plowright W, Rossdale PD, Wade JF (editors) Equine Infectious Diseases VI: Proceedings of the VI International Conference. R & W Publications, Newmarket, pp.39–43.

Khan LA, Miles RJ, Nicholas RA 2005 Hydrogen peroxide production by *Mycoplasma bovis* and *Mycoplasma agalactiae* and effect of *in vitro* passage on a *Mycoplasma bovis* strain producing high levels of H_2O_2. Veterinary Research Communications 29: 181–188

Krause DC, Balish MF 2004 Cellular engineering in a minimal microbe: structure and assembly of the terminal organelle of *Mycoplasma pneumoniae*. Molecular Microbiology 51: 917–924

Kuhnert P, Berthoud H, Straub R et al 2003 Host cell specific activity of RTX toxins from haemolytic *Actinobacillus equuli* and *Actinobacillus suis*. Veterinary Microbiology 92: 161–167

Lancefield RC 1933 A serological differentiation of human and other hemolytic streptococci. Journal of Experimental Medicine 57: 571–595

Lindmark H, Guss B 1999 SFS, a novel fibronectin-binding protein from *Streptococcus equi*, inhibits the binding between fibronectin and collagen. Infection and Immunity 67: 2383–2388

Lindmark H, Jacobsson K, Frykberg L et al 1996 Fibronectin-binding protein of *Streptococcus equi* subsp. *zooepidemicus*. Infection and Immunity 64: 3993–3999

Lindmark H, Nilsson M, Guss B 2001 Comparison of the fibronectin-binding protein FNE from *Streptococcus equi* subspecies *equi* with FNZ from *S. equi* subspecies *zooepidemicus* reveals a major and conserved difference. Infection and Immunity 69: 3159–3163

Meyer JC, Koterba A, Lester G et al 1992 Bacteraemia and pneumonia in a neonatal foal caused by *Streptococcus pneumoniae* type 3. Equine Veterinary Journal 418: 407–410

Muhktar MM, Timoney JF 1988 Chemotactic response of equine polymorphonuclear leucocytes to *Streptococcus equi*. Research in Veterinary Science 45: 225–229

Newton JR, Wood JL, Dunn KA et al 1997 Naturally occurring persistent and asymptomatic infection of the guttural pouches of horses with *Streptococcus equi*. Veterinary Record 140: 84–90

Newton JR, Verheyen K, Talbot NC et al 2000 Control of strangles outbreaks by isolation of guttural pouch carriers identified using PCR and culture of *Streptococcus equi*. Equine Veterinary Journal 32: 515–526

Newton JR, Wood JL, Chanter N 2003 A case control study of factors and infections associated with clinically apparent respiratory disease in UK Thoroughbred racehorses. Preventative Veterinary Medicine 60: 107–132

Oikawa M, Kamada M, Yoshikawa Y et al 1994 Pathology of equine pneumonia associated with transport and isolation of *Streptococcus equi* subsp. *zooepidemicus*. Journal of Comparative Pathology 111: 205–212

Peremans K, Verschooten F, De Moor A et al 1991 Monoarticular infectious arthritis in the horse. Journal of Equine Veterinary Science 11: 27–32

Prescott JF, Srivastava SK, deGannes R et al 1982 A mild form of strangles caused by an atypical *Streptococcus equi*. Journal of the American Veterinary Medical Association 180: 293–299

Pusterla N, Watson JL, Affolter VK et al 2003 Purpura haemorrhagica in 53 horses. Veterinary Record 153: 118–121

Quinn PJ, Carter ME, Markey BK et al 1994 Clinical Veterinary Microbiology, 1st edn. Mosby-Year Book Europe, London.

Racklyeft DJ, Love DN 2000 Bacterial infection of the lower respiratory tract in 34 horses. Australian Veterinary Journal 78: 549–559

Raisis AL, Hodgson JL, Hodgson DR 1996 Equine neonatal septicaemia: 24 cases. Australian Veterinary Journal 73: 137–140

Razin SD, Yogev D, Naot Y 1998 Molecular biology and pathogenicity of mycoplasmas. Microbiology and Molecular Biology Reviews 62: 1094–1156

Robinson NE 1997 Pathogenesis and management of airway disease. Proceedings of the American Association of Equine Practitioners 43: 106–115

Robinson NE 2003 Inflammatory airway disease: defining the syndrome. Conclusions of the Havemeyer Workshop. Equine Veterinary Education 15: 61–63

Rycroft AN, Garside LH 2000 *Actinobacillus* species and their role in animal disease. Veterinary Journal 159: 18–36

Seltzer KL, Byars TD 1996 Prognosis for return to racing after recovery from infectious pleuropneumonia in Thoroughbred horses: 70 cases (1984–1989). Journal of the American Veterinary Medical Association 208: 1300–1301

Sheoran AS, Sponseller BT, Holmes MA et al 1997 Serum and mucosal antibody isotype responses to M-like protein (SeM) of *Streptococcus equi* in convalescent and vaccinated horses. Veterinary Immunology and Immunopathology 59: 239–251

Smith H 1994 Reactions to strangles vaccination. Australian Veterinary Journal 71: 257–258

Spoormakers TJ, Ensink JM, Goehring LS et al 2003 Brain abscesses as a metastatic manifestation of strangles: symptomatology and the use of magnetic resonance imaging as a diagnostic aid. Equine Veterinary Journal 35: 146–151

Sternberg S, Brandstrom B 1999 Biochemical fingerprinting and ribotyping of isolates of *Actinobacillus equuli* from healthy and diseased horses. Veterinary Microbiology 66: 53–65

Sweeney CR, Whitlock RH, Meirs DA et al 1987 Complications associated with *Streptococcus equi* infection on a horse farm. Journal of the American Veterinary Medical Association 191: 1446–1448

Sweeney CR, Benson CE, Whitlock RH et al 1989 Description of an epizootic and persistence of *Streptococcus equi* infections in horses. Journal of the American Veterinary Medical Association 194: 1281–1286

Sweeney CR, Timoney JF, Newton JR et al 2005 *Streptococcus equi* infections in horses: Guidelines for treatment, control and prevention of strangles. Journal of Veterinary Internal Medicine 19: 123–134

Takai S, Anzai T, Yashiro H et al 2000 Detection of DNA restriction fragment polymorphisms in *Streptococcus equi*. Veterinary Record 146: 159–161

Timoney JF 1988a Protecting against strangles: a contemporary view. Equine Veterinary Journal 20: 392–394

Timoney JF 1988b Shedding and maintenance of *Streptococcus equi* in typical and atypical strangles. In: Powell DG (editor) Equine Infectious Diseases V: Proceedings of the V International Conference. The University Press of Kentucky, Lexington, Kentucky, pp.28–33

Timoney JF 1993 Strangles. Veterinary Clinics of North America; Equine Practice 9: 365–374

Timoney JF 1999 Equine strangles: 1999. Proceedings of the Association of American Equine Practitioners 45: 31–37

Timoney JF, Artiushin SC 1997 Detection of *Streptococcus equi* in equine nasal swabs and washes by DNA amplification. Veterinary Record 141: 446–447

Timoney JF, Artiushin SC, Boschwitz JS 1997 Comparison of the sequences and functions of *Streptococcus equi* M-like proteins SeM and SzPSe. Infection and Immunity 65: 3600–3605

Todd AG 1910 Strangles. Journal of Comparative Pathology and Therapeutics 28: 212–229

Tu AH, Clapper B, Schoeb TR et al 2005 Association of a major protein antigen of *Mycoplasma arthritidis* with virulence. Infection and Immunity 73: 245–249

Verheyen K, Newton JR, Talbot NC et al 2000 Elimination of guttural pouch infection and inflammation in asymptomatic carriers of *Streptococcus equi*. Equine Veterinary Journal 32: 527–532

Waldo RH, Jordan JL, Krause DC 2005 Identification and complementation of a mutation associated with loss of *Mycoplasma pneumoniae* virulence-specific proteins B and C. Journal of Bacteriology 187: 747–751

Walker JA, Timoney JF 1998 Molecular basis of variation in protective SzP proteins of *Streptococcus zooepidemicus*. American Journal of Veterinary Research 59: 1129–1133

Walker JA, Timoney JF 2002 Construction of a stable non-mucoid deletion mutant of the *Streptococcus equi* Pinnacle vaccine strain. Veterinary Microbiology 89: 311–321

Walker RL, Runyan CA 2003 Identification of variations in SzP proteins of *Streptococcus equi* subspecies *zooepidemicus* and the relationship between protein variants and clinical signs of infection in horses. American Journal of Veterinary Research 64: 976–981

Ward CL, Wood JLN, Houghton SB et al 1998 *Actinobacillus* and *Pasteurella* species isolated from horses with lower airway disease. Veterinary Record 143: 277–279

Whatmore AM, King SJ, Doherty NC et al 1999 Molecular characterization of equine isolates of *Streptococcus pneumoniae*: natural disruption of genes encoding the virulence factors pneumolysin and autolysin. Infection and Immunity 67: 2776–2782

Wilson DW 1988 *Streptococcus equi* infections (strangles) in horses. Equine Practice 10: 12–25

Wood JLN, Burrell MH, Roberts CA et al 1993 Streptococci and *Pasteurella* spp. associated with disease of the equine lower respiratory tract. Equine Veterinary Journal 25: 314–318

Wood JL, Chanter N, Newton JR et al 1997 An outbreak of respiratory disease in horses associated with *Mycoplasma felis* infection. Veterinary Record 140: 388–391

Wood JL, Newton JR, Chanter N et al 2005 Association between respiratory disease and bacterial and viral infections in British racehorses. Journal of Clinical Microbiology 43: 120–126

Woolcock JB 1975 Studies in atypical *Streptococcus equi*. Research in Veterinary Science 19: 115–119

Yelle MT 1987 Clinical aspects of *Streptococcus equi* infection. Equine Veterinary Journal 19: 158–162

Yoshikawa H, Yasu T, Ueki H et al 2003 Pneumonia in horses induced by intrapulmonary inoculation of *Streptococcus equi* subsp. *zooepidemicus*. Journal of Veterinary Medical Science 65: 787–792

24

Rhodococcus equi Foal Pneumonia

Noah D Cohen and Ronald J Martens

Introduction

Pneumonia is an important cause of disease and death in foals. Although conflicting data exist, pneumonia caused by *Rhodococcus equi* is considered to be the most common cause of severe pneumonia among foals from 1 to 6 months of age (Hoffman et al 1993, Takai 1997). The disease is of veterinary importance for a number of reasons:

- Prevalence and case-fatality rates can be high (Chaffin et al 2003a).
- Treatment is generally prolonged, expensive, associated with adverse effects, and not uniformly successful (Giguere 2001).
- *Rhodococcus equi* pneumonia may negatively impact future athletic performance (Ainsworth et al 1998).
- Breeding farms reputed to have a problem with *R. equi* pneumonia often suffer loss of clients.

The purpose of this chapter is to review the epidemiology, clinical signs, diagnosis, treatment, prognosis, and control and prevention for this disease.

Epidemiology

Rhodococcus equi is a Gram-positive, soil-saprophytic, facultatively intracellular pathogen that survives and proliferates in alveolar and other macrophages. The bacterium has a worldwide distribution. It shares many microbiologic and pathogenic characteristics with *Mycobacterium tuberculosis*, the causative agent of tuberculosis in humans (Prescott 1991). Similar to *M. tuberculosis*, the ability of *R. equi* to replicate within, and destroy, macrophages is the basis for its pathogenicity and for the resulting granulomatous lesions. The presence of an 85–90-kilobase (kb) plasmid has been strongly associated with virulence, on the basis of clinical and experimental observations (Takai et al 1991a, Takai 1997). This plasmid encodes the production of several virulence-associated protein antigens (e.g. VapA and VapC–VapH) (Byrne et al 2001). VapA is surface-expressed, while VapC, VapD, and VapE are secreted. The expression characteristics of VapF, VapG, and VapH have not yet been described. VapA, which has been most extensively studied, is associated with virulence, but its

presence without the remainder of the plasmid is not responsible for virulence (Giguere et al 1999). The 85–90-kb virulence-associated plasmid is not commonly associated with *R. equi* isolated from diseased, non-equine hosts, including human patients with acquired immunodeficiency syndrome.

Pneumonia caused by *R. equi* appears to occur sporadically at some breeding farms, other farms appear to have regular recurrent problems with the disease (hereafter referred to as endemic farms), while some breeding farms do not experience problems with the disease (Prescott 1991). Although data regarding temporal fluctuations in prevalence of *R. equi* pneumonia at a large number of endemic farms are lacking, evidence exists of year-to-year variation in the prevalence. There are a number of putative explanations for changes in the prevalence of *R. equi* pneumonia between years at a farm, including health and other management practices and climatic conditions. The variation in prevalence among farms and years makes it difficult to obtain accurate estimates of the prevalence of the disease. The median prevalence at 32 affected farms in Texas was 7% (range 1–100%), and more than half the farms had ≤10% of foals affected; the case mortality proportion was 29% (Chaffin et al 2003a). The prevalence at 65 affected farms in the USA (including Texas) among 5,230 foals was 13%, and the case mortality rate was only 8%.

The reasons that some farms are affected recurrently and more severely than others remain unclear. Epidemiology of infectious diseases is often considered in light of the effects of the environment, agent, and host. A possible explanation for variation in prevalence of disease among farms is that environmental exposure is greater at affected than unaffected farms. However, conflicting data exist regarding this hypothesis. The prevalence of *R. equi* in the feces of mares was similar for two farms with recent cases of *R. equi* (14%) and an unaffected control farm (31%) in Kansas (Debey & Bailie 1987). In a study of two breeding farms in Japan, prevalence of virulent organisms was greater among foals at an *R. equi*-endemic farm than among foals at an unaffected farm, but there was no difference in the prevalence of virulent isolates in the feces of dams of foals from the two farms (14% and 16%, respectively) (Takai et al 1991b). In a study of 66 farms in

Texas, *R. equi* was isolated from the soil of 85% of affected farms and 73% of unaffected farms, and virulent *R. equi* was isolated from the soil of 32% of affected farms and 21% of unaffected farms (Martens et al 2000). These differences were not significant, indicating that culturing soil to recover *R. equi* or virulent *R. equi* could not be used to differentiate affected from unaffected farms. This study, however, did not quantify the *number* of organisms in soil samples. In Japan, studies using the combination of quantitative culture and subsequent detection of either the VapA protein or its plasmid indicate that there is greater environmental contamination at endemic farms than at unaffected farms (Takai et al 1991b). Although it is plausible that increased environmental exposure increases risk of infection and disease, one must also consider that infected animals are more likely to shed the organism in their feces and that greater environmental contamination may be a result rather than a cause of disease. The timing of assessment of such exposure is important because there appear to be seasonal influences on shedding and the organism is amplified in the intestinal tract of foals, and their shedding increases with age (Takai 1997).

The environment and characteristics of affected farms differ from those of unaffected farms. Generally, affected farms are larger in size, population, and, often, stocking density than unaffected farms (Chaffin et al 2003a); however, this may simply indicate that these farms have more opportunity to have individuals affected with any disease. The desirable health management practices (such as routinely testing and treating foals for failure of passive transfer of immunity) that are commonly used at breeding farms are not effective for controlling or preventing *R. equi* pneumonia (Chaffin et al 2003b). To date, evidence indicates that soil geochemistry does not differ between affected and unaffected farms (Martens et al 2002a).

Isolates of *R. equi* have been compared using DNA fingerprinting (genotyping) methods. Restriction fragment length polymorphisms have been used to identify geographic differences in the distribution of virulence plasmids of *R. equi* isolates from five countries. While this method may be useful for molecular epidemiologic studies of virulent *R. equi*, it is not highly discriminating and thus cannot be used to provide strong evidence of a link between isolates from a given region. Using pulsed-field gel electrophoresis (a more discriminating genotyping method), it is generally not possible to establish a genotypic link among isolates on the basis of source, time-point, or location (Cohen et al 2003). Isolates from different continents may be as similar to one another as isolates from the same country or region within the country. Furthermore, isolates from the same farm are rarely identical over time and the isolates found at farms may change over time. Thus it appears that diverse arrays of isolates of *R. equi* that are capable of causing disease are widely distributed. Consequently, it will rarely be possible to

link infections to a given site or region on the basis of analysis of isolates by use of genotyping of chromosomal DNA.

Considered together, the preceding findings indicate that host factors rather than environmental factors or farm-specific virulent strains may explain why some foals are affected while others remain unaffected at farms in which there is exposure of foals to virulent (VapA-positive) isolates. Data regarding foal-level risk factors associated with *R. equi* pneumonia are scant. It has been proposed that percutaneous invasion by third-stage larvae of *Strongyloides westeri* facilitates invasion by the bacterium and subsequent disease development. To date, studies of foal-level factors have only identified characteristics that relate to farm management as predisposing to disease (Chaffin et al 2003c). Given that horses older than foals appear to be resistant to infection, and given that *R. equi* is most often an opportunistic infectious disease in other species, it is plausible that the immunity of affected foals is different from that of foals that do not develop *R. equi* pneumonia. Recently, it was determined that foals with ratios of peripheral blood $CD4^+ : CD8^+$ lymphocytes that were < 3 during the first month of life were significantly more likely to develop *R. equi* pneumonia than foals from the same farm that had ratios ≥ 3 (Chaffin et al 2004). Further studies are needed to elucidate underlying immunologic differences between affected and unaffected foals.

Anecdotally, some mares at endemic farms are known to have multiple affected foals. If true, this observation has at least two explanations: (1) there is a genetic contribution to predisposition to susceptibility; or (2) mares may be a source of infection for their foals. Evidence exists that iron is important for the growth of *R. equi* and for expression of the virulence factor VapA. Sources from which, and mechanisms by which, *R. equi* acquires iron *in vivo* may contribute to the pathogenesis of infection. Evidence exists that transferrin genotypes differ between affected and unaffected foals. Polymorphisms in certain genes such as the *Nramp1* gene have been associated with susceptibility to intracellular bacteria, including organisms closely related to *R. equi* such as mycobacteria. However the authors have recently demonstrated that mice in which the *Nramp1* gene is functionally deleted were not more susceptible to infection with virulent *R. equi* than wild-type mice possessing the gene. Polymorphisms in the *Nramp1* gene have been described in horses, and it is possible that other genes related to the host immune response are associated with predisposition to *R. equi* pneumonia.

It is also possible that mares are a source of *R. equi* for their foals. *Rhodococcus equi* can be isolated commonly from the feces of mature horses and the organism can multiply in the feces of horses (Takai 1997). Although the bacterium can survive and multiply in soil, it has been proposed that *R. equi* is maintained primarily in the intestinal tract of horses. In a study of 43 foals from two farms in Japan (Takai et al 1986), the prevalence of fecal shedding

was high among foals both from an *R. equi*-endemic farm (94%) and a farm with no history of *R. equi* pneumonia (73%). Evidence exists that there can be seasonal variations of fecal shedding of *R. equi* by mares, with increases occurring during the spring. The mean number of organisms shed by mares during the five 1-week periods before and after foaling was similar. Given the high prevalence of fecal shedding by dams, the large volume of feces passed by adult horses, the increased prevalence of shedding by mares reported in the spring (foaling season), and the fact that many foals are coprophagic, it is plausible that the dam may be an important source of infection for foals.

The age at which foals become infected is unknown. Knowing this information is important because it has implications for control and prevention. Current dogma asserts that foals become infected at around the time maternal antibody wanes. The principal rationales for this hypothesis are that administration of hyperimmune plasma is partially protective (see section on Control and Prevention, p. 362) and that the distribution of ages at onset of *R. equi* is similar to the timing of declining maternal antibody concentrations in foals. Although this thinking is plausible and possibly correct, it may also be wrong. Evidence exists that immunoglobulin can modulate cell-mediated immunity, such that the protective mechanism of hyperimmune plasma administration might not relate to strictly humoral effects. Although the typical age at onset of pneumonia caused by *R. equi* is coincident with the age at which maternal antibody is approaching its nadir in foals, development of this pyogranulomatous disease is likely insidious and therefore infection is likely to occur weeks before the development of clinical signs. Our group has advanced an alternative hypothesis, namely that foals become infected very early in life (Horowitz et al 2001). Consistent with this hypothesis, *R. equi* can be isolated from the feces of foals < 1 week of age, indicating that foals are exposed early in life (Takai et al 1986). The concept of early infection first occurred to one of the authors (R.J.M.) when he observed that it was easier to experimentally infect foals before 2 weeks of age than it was to infect older foals, suggesting that younger foals might be particularly susceptible to infection. Irrespective of susceptibility, we have reported epidemiologic evidence that is consistent with foals being infected very early in life. This evidence was based on the distribution of the ages of onset and ages of death being logarithmically normal, consistent with infection at or around the time of birth (Horowitz et al 2001).

Clinical Signs

Rhodococcus equi is most commonly recognized as causing pneumonia in foals. Clinical signs are often insidious, such that early signs of disease are subtle, inconsistent, and non-specific, including low-grade fever, exercise intolerance, and a mildly increased respiratory rate. The disease
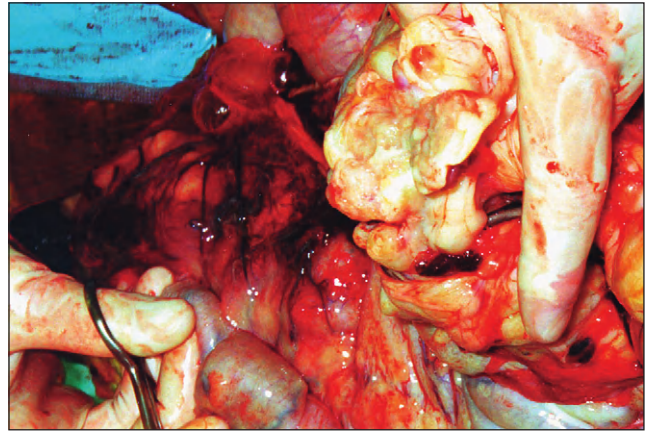
Fig. 24.1. Mesenteric pyogranuloma caused by *Rhodococcus equi* in a foal (being held by the surgeon's left hand). These lesions often progress in the face of appropriate antimicrobial therapy, and foals with intra-abdominal pyogranulomas generally have a poor prognosis.

generally appears to progress over a period of weeks. Foals may become lethargic, lose their appetite, become febrile, and have increases in respiratory rate and effort. The presence of cough and nasal discharge is inconsistent. Some foals appear to experience either a more rapid progression of the disease or a more rapid progression to severe pulmonary disease, sometimes in association with concurrent infection with *Pneumocystis carinii*.

Extrapulmonary disorders also commonly occur with *R. equi* infection of foals. Approximately two-thirds of affected foals admitted to a teaching hospital had at least one extrapulmonary disorder (Chaffin & Martens 1997). Intra-abdominal problems are among the more common extrapulmonary disorders, and include intra-abdominal lymphadenitis, granulomatous enterocolitis/typhlitis, diarrhea, and peritonitis. Approximately 50% of affected foals that were examined post-mortem had intra-abdominal lymphadenitis/lymphatic abscessation (Fig. 24.1) (Zink et al 1986). Clinical signs associated with intra-abdominal abscesses are often non-specific, and include failure to thrive, lethargy, fever, and diarrhea. Intra-abdominal lesions caused by *R. equi* may progress in the face of successful treatment of pneumonia caused by the bacterium.

Another common extrapulmonary disorder is polysynovitis (Fig. 24.2), possibly as the result of immune-complex deposition, and most commonly involving the tarsocrural, femoropatellar and carpal joints. Lameness is generally mild such that joint effusion is often the only clinical sign observed. Dissemination (presumably hematogenous) can occasionally result in osteomyelitis or septic arthritis. Clinical signs are referable to the site of the affected bone; for example, ataxia has been reported in association with vertebral body osteomyelitis in foals and cauda equina syndrome has been reported in association with diskospondylitis and a paravertebral abscess in a foal.

Fig. 24.2. Polysynovitis affecting the tarsocrural joints of a foal with *Rhodococcus equi* pneumonia.

Table 24.1. Extrapulmonary disorders occurring with *Rhodococcus equi* infection

Common
Abdominal lymphadenitis
Granulomatous enterocolitis/typhlitis with or without diarrhea
Peritonitis

Less common
Osteomyelitis
Septic arthritis
Diskospondylitis
Paravertebral abscessation
Pleuritis
Uveitis/keratouveitis/panophthalmitis
Mediastinal lymphadenitis
Hepatic pyogranuloma
Immune-mediated hemolytic anemia
Immune-mediated thrombocytopenia
Pericarditis
Guttural pouch empyema
Granulomatous laryngitis
Septic sinusitis
Pyogranulomatous dermatitis
Telogen effluvium
Pyelonephritis

A number of other extrapulmonary disorders have been reported (see Table 24.1).

One important aspect of extrapulmonary disorders is that they may become manifest before signs of respiratory tract disease; extrapulmonary disorders for which this is most common include immune-mediated polysynovitis, diarrhea, osteomyelitis, and septic arthritis. Extrapulmonary disorders may also be identified during the course of diagnostic evaluation or treatment, and some are only identified at necropsy. Some extrapulmonary disorders ultimately result in death or euthanasia, even if the associated pneumonia has been treated successfully.

Diagnosis of *R. equi* Pneumonia

Determining a diagnosis of *R. equi* pneumonia can be accomplished in a number of ways, depending on the status of the individual foal (severity of disease), the disease history of the farm, the resources and needs of the farm, and the resources and needs of the veterinarian. Diagnostic methods include non-specific and *R. equi*-specific approaches.

Non-specific testing

Results of non-specific diagnostic methods may provide an indication for specific diagnostic testing. Non-specific findings include the signalment, history, physical examination findings, clinicopathologic testing, and diagnostic imaging of the thorax. The disease is most commonly reported in foals aged 1–3 months, but we and others have seen cases with mild lesions in foals as young as 10–17 days of age (Prescott et al 1989, Horowitz et al 2001). Farm history is important because it will influence the probability that a foal with certain findings has *R. equi* pneumonia, and consequently, the extent of diagnostic efforts by the veterinarian. For example, typical radiographic findings in a foal from an endemic farm may be considered by a given veterinarian to be sufficient basis for a diagnosis of the disease in that foal. Extrapulmonary findings often occur with *R. equi* pneumonia, and their occurrence can provide an indication for further testing. Abnormal lung sounds are, in our experience, an insensitive diagnostic method, particularly early in the course of disease. This lack of sensitivity is likely because the disease is insidious such that abnormal lung sounds may not be found until infection is quite advanced.

Non-specific diagnostic testing should include a complete blood count because many, but by no means all, foals with *R. equi* pneumonia have leukocytosis, hyperfibrinogenemia or both (Giguere et al 2003, Chaffin et al 2004). Absence of these findings, however, would not preclude a diagnosis of the disease.

Diagnostic imaging should include ultrasonography, radiography, or both. Each method has strengths and limitations. Radiography of foals between 1 and 3 months of age can be performed in the field. Radiography can
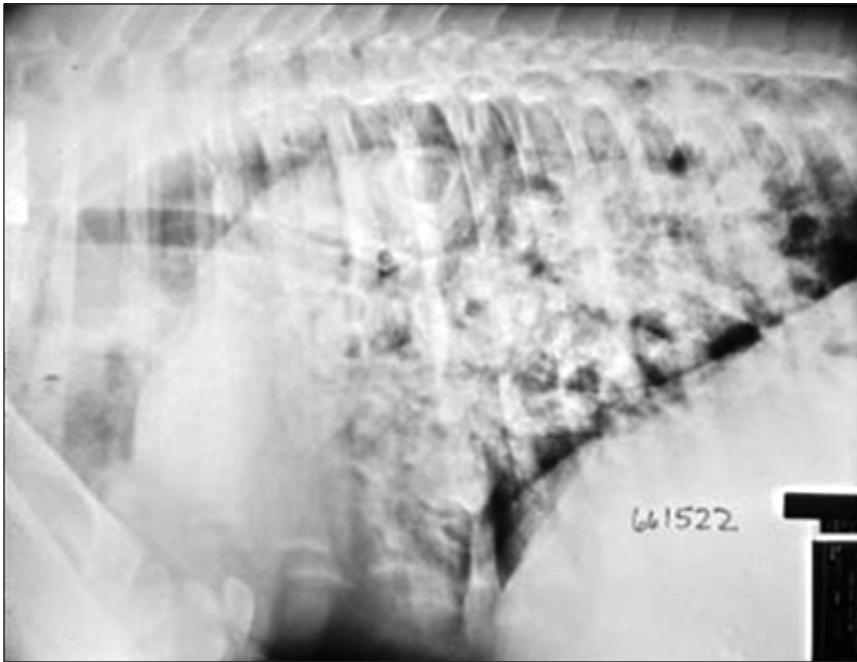
Fig. 24.3. Radiographic findings in a foal with severe *Rhodococcus equi* pneumonia. Numerous interstitial opacities caused by pyogranulomas appear throughout the lungs. The prognosis for foals with such radiographic findings is poor.

detect lesions that are either axial or abaxial (Fig. 24.3). Disadvantages of radiography include the need for special equipment and adequate personnel, exposure of personnel to radiation, costs associated with the procedure, the time required to perform and evaluate the results, technical limitations of performing the technique in larger foals, and the finding that early radiographic lesions can be subtle and less characteristic of *R. equi* pneumonia than more advanced cases. Ultrasonography may reveal abnormalities of the abaxial lung (Fig. 24.4). With experience, thoracic ultrasonography of a foal can be performed in a matter of minutes and, when the peripheral lung is involved, ultrasonography may be more sensitive than radiography. Disadvantages of ultrasonography include the potential to miss central pulmonary lesions surrounded by normal aerated lung, the need for experience to perform the technique with diagnostic accuracy, the need for special equipment and adequate personnel, costs associated with the procedure, and the time required to perform (including clipping a foal's coat when necessary) and evaluate the results. Radiographic or sonographic findings can be strongly suggestive of *R. equi* pneumonia and often provide a basis for more specific testing.

Rhodococcus equi-specific testing

Because they are relatively non-invasive, a variety of serologic tests have been developed, including enzyme-linked immunosorbent assays, an indirect hemagglutination inhibition test, agar gel immunodiffusion, and synergistic hemolysis inhibition tests. Recent evidence indicates that serologic testing of single or paired samples is

of no value for either diagnosis or early detection of *R. equi* pneumonia (Martens et al 2002b). There are a number of reasons why these tests have poor diagnostic accuracy. They measure exposure, and thus cannot differentiate foals that are exposed from foals that are actively infected. Given the ubiquitous nature of the organism and the fact that it can be found at affected and unaffected farms, exposure is widespread. Furthermore, the tests cannot differentiate between antibodies that are maternally derived and those

Fig. 24.4. Ultrasonographic image of a small, abaxial pyogranuloma (circular hypoechoic area) in the right eighth intercostal space of a foal with *Rhodococcus equi* pneumonia. The top of the figure is at the foal's skin surface (abaxial) and the bottom of the image is axial; dorsal is to the left and ventral to the right of the figure.

produced by the foal. Indeed, many mature horses have serologic evidence of exposure to the organism. Furthermore, paired titers may be of less value for an insidious disease like *R. equi* pneumonia because foals will have been infected for weeks or months before signs become apparent.

It is generally considered that detection of *R. equi* [either by microbiologic culture or by polymerase chain reaction (PCR)] from fluid collected by tracheobronchial aspiration (TBA) in combination with cytologic evidence of sepsis in the TBA fluid is needed to definitively diagnose *R. equi* pneumonia. This approach likely provides strong evidence for the disease, but it is important to consider limitations in the sensitivity and specificity of these methods. Microbiologic culture cannot differentiate between virulent and avirulent isolates. Reported sensitivities of TBA culture in foals with spontaneous disease, fatal disease, or experimental infection range from 57% to 100%. Foals without disease that are exposed to *R. equi* may yield positive results on culture. In one study, 35% (77/216) of foals had culture-positive TBA fluid, but lacked clinical signs of respiratory disease, suggesting low specificity (Ardans et al 1986); however, the extent to which these foals might have had subclinical pneumonia is unknown. Cytologic evidence of septic pneumonia (large number of degenerate neutrophils with intracellular rod-like organisms) and Gram-stain results consistent with *R. equi* pneumonia (large number of pleomorphic, Gram-positive rods) should be considered in establishing a diagnosis. Consideration of farm history and findings of diagnostic imaging can also help influence the probability that a positive TBA result is truly indicative of disease. Evidence exists that gene amplification of DNA extracted from TBA fluid using the PCR and primers for a segment of the *VapA* gene (specific for virulent organisms) may be more sensitive than culture (Sellon et al 2001). Limitations of this method include its limited availability and the fact that the high sensitivity may result in amplification of environmental "contaminants" that can be found at both affected and unaffected farms.

Performing a percutaneous TBA can pose significant risk to foals with marked respiratory compromise. Performing a TBA via endoscopy may be less stressful for some foals and technically easier for veterinarians (regardless of the status of the foal). Double-guarded, triple-lumen transendoscopic TBA catheters are commercially manufactured for obtaining specimens for microbiologic culture. To date, it appears that culturing the organism or using PCR for detection from nasal swabs or nasopharyngeal swabs are not reliable for diagnosis of *R. equi* pneumonia (Sellon et al 2001).

Diagnosis of *R. equi* Extrapulmonary Disorders

Some of the extrapulmonary disorders are directly attributable to infection (e.g. a large intra-abdominal abscess) whereas others may be immune-mediated (e.g. poly-

synovitis, uveitis, anemia). Presence of extrapulmonary disorders should prompt testing for pneumonia as well as specific testing to determine the causes of the extrapulmonary lesion(s). There is no reason to believe that serologic testing will be more useful for an extrapulmonary disorder than for *R. equi* pneumonia.

Culturing *R. equi* from the feces of foals is not reliable for diagnosing enteric infection (Takai et al 1986). Although serial quantitative culturing has been advocated, the reliability of this approach has not been tested, and it is not very practical to sequentially culture feces. Furthermore, culturing feces does not appear to be reliable for diagnosing *R. equi* pneumonia, as only 17% (5/30) of foals with *R. equi* pneumonia were fecal-culture positive in one study (Ardans et al 1986). Detecting the organism in aspirates of intra-abdominal abscesses and infected bone can be diagnostic. For suspected immune-mediated disorders, such as polysynovitis, cytologic or microbiologic culture results generally do not provide evidence of sepsis. Indeed, absence of evidence of sepsis is a basis for considering these lesions to be non-septic.

Treatment of *R. equi* Pneumonia

Antimicrobials

In vitro, a wide variety of antimicrobials is effective against *R. equi*. However, because of the intracellular location of *R. equi* and its development within pyogranulomatous lesions, drugs that are effective *in vitro* may not be effective *in vivo*. The efficacy of any antimicrobial for treatment of this disease has not been demonstrated using a controlled trial. There are retrospective data to suggest that the combination of penicillin and gentamicin is therapeutically ineffective (Sweeney et al 1987).

Recommended doses of antimicrobials for treatment of *R. equi* are summarized in Table 24.2. Erythromycin in combination with rifampin is considered the standard treatment for *R. equi* (Hillidge 1987, Sweeney et al 1987). Their combination is synergistic *in vitro* and *in vivo*, and the combination reduces the likelihood of resistance to either drug. Doses of rifampin range from 5 to 10 mg/kg by mouth (PO) once or twice daily. In the USA, the drug is available as capsules for human use. Doses for erythromycin range from 15–25 mg/kg four times a day to 25–37.5 mg/kg twice a day (Lakritz & Wilson 2002). There are different formulations of erythromycin for oral use; intravenous (IV) use is not recommended because of gastrointestinal and other side effects. Erythromycin ethylsuccinate is not well absorbed in foals and should be avoided. Erythromycin estolate is bioavailable in foals. In the USA, erythromycin phosphate is manufactured as a powder for use in poultry. It is relatively inexpensive, and at least as bioavailable as erythromycin estolate. Erythromycin stearate appears to be well absorbed in adult horses,

Table 24.2. Summary of recommended doses of antimicrobials for treatment of *Rhodococcus equi*		
Drug	**Dosage**	**Comments**
Erythromycin (base and salts)	15–25 mg/kg; PO; q 6–8 h	Many formulations are effective (see text), but ethylsuccinate should be avoided
Erythromycin (base and salts)	37.5 mg/kg; PO; q 12 h	Side effects of erythromycin include an environmentally mediated hyperthermia, diarrhea in the foal, and diarrhea in the dam
Azithromycin	10 mg/kg; PO; q 24 h for 5–7 days; then 10 mg/kg; PO; q 48 h	Can cause similar side effects as erythromycin; some people use q 24 h for duration of treatment
Clarithromycin	7.5 mg/kg; PO; q 12 h	May be more effective than other macrolides; likely to cause similar side effects to erythromycin
Rifampin	5–10 mg/kg; PO; q 12 h	Must be used in combination with a macrolide

and is less expensive than the estolate formulation. Erythromycin base is available as enteric-coated tablets made for human beings. Microencapsulated base in gelatin granules is reported to have better bioavailability than the coated tablets. The volume of the suspension can be large and this formulation is expensive. However, it has desirable properties, including good bioavailability, the pro-drug is absorbed intact, and it may produce enhanced intracellular concentration of the active metabolite (erythromycin A).

Side effects associated with erythromycin include environmentally modulated hyperthermia and diarrhea in foals, and diarrhea in the dam; the latter can be severe and often fatal (Gustafsson et al 1997, Stratton-Phelps et al 2000). Resistance to both erythromycin and rifampin has been reported for isolates of *R. equi* from both foals and human beings.

Use of azithromycin (10 mg/kg; PO; q 24 h for 5–7 days then q 48 h) has greatly increased. Advantages of the drug include once daily or every-other-day dosing and persistently high intracellular concentrations. Often, it is used in combination with rifampin. If it proves to be more effective than erythromycin, it is conceivable that the duration of treatment might be reduced. The drug is a human pharmaceutical, is expensive, and adverse reactions include diarrhea, elevated liver enzymes, and, rarely, hyperthermia. Additionally, the syndrome of inappropriate antidiuretic hormone secretion attributed to azithromycin has been reported in a human patient. Clarithromycin (7.5 mg/kg; PO; q 12 h) is another macrolide that has been reported to be effective for treating *R. equi* pneumonia (Giguere 2001). Anecdotally, data from a retrospective study indicate that clarithromycin may be more effective than other macrolides for treating *R. equi* pneumonia (Giguere et al 2004). It may be combined with rifampin, and side effects include diarrhea. Because its use has been limited to date, it is unclear whether other common macrolide-associated side effects,

such as elevations in liver enzymes or hyperthermia, should be expected with clarithromycin.

Newer classes of antimicrobials may become available or necessary as resistance to other antimicrobials develops. For example, linezolid is a member of a new class of antimicrobials, the oxazolidinones. Pharmacokinetics in horses or foals are lacking for this drug. It is used to treat *R. equi* and other Gram-positive infections in human beings, including methicillin-resistant *Staphylococcus aureus*, and consequently its use in veterinary medicine may be restricted or discouraged.

Duration of treatment of *R. equi* is generally prolonged. The earlier the infection is diagnosed, the shorter the duration of treatment is likely to be (this is the rationale for screening). Foals with radiographically apparent lesions should be treated for a minimum of 3 weeks. Monitoring cases with radiography or ultrasonography can be helpful. In general, treatment should be extended at least a few days past resolution of ultrasonographic or radiographic lesions. Monitoring the white blood cell count or fibrinogen concentration can be used, but these are imprecise, and we recommend treating at least 2 weeks beyond reduction of either parameter to the reference range and evidence that the foal is clinically healthy. Serologic testing is of no value for monitoring affected foals. There is some evidence that monitoring serum amyloid A is of value.

Other treatments

There are also ancillary treatments that can be administered to affected foals. The benefits of hyperimmune plasma as adjunctive therapy remain unknown, but should be considered. The use of non-steroidal anti-inflammatory drugs to reduce fever and pulmonary inflammation may be of benefit for some foals. Administration of intranasal oxygen may help foals with severe respiratory distress. The benefit of bronchodilators for treating foals with

this disease is unknown. Nebulization of antimicrobials, bronchodilators, and mucolytic agents may be of benefit for some foals. Combinations of some medications (e.g. aminophylline and erythromycin) may have interactions that result in adverse effects. Avoiding exposure of foals to high heat and humidity (if possible) may help to avoid macrolide-associated hyperthermia, and also may make pneumonic foals more comfortable.

Treatment of Extrapulmonary Disorders

Treatment with erythromycin or another macrolide in combination with rifampin is indicated for intra-abdominal pyogranulomas; however, many of these lesions appear to progress in the face of treatment that is effective for *R. equi* pneumonia. The prognosis for intra-abdominal pyogranulomas is generally poor. Typically, polysynovitis lesions resolve with successful treatment of the accompanying pneumonia. The authors have occasionally used systemically administered corticosteroids as adjunctive therapy. Limiting, but not eliminating, exercise may be of benefit for affected foals. Appropriate therapy for other septic conditions, such as drainage of abscesses or curettage for osteomyelitis, may be needed. Some manifestations such as uveitis may be immune-mediated or non-responsive to antimicrobials, but may resolve solely with successful treatment of the accompanying pneumonia.

Prognosis for *R. equi* Infections

Prognosis for foals with *R. equi* infection varies with the severity and anatomic location of the infection, and appears to vary among farms and admitting clinics (Ainsworth et al 1998, Chaffin et al 2003a). Generally, case fatality proportions are low (<30%) but may be considerably higher at some farms. Early detection of disease and prompt implementation of treatment will improve prognosis for foals affected with *R. equi* pneumonia. Foals admitted to a group of teaching hospitals that were tachycardic and in respiratory distress at admission had a poorer prognosis (Ainsworth et al 1998). Foals with more severe radiographic abnormalities are less likely to survive (Ainsworth et al 1998). The prognosis for foals with intra-abdominal lymphadenitis and granulomatous enterocolitis/typhlitis is guarded to poor (Chaffin & Martens 1997), but treatment may occasionally be successful.

Foals that survive *R. equi* appear to be less likely than the general population of contemporary thoroughbred or standardbred foals in the USA to start at least one race. However, standardbred and thoroughbred foals that survive and race do not appear to have reduced performance (Ainsworth et al 1998). The impact of *R. equi* on activities other than racing has not been systematically evaluated.

Control and Prevention

For farms with foals affected by *R. equi* pneumonia, we have suggested an approach to control and prevention that addresses three areas, namely:

- treatment of affected foals
- screening for early detection of infection or disease in foals without clinical signs
- preventing infection or disease in foals.

This approach to control and prevention entails considering the farm, rather than individual foals, as the unit for evaluation. It is important to consider that programs for control and prevention must be developed to accommodate the specific needs and resources of a given farm. Resources that a farm is willing and able to commit will vary, and the duration and extent of the pneumonia problem will influence how much money and other resources owners/managers will be willing to expend. Nevertheless, the first occurrence of *R. equi* pneumonia on a farm could represent the beginning of recurrent problems. Veterinarians should consider a foal with *R. equi* pneumonia as an individual patient in need of care, as a source of virulent organisms, and as a signal that the farm of origin may experience new and/or ongoing problems with the disease. Owners and farm managers should be advised that, as is the case for treatment, no program for control and prevention should be expected to be 100% effective.

Treatment of affected foals

Treatment of affected foals has been discussed previously. Whether affected foals should be isolated from other foals and pregnant mares is unclear. As noted, virulent isolates of the organism can be found in the soil of farms with or without a history of *R. equi* pneumonia (Martens et al 2000), and the source of infection for foals remains uncertain. Exposure is likely widespread at affected farms and has probably occurred for many foals by the time cases of *R. equi* pneumonia are identified. However, foals can amplify the organism in their intestinal tracts, thereby enhancing contamination of the environment. Because such environmental contamination may increase exposure to the agent, it may be advisable to isolate affected foals. In the case of farms with resident and transient populations, it seems appropriate to minimize mixing of groups so that affected transient horses and foals do not contaminate the environment of resident horses and vice versa.

Screening for earlier detection of infection or disease

A variety of methods exist for screening foals at farms with a history of *R. equi* pneumonia to detect disease or infection at an earlier point in time; however, none of them is ideal

in terms of accuracy, cost, and labor. The rationale for screening is the belief that earlier initiation of specific treatment will improve the prognosis for recovery. The various methods for screening often need to be applied repeatedly. The individual screening methods can be employed sequentially, individually, or in parallel, depending on the needs and resources of the individual farm. Using a single screening test and initiating treatment on the basis of a positive result might be best for a farm with recurrent high prevalence of disease or one with limited resources. In contrast, farms with greater resources or more sporadic disease occurrence might use a sequence of screening tests to ensure a higher probability that a foal selected for treatment has the disease. Variation in resources and preferences renders it impossible either to prescribe methods for control and prevention that are suitable for all farms or to predetermine the approach to screening that is best for a particular farm. The plan for each farm must be determined after consideration of the specific needs and wishes of each farm, and following consultations between the veterinarian, farm owners, and farm managers.

Screening tests are not the same as diagnostic tests

The distinction between a diagnosis based on a screening test (an epidemiologic diagnosis) and a diagnosis based on definitive diagnostic testing (a clinical diagnosis) should be explained to all interested parties. Positive results of a screening test for early detection of *R. equi* would be a basis for initiating treatment for *R. equi*; however, positive results of a screening test do not represent a definitive diagnosis of either *R. equi* infection or its disease. A useful screening test is one in which the probability of disease is very high among those with a positive test result (i.e. high positive predictive value) and very low among those with negative test results (i.e. high negative predictive value). The higher the prevalence of disease at a farm, the higher the positive predictive value of a screening test's result. Thus, a positive result for a screening test, such as leukocytosis, for *R. equi* pneumonia would need to be interpreted differently for a foal at a farm with a recurrent history of pneumonia caused by *R. equi* than it would be for a foal at a farm without a history of *R. equi* pneumonia. For the former, but not the latter, the result would be a reasonable basis for treating the foal for *R. equi* pneumonia.

Visual inspection of foals at an affected farm is recommended as an initial screening test. Pneumonia caused by *R. equi* is insidious and clinical signs are often absent in foals with early infection or disease; however, foals that have increased respiratory effort or lethargy should be further evaluated. Visual inspection may also reveal extrapulmonary disorders such as polysynovitis. These extrapulmonary disorders are often the initial clinical signs detected in affected foals (Chaffin & Martens 1997). Rectal temperatures of foals can be monitored twice daily. Febrile foals should be either treated or tested further. Performing physical examination (including thoracic auscultation) twice weekly has been demonstrated to be effective for early recognition and reduction of mortality attributed to *R. equi* pneumonia at an endemic farm (Prescott et al 1989). However, in our experience, thoracic auscultation alone is an insensitive tool for early detection of foals with *R. equi* pneumonia.

Results of complete blood counts can be used for screening purposes. Foals with leukocytosis (i.e. >13,000 white blood cells per liter) or increased concentration of fibrinogen (>400 mg/dl) should be considered either for further testing or for treatment. Evidence exists that fibrinogen concentration is a relatively insensitive indicator of *R. equi* infection (Giguere et al 2003). Serologic tests lack sensitivity and specificity both for screening (early detection) and diagnosis of *R. equi* pneumonia (Martens et al 2002b).

Radiography, ultrasonography, or both can be useful for screening to detect foals with early or subclinical *R. equi* pneumonia. Diagnostic imaging is relatively specific because it may reveal pulmonary lesions. The radiographic appearance of pulmonary lesions is often strongly suggestive of *R. equi* pneumonia, and in any case antimicrobial therapy for *R. equi* pneumonia is generally effective for streptococcal organisms which are the other common cause of lung abscesses in foals (Hoffman et al 1993).

A positive result of a screening test would be interpreted as an indication for either further screening or initiating treatment. The decision to initiate treatment must include consideration of the aforementioned adverse effects of antimicrobials. An example of a sequential screening program would be one in which foals were visually inspected and had rectal temperatures recorded twice daily; any foal that appeared abnormal or was febrile would have a complete blood count performed; any foal with a leukocytosis or hyperfibrinogenemia would be treated with antimicrobials (or subjected to radiography or ultrasonography). However, some veterinarians may prefer to subject foals that are identified as positive by screening tests to more definitive diagnostic testing to reduce the rate of false-positive screening results. It should be recognized, however, that the goal of screening is not to obtain a definitive diagnosis, and accurate diagnosis early in the course of infection is difficult and false-negative results can occur in more definitive diagnostic testing.

Preventing Disease

Hyperimmune plasma

To date, the only method proven to prevent pneumonia caused by *R. equi* is transfusion of hyperimmune plasma (Martens et al 1989), but it is not without limitations.

Plasma administration is costly and labor-intensive, is associated on rare occasions with transfusion reactions or injury to the foal during handling, and is not universally successful. The volume of plasma that should be administered and the time(s) of administration for optimal protection are unknown. We recommend administration of 1 or 2 liters of hyperimmune plasma to a foal during the first few days of life and again during the third week of life. The rationale for this approach is that we believe exposure and infection occur early in life and that younger foals are more susceptible to infection than older foals (see above).

Although hyperimmune plasma is expensive, the costs of prophylactic administration of plasma to many foals can be lower for some farms than the costs of treatment and the lost value of any foals that succumb to the disease. Assessing the cost–benefit ratio for use of plasma for prevention may be beneficial, using methods such as decision-tree analysis. Such an analysis requires estimation of the average value of foals produced, the expected prevalence of disease, the case fatality rate, and the average cost of treating *R. equi* pneumonia. Farms with a high prevalence of disease, high case fatality rate, or both will often have a net financial benefit from administration of 1 or 2 liters of hyperimmune plasma to all foals.

Vaccination

To date, evidence is lacking that vaccination of mares or oral administration of colostrum from *R. equi*-immunized mares to neonatal foals is effective for preventing disease. A product containing concentrated serum which was developed to prevent failure of passive transfer was reported to increase serum antibody titers against *R. equi* and to delay by 2–3 weeks the time of seroconversion to *R. equi* among foals (Davis et al 1995). Controlled trials demonstrating the efficacy of this product have not been reported. Given that colostrum from immunized mares is not protective (Martens et al 1991, Mueller & Madigan 1992), claims of efficacy for oral products should be viewed with considerable caution. Vaccination of foals or dams has not been demonstrated in controlled trials to be effective for preventing *R. equi* pneumonia. Novel strategies for vaccination, such as development of DNA vaccines to promote mucosal immunity, may eventually prove to be effective. Alternatively, vaccination may be ineffective if foals are infected very early in life such that infection would have occurred before the development of an effective immune response.

Environmental and management considerations

Environmental factors often influence the risk of infectious diseases. A number of management factors have been implicated in relation to *R. equi* pneumonia but evidence is generally lacking. In the USA, the disease is not associated with poor health management practices. Rather, the disease often occurs on farms that provide health management deemed to be desirable, indicating that these strategies are inadequate for preventing *R. equi* pneumonia (Chaffin et al 2003b).

Removing manure from foaling stalls, pens, and paddocks could decrease environmental contamination and the level of exposure of susceptible foals because *R. equi* is shed in the feces of mares and foals and multiplies within feces (Takai 1997). Frequent cleaning of foaling stalls may help to decrease contamination and the opportunity for infection; however, the organism is a soil saprophyte that can be found both at farms affected with the disease and at farms not affected with the disease. The organism is seldom recovered from soil at a depth greater than 30 cm (Takai 1997). Removal and replacement of topsoil in stalls and pens is impractical and soil might rapidly become re-contaminated.

Methods used for disposal of manure might reduce exposure to *R. equi*. Directly spreading feces containing virulent *R. equi* on pastures may increase contamination of the environment. Composting manure before spreading it on pastures or disposing of manure without applying it to pastures is recommended; however, evidence to support these recommendations is lacking.

The stocking density of horses, particularly the density of foaling mares and foals, is likely to increase the risk of *R. equi* pneumonia. Efforts to reduce the stocking density of mares and foals should be considered on farms that have a high density of breeding horses and endemic *R. equi* pneumonia. A systematic cost–benefit analysis would have to be conducted to determine whether the financial benefits of decreased disease and death outweigh the costs of lost revenue associated with reducing the number of horses or increasing the pasture area.

Keeping resident and transient populations separated may help to reduce transmission. Although evidence that this approach specifically prevents transmission and spread of *R. equi* pneumonia is lacking, it is a generally useful principle for control of infectious diseases at farms with resident and transient populations. Whether there is benefit in isolating mares and foals returning to unaffected farms from *R. equi*-endemic farms is unclear. Virulent isolates of this organism can be found in the soil of unaffected farms, and duration or pattern of shedding by infected horses is ill-defined. As a general principle for control of infectious diseases, isolating mares and foals returning from facilities where there are horses of diverse origins and ages is advisable.

As inhalation is considered the principal mode of transmission of *R. equi* pneumonia, improving ventilation may decrease the risk of *R. equi* pneumonia. Anecdotally and in a recent survey of farms in the USA, a dusty environment was associated with farms having *R. equi* infections.

Diminishing dust in the environment may help to reduce the risk of *R. equi* pneumonia. Applying water sprinklers to pens and small paddocks can reduce dust. Rotation of paddocks to avoid overgrazing, reducing the density of horses (and foals) in paddocks or pens, and reseeding paddocks or pens may diminish dust levels by promoting grass growth.

Chemoprophylaxis

Chemoprophylaxis for *R. equi* pneumonia has not been evaluated in foals. Given that hyperimmune plasma is not completely effective and has the other aforementioned limitations, and that vaccination is unlikely to be effective if foals are infected early in life, this approach merits further investigation.

REFERENCES

Ainsworth DM, Eicker SW, Yeager AE et al 1998 Associations between physical examination, laboratory, and radiographic findings and outcome and subsequent racing performance of foals with *Rhodococcus equi* infection: 115 cases (1984–1982). Journal of the American Veterinary Medical Association 213: 510–515

Ardans AA, Hietala SK, Spensley MS et al 1986 Studies of naturally occurring and experimental *Rhodococcus equi*. Proceedings of the American Association of Equine Practitioners 32: 129–144

Byrne BA, Prescott JF, Palmer GH et al 2001 Virulence plasmid of *Rhodococcus equi* contains inducible gene family encoding secreted proteins. Infection and Immunity 69: 650–656

Chaffin MK, Martens RJ 1997 Extrapulmonary disorders associated with *Rhodococcus equi* pneumonia in foals: retrospective study of 61 cases (1988–1996). Proceedings of the American Association of Equine Practitioners 43: 79–80

Chaffin MK, Cohen ND, Martens RJ 2003a Evaluation of equine breeding farm characteristics as risk factors for development of *Rhodococcus equi* pneumonia in foals. Journal of the American Veterinary Medical Association 222: 467–475

Chaffin MK, Cohen ND, Martens RJ 2003b Evaluation of equine breeding farm management and preventative health practices as risk factors for development of *Rhodococcus equi* pneumonia in foals. Journal of the American Veterinary Medical Association 222: 476–485

Chaffin MK, Cohen ND, Martens RJ et al 2003c Foal-related risk factors associated with development of *Rhodococcus equi* pneumonia on farms with endemic infection. Journal of the American Veterinary Medical Association 223: 1791–1799

Chaffin MK, Cohen ND, Martens RJ et al 2004 Hematologic and immunophenotypic factors associated with development of *Rhodococcus equi* pneumonia of foals at equine breeding farms with endemic infection. Veterinary Immunology and Immunopathology 100: 33–48

Cohen ND, Smith KE, Ficht TA et al 2003 Epidemiologic study of pulsed-field gel electrophoresis of isolates of *Rhodococcus equi* obtained from horses and horse farms. American Journal of Veterinary Research 64: 153–161

Davis J, Gaskin JM, Hietala SK et al 1995 Oral administration of concentrated equine serum to newborn foals prevents failure of passive transfer of immunoglobulin and provides passive transfer of immunity for *Rhodococcus equi*. Proceedings of the American Association of Equine Practitioners 41: 176–177

Debey MC, Bailie WE 1987 *Rhodococcus equi* in fecal and environmental samples from Kansas horse farms. Veterinary Microbiology 14: 251–257

Giguere S 2001 *Rhodococcus equi* pneumonia. Proceedings of the American Association of Equine Practitioners 47: 456–467

Giguere S, Hondalus MK, Yager JA et al 1999 Role of the 85-kilobase plasmid and plasmid-encoded virulence-associated protein in intracellular survival and virulence of *Rhodococcus equi*. Infection and Immunity 67: 3548–3557

Giguere S, Hernandez J, Gaskin J et al 2003 Evaluation of white blood cell concentration, plasma fibrinogen concentration, and an agar gel immunodiffusion test for early identification of foals with *Rhodococcus equi* pneumonia. Journal of the American Veterinary Medical Association 222: 775–781

Giguere S, Jacks S, Roberts GD et al 2004 Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with *Rhodococcus equi* pneumonia. Journal of Veterinary Internal Medicine 18: 568–573

Gustafsson A, Baverud V, Gunnarson A et al 1997 The association of erythromycin ethylsuccinate with acute colitis in horses in Sweden. Equine Veterinary Journal 29: 314–318

Hillidge CJ 1987 Use of erythromycin-rifampin combination in treatment of *Rhodococcus equi* pneumonia. Veterinary Microbiology 14: 337–342

Hoffman AM, Viel L, Prescott JF et al 1993 Association of microbiologic flora with clinical, endoscopic, and pulmonary cytologic findings in foals with distal respiratory tract infection. American Journal of Veterinary Research 54: 1615–1622

Horowitz ML, Cohen ND, Takai S et al 2001 Application of Sartwell's model (lognormal distribution of incubation periods) to age at onset and age at death of foals with *Rhodococcus equi* pneumonia as evidence of perinatal infection. Journal of Veterinary Internal Medicine 15: 171–175

Lakritz J, Wilson WD 2002 Erythromycin and other macrolide antibiotics for treating *Rhodococcus equi* pneumonia in foals. Compendium on Continuing Education for Practicing Veterinarians 24: 256–261

Martens RJ, Martens JG, Fiske RA 1989 *Rhodococcus equi* foal pneumonia: protective effects of immune plasma in experimentally infected foals. Equine Veterinary Journal 21: 249–255

Martens RJ, Martens JG, Fiske RA 1991 Failure of passive immunization by ingestion of colostrum from immunized mares to protect foals against *Rhodococcus equi* pneumonia. Equine Veterinary Journal 12 (suppl): 19–22

Martens RJ, Takai S, Cohen ND et al 2000 Association of disease with isolation and virulence of *Rhodococcus equi* from farm soil and foals with pneumonia. Journal of the American Veterinary Medical Association 217: 220–225

Martens RJ, Cohen ND, Chaffin MK et al 2002a Association of *Rhodococcus equi* foal pneumonia with farm soil geochemistry. American Journal of Veterinary Research 63: 95–98

Martens RJ, Cohen ND, Chaffin MK et al 2002b Evaluation of 5 serologic assays to detect *Rhodococcus equi* pneumonia in foals. Journal of the American Veterinary Medical Association 221: 825–833

Mueller NS, Madigan JE 1992 Methods of implementation of an immunoprophylaxis program for the prevention of *Rhodococcus equi* pneumonia: results of a 5-year field study. Proceedings of the American Association of Equine Practitioners 38: 193–201

Prescott JF 1991 *Rhodococcus equi*: an animal and human pathogen. Clinical Microbiology Reviews 4: 20–34

Prescott JF, Machangu R, Kwiecien J et al 1989 Prevention of foal mortality due to *Rhodococcus equi* pneumonia on an endemically affected farm. Canadian Veterinary Journal 30: 871–875

Sellon DC, Besser TE, Vivrette SL et al 2001 Comparison of nucleic acid amplification, serology, and microbiologic culture for diagnosis of *Rhodococcus equi* pneumonia in foals. Journal of Clinical Microbiology 39: 1289–1293

Stratton-Phelps MS, Wilson WD, Gardner IA 2000 Risk of adverse effects in pneumonic foals treated with erythromycin versus other antibiotics: 143 cases (1986–1996).

Journal of the American Veterinary Medical Association 217: 68–73

Sweeney CR, Sweeney RW, Divers TJ 1987 *Rhodococcus equi* pneumonia in 48 foals: response to antimicrobial therapy. Veterinary Microbiology 14: 326–329

Takai S 1997 Epidemiology of *Rhodococcus equi* infections: a review. Veterinary Microbiology 56: 167–176

Takai S, Timori S, Tsubaki S 1986 Quantitative fecal culture for early diagnosis of *Corynebacterium* (*Rhodococcus*) *equi* enteritis in foals. Canadian Journal of Veterinary Research 50: 479–484

Takai S, Sekizaki T, Ozawa T et al 1991a Association between a large plasmid and 15- to 17-kilodalton antigens in virulent *Rhodococcus equi.* Infection and Immunity 59: 4056–4060

Takai S, Ohbushi S, Koike K et al 1991b Prevalence of virulent *Rhodococcus equi* in isolates from soil and feces of horses from horse-breeding farms with and without endemic infections. Journal of Clinical Microbiology 29: 2887–2889

Zink MC, Yager JA, Smart NL 1986 *Corynebacterium equi* infections in horses: 1958–1984: a review of 131 cases. Canadian Journal of Veterinary Research 27: 213–217

25 Diseases of the Nasal Cavities

Jim Schumacher and Padraic M Dixon

High Blowing (False Nostril Flutter)

High blowing is a loud, vibratory expiratory noise that is associated with turbulence in the nasal vestibule caused by vibration of both the true and false nostrils. Some horse may produce high blowing noises at the start of exercise or when excited. These noises are not associated with respiratory impairment, and so are not clinically important. Some owners even find them desirable.

Sometimes, while high blowing, the horse's nostrils can be seen to vibrate. High blowing noises usually disappear when the horse is strenuously exercised. Although the noise caused by high blowing is not clinically important, it must be recognized and differentiated from abnormal respiratory noise caused by disease.

Nasal Paralysis

The nostrils are comma-shaped when the horse is at rest, but during strenuous exercise the nostrils become circular as the musculature of the nostrils contracts to increase the size of the nasal vestibule. Nasal collapse is usually the result of trauma to one or both dorsal buccal branches of cranial nerve VII (i.e. facial nerves) caused by trauma to the side of the head or improper padding beneath the head when the horse is anesthetized and positioned in lateral recumbency. Damage to a facial nerve resulting in nasal collapse can also be caused by mycosis of a guttural pouch or by temporohyoid osteoarthropathy. Unilateral nasal collapse can occur after injury to the dorsal buccal branch of cranial nerve VII during dental extraction performed through a buccotomy. Mild, bilateral paralysis can be caused by harness that is too tight or by a bitless bridle. Severe, bilateral facial paralysis causing bilateral nasal collapse can occur in association with disorders causing generalized neuromuscular dysfunction, such as botulism or lead poisoning.

Clinical signs

Horses suffering from unilateral or bilateral nasal collapse exhibit exercise intolerance and make an abnormal respiratory noise that can be localized to the nostrils. A unilaterally affected horse may have a detectable decreased airflow from the affected naris while at rest. Failure to dilate the nostrils and nasal collapse during inspiration may be apparent when the horse exercises.

Diagnosis

Unilateral nostril paralysis is usually obvious, because the horse has a deviated muzzle and sometimes a dropped ear and ptosis on the affected side, depending on the level of the site of nerve damage. Mild, bilateral nasal collapse caused by bilateral facial paralysis may be more difficult to recognize. The condition may be confirmed if the horse's tolerance to exercise improves and abnormal noises disappear when the nostrils are dilated by abducting the alar cartilages with sutures (Beard 1996). An affected nostril may be seen to collapse when the contralateral nostril is occluded to increase inspiratory pressure (Torre 2000, 2003). Severe, bilateral facial paralysis associated with generalized neuromuscular dysfunction is usually associated with other clinical signs, such as generalized muscular weakness.

Treatment and prognosis

Nasal collapse resulting from neuropraxia of cranial nerve VII or its branches caused by a blunt trauma to the side of the head usually resolves spontaneously within days to months. Anti-inflammatory therapy, with corticosteroids and/or non-steroidal anti-inflammatory drugs, may hasten recovery. If nasal collapse does not resolve, airflow through the affected nostril can be improved by removing the alar fold (see Alar Fold Stenosis, p. 373) and/or by implanting a prosthesis to provide rigid support to the naris (Hawkins et al 1995). Prostheses used to prevent nasal collapse include autogenous auricular cartilage (Torre 2003) and stainless steel mesh (Torre 2000). Removing the lateral alae or creating a permanent tracheostomy may also improve the horse's respiratory capacity, but these procedures are disfiguring.

Vasomotor Rhinitis

Vasomotor rhinitis is a non-inflammatory, non-allergic, physiological disorder of the nasal mucosa characterized by a bilateral watery nasal discharge, sneezing, obstruction

Fig. 25.1. The nostril of a horse suffering from grass sickness that has developed rhinitis sicca, with excoriation and crusting of the nasal mucosa.

to nasal airflow, nasal pruritus, and loss of sense of smell (Lane & Mair 1987, McGorum & Dixon 1990, Ayars 2000). The disorder may be caused by an imbalance in the autonomic control of nasal mucosal function that results in hyperreactivity of the nasal mucosa to exogenous and endogenous stimuli. The disorder occurs commonly in human beings, but apparently horses are seldom affected.

Clinical signs and diagnosis

Clinical signs of vasomotor rhinitis include bilateral serous nasal discharge, nose rubbing, snorting, and head shaking (Lane & Mair 1987, McGorum & Dixon 1990). Clinical signs in human beings may be triggered by changes in temperature and humidity, certain odors, and physical or emotional stress, and the same may be true of horses. In a report of an affected horse, clinical signs were induced by exercise or by stress (McGorum & Dixon 1990). Clinical signs of unilateral vasomotor rhinitis in another horse were associated with unilateral Horner syndrome and probably resulted from damage to the sympathetic inner-vation of the nasal mucosa (Lane & Mair 1987). Diagnosis of vasomotor rhinitis is based on clinical signs, elimination of other causes of chronic rhinitis, and remission of signs after treatment.

Treatment

Horses affected with vasomotor rhinitis can be treated by nebulization with an adrenergic agonist, such as xylometazoline, or with sodium cromoglycate (McGorum & Dixon 1990). Adrenergic agents may reduce nasal congestion and discharge by constricting the nasal

vasculature. Sodium cromoglycate stabilizes mast cells and may reduce nasal inflammation by preventing excessive release of histamine. Nasal administration of drugs is often resented by horses, and long-term nasal administration of an adrenergic agonist may eventually result in desensitiza-tion of the nasal vasculature to the adrenergic agent.

Rhinitis Sicca

Rhinitis sicca is a common disorder of horses suffering from chronic grass sickness and is characterized by swelling, excoriation, and exudation of the nasal mucosa, presumably as a result of disturbed autonomic control of the nasal vasculature and mucosal glands. The crusted and inflamed nasal mucosa is usually visible at the nostrils (Fig. 25.1). Depending on the degree of nasal obstruction, affected horses may make "snuffling" respiratory sounds at rest, and occasionally horses with severe nasal obstruc-tion need a tracheostomy. Rhinitis sicca may adversely affect olfaction, which may further depress appetite and, therefore, worsen the prognosis for severely affected horses. The disorder resolves over a period of months, if the horse survives (Milne et al 1994).

Apical Infection of a Rostral Cheek Tooth with Intranasal Drainage

The apices of the first, second, and third cheek teeth (Triadan 106–108 and 206–208) are embedded in the rostral portion of the maxillary bone (Fig. 25.2) and lie completely or partially outside the paranasal sinuses. Periapical infection of these teeth usually results in facial swelling and sometimes in a discharging tract on the side of
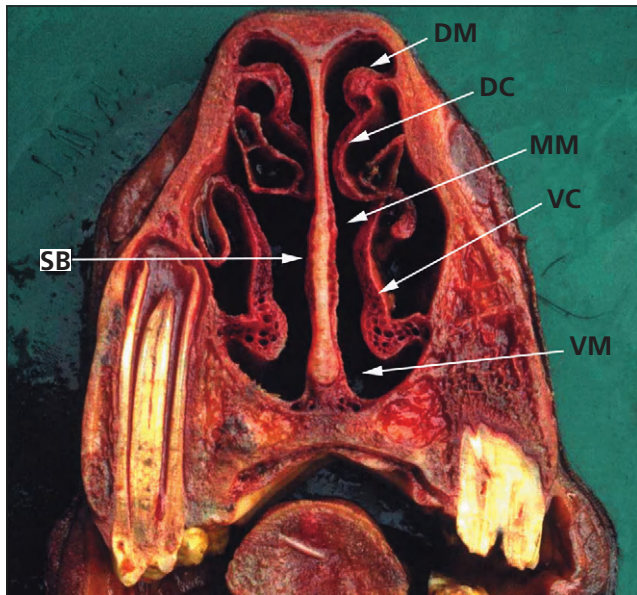
Fig. 25.2. Transverse section of an equine skull at the level of the second cheek teeth (Triadan 07) showing the dorsal meatus (DM), dorsal concha (DC), middle meatus (MM), ventral concha (VC), ventral meatus (VM), and swell body (SB). The swell body is distended by blood in the live horse and protrudes into the middle meatus.

the face. It may also occasionally result in formation of a tract that drains medially into the nasal cavity, leading to a unilateral, purulent, foul-smelling nasal discharge and sometimes formation of a nasal granuloma (Tremaine & Dixon 2001a,b).

Clinical signs

Clinical signs of a nasal tract caused by periapical infection of a maxillary cheek tooth include halitosis, purulent nasal discharge, and sometimes, rostral maxillary swelling. Pain associated with disease of the clinical crown of the affected tooth may cause quidding in a small proportion of affected horses. A large nasal granuloma may cause a detectable decrease in airflow from the affected nasal cavity. Nasal endoscopy may reveal exudate, feed material, or a granuloma within the rostrolateral aspect of the nasal cavity. The tall reserve crowns of young horses may cause the draining tract to be obscured by the ventral concha.

Diagnosis

Careful oral examination may reveal an abnormality of the crown of one of the maxillary premolars, such as occlusal exposure of the pulp, infundibular caries, or fracture. Radiographic examination of the dental apices is necessary to confirm periapical infection but when radiographic changes are equivocal, scintigraphy is useful.

Treatment

Treatment involves extraction of the affected tooth and sealing of the oral aspect of the oronasal fistula, using a material such as bone cement (polymethylmethacrylate) or dental wax. Some surgeons have attempted endodontic treatment of affected horses, but the efficacy of this treatment has not been critically assessed. If the intranasal granuloma is large, it should be excised. The draining tract soon resolves after the source of infection has been removed.

Oronasal Fistula

An oronasal fistula is characterized by the presence of a direct, epithelial-lined communication between the oral and nasal cavities that allows the ingress of oral contents into the nasal cavity. The usual cause of an oronasal fistula is failure of the alveolus of a rostral cheek tooth to heal completely after the tooth has been repulsed. The non-healing alveolus often contains dental or alveolar remnants and is incompletely filled with infected granulation tissue and feed.

Affected horses have a unilateral, malodorous, purulent nasal discharge that often contains feed material. Some affected horses have a rostral maxillary swelling on the affected side. During oral examination, the non-healed alveolus appears as a deep cavity, and a metal probe inserted into the oral aspect of the alveolus can be observed or palpated where it emerges in the nasal cavity. Digital palpation of the rostrolateral wall of the affected nasal cavity may reveal a tract or a granuloma. Rhinoscopy may be useful in identifying the nasal aspect of the fistula or associated food and pus (Fig. 25.3), if the fistula is situated beyond the reach of a finger. Dental or alveolar remnants can often be observed radiographically in the unhealed alveolus.

Treatment

Treatment is initially directed at promoting alveolar healing by removing dental and osseous fragments and the epithelial lining of the tract using a long-handled, right-angled curette inserted *per os*. Intranasal granulation tissue is removed by curettage using a straight-handled curette inserted *per nasum*. A wax plug can be placed several centimeters into the oral portion of the alveolus to prevent the ingress of feed into the alveolus. An acrylic prosthesis is better suited for this purpose because it can be attached onto the tooth in front or behind the vacated alveolus. The restriction of hay or haylage in the diet for a few weeks can reduce the amount of time spent masticating and so prevent dislodgement of the alveolar prosthesis. If the alveolus fails to heal using these measures, the fistula can be closed using a plug of transposed muscle (Orsini et al 1992) or a sliding mucoperiosteal flap (Barakzai & Dixon 2005) (Fig. 25.3).
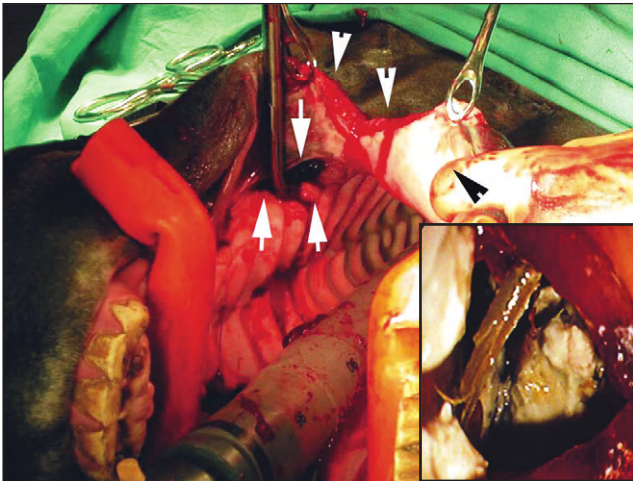
Fig. 25.3. The larger image shows creation of a sliding muco-periosteal hard palate flap to repair a chronic oronasal fistula (arrows) that developed after repulsion of two cheek teeth. The commissures of the lips have been incised (arrowheads) for surgical access. The smaller image shows inspissated pus and feed material seen during nasal endoscopy of a similarly affected horse.

Epidermal Inclusion Cysts of the Nasal Diverticulum

Epidermal inclusion cysts (or epidermoid cysts) of the nasal diverticulum are uncommonly encountered, spherical cystic structures, lined by epithelium, located between the skin and the mucous membrane in the dorsocaudal aspect of the nasal diverticulum (i.e. false nostril), rostral to the nasoincisive notch (Head & Dixon 1999, Tremaine et al 1999, Tremaine and Dixon 2001a). These lesions are a congenital malformation resulting from aberrant location of epithelial tissue. They are sometimes erroneously identified as an atheroma (i.e. a sebaceous cyst) because they contain a thick, gray, greasy material that resembles sebum (Tremaine et al 1999). Epidermal inclusion cysts can occur elsewhere in the body other than the nasal diverticuli, and an occasionally reported site is the brain (Kelly & Watson 1976, Gordon 1978).

Clinical signs

Although present at birth, a cyst may only become apparent when continuous exfoliation of squamous cells from its lining causes the cyst to slowly or rapidly expand. A cyst is usually first noted by the time the affected horse is 2 years old. Cysts range from 2 to 5 cm in diameter and usually bulge laterally to distort the contour of the nostril, rather than medially into the lumen of the nasal diverticulum (Fig. 25.4) (Robertson & Rooney 1997). They are soft, fluctuant, and mobile in the subcutaneous tissue, and painless on palpation. They do not obstruct respiration and are of cosmetic significance only (Lane 1998).
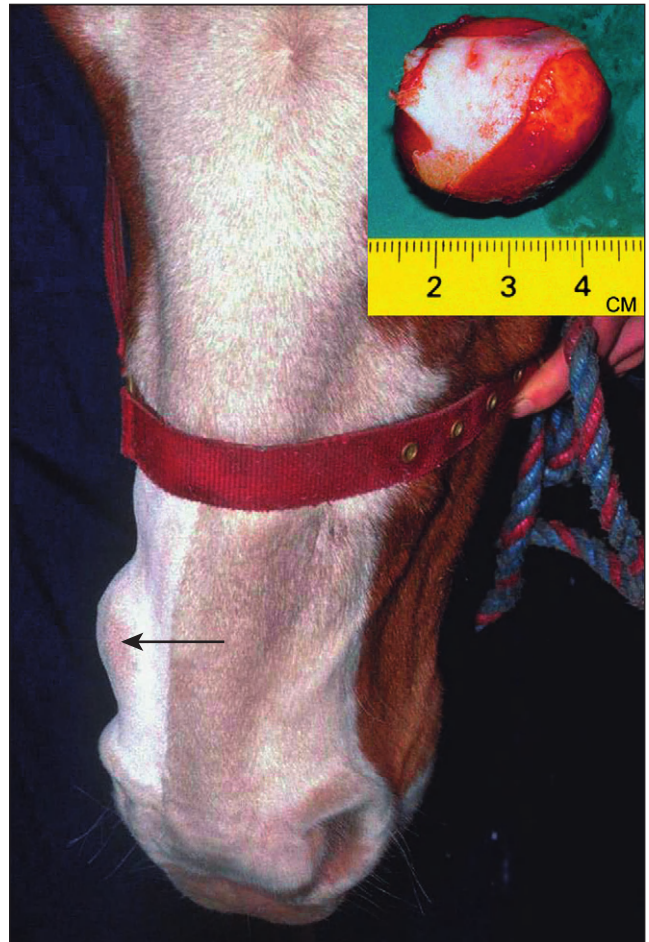
Fig. 25.4. Epidermal inclusion cyst (i.e. false nostril cyst) in the right nasal diverticulum (i.e. false nostril). The smaller image shows the intact, excised cyst.

Pathology

Histologic examination of an epidermal inclusion cyst reveals a well-differentiated, stratified squamous epithelium, 4–30 cells deep, with a thick band of surface keratin and acellular debris (Gordon 1978, Head & Dixon 1999). The subepithelial connective tissue is infiltrated with a mixed population of cells, mainly lymphocytes (Tremaine et al 1999). Keratinized and non-keratinized squamous cells are seen during microscopic examination of the contents of the cyst.

Diagnosis

Diagnosis is based on the pathognomonic location and gross appearance of the lesion and is confirmed by aspirating its contents, which are odorless, greasy, and dark-to-light gray (Robertson & Rooney 1997, Head & Dixon 1999).
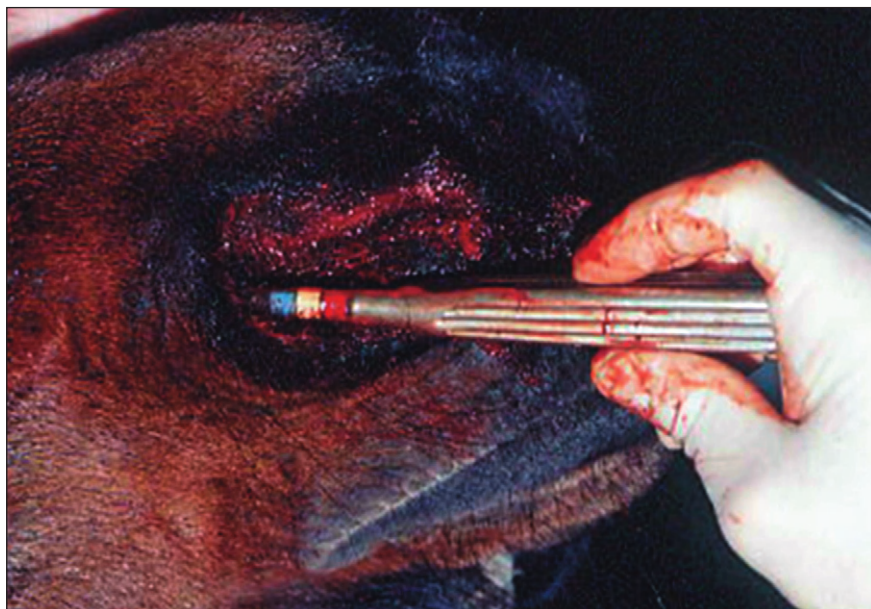
Fig. 25.5. An epidermal inclusion cyst being removed with a laryngeal burr.

Treatment

Affected horses are treated only to improve cosmesis. The usual treatment is excision of the entire cyst with the horse sedated and standing, using local anesthesia, through a skin incision created over the cyst. The cyst is thin-walled, and inadvertent perforation of the cyst hampers complete excision.

Another technique of excision that does not require meticulous dissection is to remove the cyst using a laryngeal burr (Schumacher et al 1997). The technique is performed after anesthetizing the infraorbital nerve of the affected side or after infiltrating local anesthetic solution into the subcutaneous tissue overlying the rostral aspect of the cyst within the nasal diverticulum. After scrubbing the nasal diverticulum, a 1-cm stab incision is made into the rostroventral aspect of the cyst, and the cyst's contents are expressed into the cavity of the false nostril. A laryngeal burr is inserted into the lumen of the cyst and rotated to engage the cyst's lining (Fig. 25.5). The burr is retracted, everting the wall of the cyst, which is then excised. If the thin cyst lining tears, the burr is reinserted, rotated, and retracted to remove residual tissue. The incision is left unsutured to heal by second intention.

The cyst can also be removed by chemically destroying its lining. One such technique is to swab the cavity of the cyst daily with a sclerosing agent, such as tincture of iodine, through an incision in the rostroventral aspect of the cyst (Haynes 1984). Recurrence of the cyst is a frequent complication of this technique. A more effective method of chemical ablation is to instill 2–4 ml of a 4% solution of formaldehyde (i.e. 10% formalin) into the lumen of the cyst after aspirating its contents (Frankeny 2003). The cyst enlarges within 24 h after injection and then, within 7 days, begins to regress until it is no longer visible. After several weeks, all that is left of the cyst is a firm, leathery mass attached to the nasal diverticulum by a stalk. The desiccated mass is removed by severing the stalk. Reported complications of the procedure are mild and temporary and include signs of nasal irritation, swelling of the cyst, and mucoid nasal discharge (Frankeny 2003).

Alar Fold Stenosis (Nasal Flutter)

The alar fold is a mucocutaneous structure located in the dorsorostral aspect of the nasal cavities (Fig. 25.6) that extends caudally from the laminar portion of the alar cartilage to the rostral aspect of the ventral nasal concha (Sisson & Grossman 1953, Boles 1979, Schummer et al 1979). It divides the external naris into a small, upper nasal diverticulum (i.e. the false nostril) and a large, lower opening (i.e. the true nostril). Its dorsolateral surface is covered with skin and forms the ventral and medial aspects of the nasal diverticulum. The mucous membrane of the ventromedial surface of the fold is continuous with the nasal mucosa.

During deep inspiration, the alar cartilage, which is attached to the alar fold, is elevated by action of the transversus nasi muscle (Foerner 1967). This tightens the alar fold, which closes the entrance to the false nostril. Malfunction of the transversus nasi muscles or excessive size of the alar folds allows air to enter the nasal diverticula, which causes obstruction of the nasal cavities, a condition referred to as alar fold stenosis. Relative obstruction of the
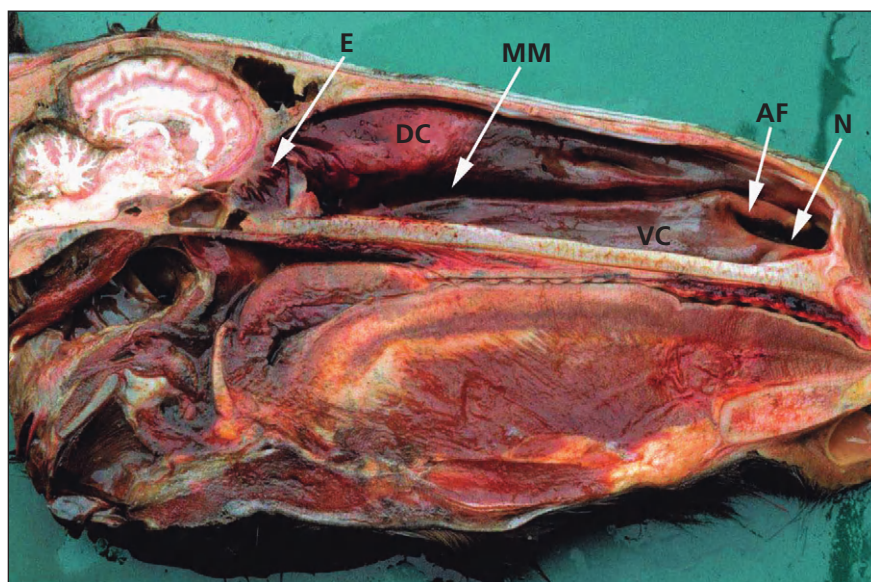
Fig. 25.6. Sagittal section of a normal equine skull with the nasal septum removed showing the ethmoturbinates (E), dorsal concha (DC), middle meatus (MM), ventral concha (VC), alar fold (AF), and (true) nostril (N).

nasal cavities by alar folds that are anatomically and functionally normal can occur if the nasal vestibula are abnormally narrow (Boles 1979, Haynes 1984).

Signalment

Flaccidity of the alar folds (i.e. alar fold stenosis) was originally described as a cause of a loud, objectionable respiratory noise that occurred during exercise, or sometimes even at rest, in some American Saddlebred horses that may have been genetically predisposed to the condition (Foerner 1967). Standardbred horses also appear to be at risk of alar fold stenosis, perhaps because of the conformation of the rostral aspect of their premaxillae and associated soft tissue structures (Hawkins et al 1995).

Noise attributed to the alar folds may also occur in young male horses, especially miniature horses, even though their alar folds are anatomically and functionally normal, because unerupted maxillary canine teeth may contribute to narrowing of the nasal vestibule (Boles 1979). After the maxillary canine teeth erupt, the tendency for the alar folds to produce abnormal noise decreases. (Note: Eruption of the cheek teeth of 2- to 4-year-old miniature horses is sometimes associated with nasal obstruction so severe that tracheostomy is necessary.)

Clinical signs

Alar fold stenosis causes a continuous, muffled rattling or vibrating noise emanating from the area of the nostrils during both inspiration and expiration, but the noise is most pronounced during expiration (Foerner 1967). Affected horses may make abnormal respiratory noise at rest, but others require intense exercise to elicit the noise. Drooping

of the alar cartilages may be evident, and the nasal diverticula may be deeper than normal (Foerner 1967). In addition to causing an abnormal respiratory noise, the condition may impair exercise tolerance (Vasey et al 1995).

Diagnosis

The diagnosis can be confirmed by temporarily retracting the alar folds with sutures and then exercising the horse to determine if this prevents the noise (Fig. 25.7). The horse is restrained with a nose twitch, and local anesthetic solution is injected into the alar folds. To retract the folds, heavy suture material is placed in a horizontal mattress pattern through each alar fold within the external nares, just caudal to the alar cartilages, and the sutures are tied together over a gauze roll situated over the nasal bones. Tension on the sutures retracts the alar folds and closes the nasal diverticula. Alleviation of noise with the folds retracted and recurrence of the noise with the sutures removed confirm the diagnosis of alar fold stenosis.

Treatment

Both alar folds and the medial wall of the nasal diverticula are resected with the horse anesthetized and positioned in dorsal or lateral recumbency (Foerner 1967). The procedure can be performed through the external nares or by incising the lateral alae of the nostrils to expose the alar folds. Dorsal recumbency provides adequate access to both alar folds, if the procedure is performed through the nostrils. Lateral recumbency provides better exposure to an alar fold, if the lateral ala of the nostril is incised, but to resect both alar folds using this approach, the horse must be turned over during surgery.
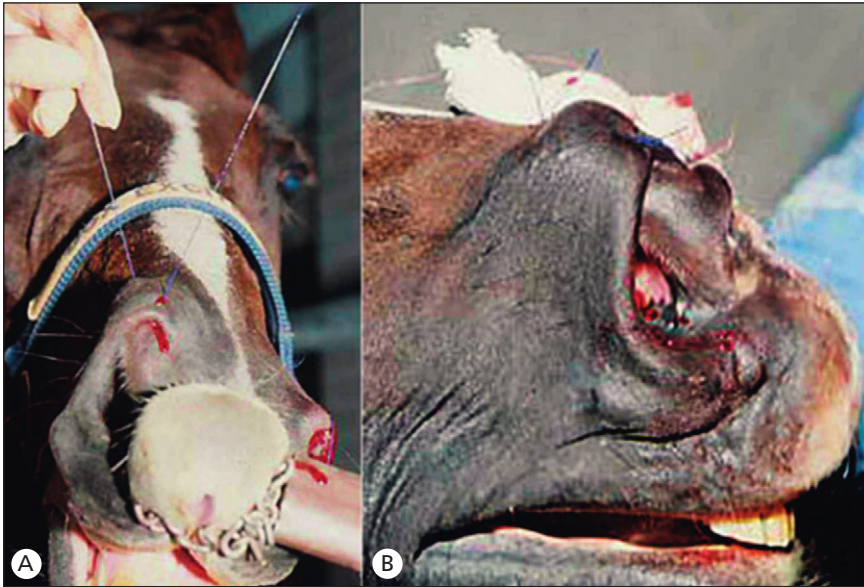
Fig. 25.7. The role of the alar folds in production of abnormal respiratory noise can be determined by temporarily retracting the alar folds with suture placed through each alar fold (A). The sutures are tied together, under tension, over a gauze roll situated over the nasal bones, to retract the alar folds (B). Remission of noise with the folds retracted confirms the diagnosis of alar fold stenosis.

The alar fold is uncurled and resected, using scissors, along its longitudinal limits, from the alar cartilage to the cartilaginous portion of the ventral concha (turbinate). Severe hemorrhage created by excision stops when the mucosal edge of the incision is joined to the corresponding skin edge with a continuous suture pattern using absorbable suture. The horse can be returned to work in approximately 2 weeks.

Prognosis

The prognosis after surgery is favorable, both for resolution of abnormal respiratory noise and return to the previous level of exercise (Vasey et al 1995). Horses with normal sized nasal cavities appear to receive the greatest benefit from resection of the alar folds (Hawkins et al 1995). If the horse is exercise intolerant and/or produces a noise at rest because of the inadequate size of the rostral portion of the nasal cavities, excision of the folds may provide some relief but should not be expected to totally relieve the signs.

Wounds of the Nostrils

Because the horse is an obligate nasal breather, its nostrils must dilate effectively during strenuous exercise, and consequently, wounds of the nostrils must be managed carefully to maintain normal nostril morphology. Obstruction to nasal airflow can result from scar formation caused by poor surgical technique in suturing a nostril laceration or from failure to surgically appose the laceration (Fig. 25.8).

Wry Nose (Campylorrhinus Lateralis)

Wry nose, or campylorrhinus lateralis, is a congenital shortening and deviation of the maxillae, premaxillae,
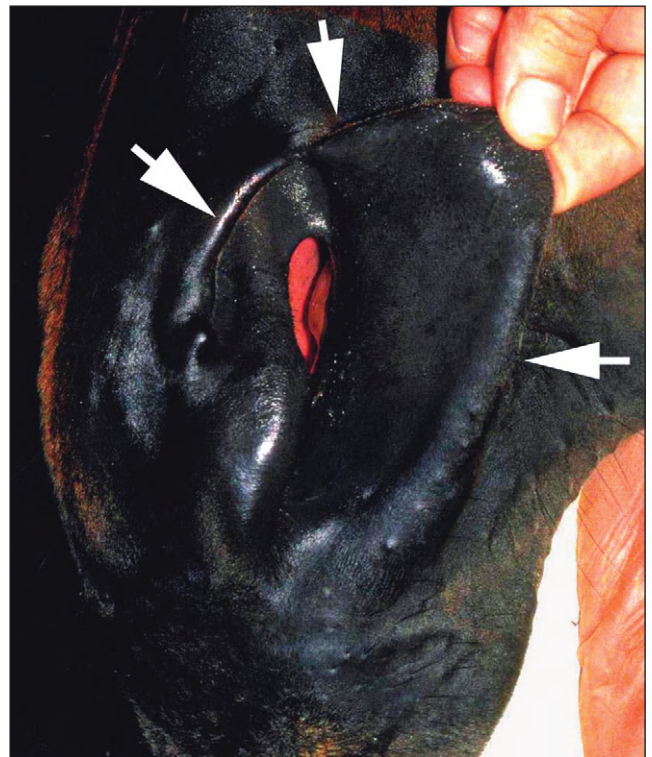
Fig. 25.8. This horse sustained a 10-cm long laceration to its left nostril that was not sutured. Consequently, the lateral, flaccid skin flap was sucked into the nostril during deep inspiration causing airflow obstruction and abnormal noise.

nasal bones, and vomer bone (Valdez et al 1978, McKellar & Collins 1993, Baker 1999, Puchol et al 2004). The deformity is accompanied by deviation of the nasal septum, which occasionally results in severe nasal obstruction. The inability of a mare, especially a primiparous mare, to

distend its uterus to accommodate a developing foal may cause fetal malpositioning, which may be responsible for the condition (Vandeplassche et al 1984).

Signalment

Wry nose occurs occasionally in all breeds, but the incidence seems to be highest in the Arabian breed, leading to speculation that the condition may be heritable (Baker 1999). Male and female foals are equally affected, and the malformation affects the right and left sides with equal frequency (Vandeplassche et al 1984).

Clinical signs and diagnosis

The deviation may be mild to severe (e.g. up to 90°) and may be accompanied by a protrusion ("hump") on one of the nasal bones (Fig. 25.9). Excessive arching of the nasal bones (i.e. Roman nose) and of the hard palate may accompany wry nose (J. Easley, personal communication). The affected foal may also suffer from cleft palate. Premaxillary deviation causes malocclusion of all or some of the incisor teeth, and all or a portion of the mandibular incisors may be visible. The tongue may protrude, and feed may be retained in the oral cavity producing a fetid odor (Puchol et al 2004). Deviation of the rostral portion of the facial bones can complicate deglutition, and the affected foal may be unable to nurse (McKellar & Collins 1993). Severe deviation may result in respiratory impairment, even when the foal is resting.

Treatment

A mildly affected foal needs no immediate treatment, but severely affected foals require intensive nursing care, and euthanasia may even be necessary. Administration of nutrition through a nasogastric tube may be necessary if the foal is unable to nurse, but often a foal unable to nurse is able to drink milk replacer from a bucket. Serum concentration of immunoglobulins should be assessed to determine if the foal has received colostrum.

A mild deformity may resolve spontaneously as the foal grows (Vandeplassche et al 1984). Surgical correction is usually performed in two stages and involves straightening the maxillae/premaxillae and nasal bones and removing the affected part of the nasal septum (Valdez et al 1978, McKellar & Collins 1993). During the first stage of repair, the premaxillae/maxillae are transected at their point of maximum curvature, and an autogenous rib graft is inserted into the space created on the concave side of the deformity when the incisors are realigned. The severed premaxillae/maxillae are stabilized with Steinmann pins inserted into their medullary cavity or with an external fixator. During the second stage of repair, usually per-

Fig. 25.9. This foal is severely affected with wry nose (campylorrhinus lateralis).

formed after the premaxillary/maxillary osteotomies have healed, airflow through the nares is improved by removing a portion of the deviated nasal septum, and the nasal bones are straightened.

Distraction osteogenesis has also been used to correct deviation of the premaxillae/maxillae (Puchol et al 2004). Using this technique, the premaxillae/maxillae are partially transected with a saw at their point of maximum curvature, through small incisions at the midpoint between the dorsal and ventral aspect of the premaxillae/maxillae. Steinmann pins are inserted perpendicularly across the premaxillae/maxillae, rostral and caudal to the osteotomies. A double connecting bar is attached on the convex side of the face, and a monolateral distraction external skeletal fixator is attached on the concave side. The rostral and caudal pins on the concave side of the face are distracted 1.0 mm apart every 24 h, and clamps that attach the

Steinmann pins with the double connecting bar on the convex side of the face are slackened and retightened every other day to accommodate the pressure generated by bone distraction on the concave side.

Prognosis

The cosmetic results of surgery are poor if the premaxillae and maxillae are abnormally short, or if removing a large portion of the nasal septum causes the nasal bones to collapse. The nasal bones are more likely to collapse after surgery if the foal is less than 6 months old (Tulleners & Raker 1983). The cosmetic result of distraction osteogenesis is reported be superior to that of conventional surgical correction (Puchol et al 2004).

Choanal Atresia

Choanal atresia, also known as posterior nasal atresia or imperforate buccopharyngeal septum, is a congenital malformation in which one or both nasal cavities fail to communicate with the nasopharynx (Crouch & Morgan 1983, Aylor et al 1984, Goring et al 1984, Hogan et al 1995, Lane 1998). The condition occurs as the result of persistence of the buccopharyngeal septum, which separates the nasal and nasopharyngeal cavities during embryonic development. The boundaries of the obstructing tissue in affected human beings, and presumably in affected foals, are the body of the sphenoid bone dorsally, the medial pterygoid lamina laterally, the caudal edge of the nasal septum medially, and the caudal aspect of the hard palate ventrally (Hengerer & Stome 1982).

Choanal atresia may be unilateral or bilateral, and the obstructing septum may be complete or incomplete, and bony or membranous (Crouch & Morgan 1983, Aylor et al 1984, Goring et al 1984, Hogan et al 1995, Lane 1998). Although the obstructing tissue of human beings is usually bony (Hengerer & Stome 1982), that of horses seems to usually be membranous, based on the few reports of the condition in this species (Hogan et al 1995). The condition results in partial or complete inability to breathe though the nose. The horse is an obligate nasal breather, and so when the condition is bilateral and complete, the foal is at immediate risk of suffocation, unless it receives a temporary tracheostomy. Choanal atresia in human beings is sometimes accompanied by other facial deformities, such as an arched hard palate and thickened vomer bone, especially if the condition occurs bilaterally (Silverman et al 1977, Hengerer & Stome 1982), but accompanying abnormalities in affected horses have not been reported.

This anomaly in human beings has sometimes been seen in multiple family members (Maniglia & Goodwin 1981). It has been induced in offspring of baboons by administering triamcinolone acetonide to pregnant females (Silverman et al 1977). The heritability of the condition in horses has not been evaluated, and teratogenic causes have not been identified.

Prevalence

The condition is rarely reported, but the scarcity of reports may not be indicative of the condition's true prevalence. Bilateral choanal atresia may often go undetected because of the rapid death of the foal after birth and failure to perform a detailed post-mortem examination. Some foals that are assumed to have been stillborn may have actually died after birth as a result of bilateral choanal atresia (Crouch & Morgan 1983).

Clinical signs

Complete, bilateral choanal atresia may not be recognized unless the birth is attended or a complete necropsy is performed. If the birth is attended, severe inspiratory difficulty and ballooning of the guttural pouches may be noted (Sprinkle et al 1984). If the condition is unilateral or bilateral but incomplete, it may go undetected until the horse is put into training and noted to be exercise intolerant or to make an abnormal respiratory noise (Lane 1998). Unilaterally affected horses may have a unilateral nasal discharge and unilateral nostril flare during exercise (Hogan et al 1995).

Diagnosis

Diagnosis is based on clinical signs, inability to advance a nasogastric tube or catheter into the nasopharynx, and endoscopic or contrast radiographic examination of the nasal cavities. During rhinoscopic examination, which is the most practical and useful means of diagnosing the condition, an obstructing membrane is seen at the level of the caudal nares, or the entrance between one or both nasal cavities and the nasopharynx may appear to be narrowed (Lane 1998). The ethmoturbinates may be atretic and distorted.

Contrast radiography, using a contrast medium introduced into the nasal cavities through the external nares, defines the caudal extent of the nasal cavities and shows the location of the obstructing tissue. Computed tomography, if available, may aid evaluation.

Treatment

Treatment of foals affected with complete and bilateral choanal atresia involves performing an emergency tracheostomy to bypass the obstruction, which can then be removed when the foal's condition is stable. The persistent septum can be removed using an intranasal approach or

through a facial bone flap (Aylor et al 1984, Goring et al 1984, Hogan et al 1995). The transpalatine approach used to access the caudal choanae of human infants is impractical for use in the horse because of the length of the horse's maxillary region (Goring et al 1984, Hogan et al 1995). The intranasal approach should be reserved for those horses with membranous obstruction (Hogan et al 1995). If the obstruction is bony, or if a membranous obstruction is exceptionally thick, the area of obstruction should be approached through a facial bone flap.

Using an intranasal approach with endoscopic guidance, obstructing membranous tissue can be perforated with a probe; destroyed by electrocoagulation or laser; or excised using laparoscopic scissors and forceps (Aylor et al 1984, Goring et al 1984, Hogan et al 1995). Cicatrization with subsequent closure, the most common complication of resection of choanal obstruction, is prevented by inserting a tube through the nasal cavity into the nasopharynx and maintaining the tube in this position for 4–6 weeks.

The obstructing membranous or bony buccopharyngeal septum can be excised through facial bone flaps, which provide surgical access to the caudal aspect of the nasal cavities, but the foal is likely to develop facial deformity and dental malocclusion after this surgery because of decreased growth of the maxillae caused by disruption of the facial suture lines (Aylor et al 1984, Goring et al 1984). Horses affected with life-threatening choanal atresia can be treated by permanent tracheostomy, if removal of the nasal obstruction is not feasible or if an attempt at removal has failed.

Deformity of the Nasal Septum

The nasal septum is a cartilaginous plate extending rostrally from the ethmoidal turbinates to the alar cartilages that separate the right and left nasal cavities (Figs 25.2 and 25.6) (Sisson & Grossman 1953, Schummer et al 1979). The septum is positioned on the midline perpendicular to the frontal and nasal bones. From its dorsal border, the parietal cartilage curves outward on each side for a short distance and lies on the lateral aspect of the nasal bones at the incisive notch. The ventral border of the nasal septum rests on the bony groove of the vomer bone and palatine processes of the premaxillae. The septum is cartilaginous, except at its caudal extent, where it becomes osseous as it blends with the perpendicular plate of the ethmoid and vomer bones. The cartilage of the septum is covered with a highly vascular mucosa.

Deformities of the nasal septum include deviation and thickening and are associated with obstruction of airflow through the nasal cavities. Causes of nasal septum thickening include congenital cystic degeneration (Tulleners & Raker 1983); hamartoma formation (Servantie & Sautet 1986); trauma from fracture of the nasal and frontal bones, amyloidosis (see Nasal Amyloidosis), or a halter that is too

tight; and bacterial infection of the septal cartilage associated with severe respiratory infection (Tulleners & Raker 1983). Rarely, the septum can be thickened as a result of mycotic infection or neoplasia. Septal deviation is caused by congenital malformation, such as wry nose, or from an expanding mass, such as a neoplasm or cyst, within the paranasal sinuses or a nasal cavity. Occasionally, nasal perforations occur that can cause continuous whistling type noises (without airflow obstruction) even at rest in affected horses.

Clinical signs

The most common clinical signs displayed by a horse with nasal septum deformity are respiratory difficulty during exercise and production of abnormal respiratory noise without apparent respiratory impairment (Tulleners & Raker 1983). Asymmetry of the face may be noted if the septal abnormality is caused by wry nose, by an expanding mass within the paranasal sinuses, or by trauma to the nasal and frontal bones.

Diagnosis

Most septal abnormalities can be seen or palpated, and an uneven flow of air through the nasal cavities can often be detected at the external nares (Tulleners & Raker 1983). Septal deformity or thickening can usually be palpated by simultaneously inserting an index finger into each nasal cavity and feeling the septum between the fingers. Rhinoscopic examination and dorsoventral radiographic projections of the nasal region can be used to determine the extent of the abnormality. Septal deformity results in narrowing of one or, usually, both nasal cavities, and difficulty may be encountered when inserting the endoscope into the nasal cavities. The septal cartilage and soft tissues surrounding it are visible on a dorsoventral radiographic projection, but precise positioning is required to observe septal deformity (Stilson et al 1985).

Treatment

Deformity of the nasal septum seldom resolves after it becomes clinically evident; therefore, the usual treatment of affected horses is excision of the accessible portion of the deformed septum. Only the rostral three-quarters of the septum is accessible for removal (Tulleners & Raker 1983). Septal deformity seldom advances to the point of being life-threatening, and so resection may not be necessary if the horse is not expected to perform athletically.

Because the horse may lose a large quantity of blood during the procedure, the hematocrit and coagulation profile should be determined before surgery. Replacing blood during surgery is usually not necessary, but if the horse is anemic or if the surgeon is inexperienced in this

procedure, having at least 4 liters of blood available for transfusion may be prudent. The horse should receive a balanced electrolyte solution intravenously during surgery to avoid hypovolemia caused by severe hemorrhage.

Both common carotid arteries can be ligated temporarily to control hemorrhage while the septum is being removed (Freeman et al 1990), but this procedure prolongs surgery, is sometimes ineffective in reducing severe hemorrhage, and may damage one or both recurrent laryngeal nerves or vagosympathetic trunks, which lie adjacent to the common carotid arteries (Greet 1992, Tremaine & Dixon 2001b).

The nasal septum is excised with the horse anesthetized and positioned in lateral recumbency. A small trephine hole (i.e. 15–25 mm in diameter) is made on the midline of the face through the nasal bones at a site just rostral to the conchofrontal sinuses. This site is located where the nasal bones begin to diverge and is several centimeters caudal to an imaginary line drawn between the rostral aspects of the facial crests. The nasal bones can be exposed through a curvilinear incision through the skin and periosteum, which are reflected. Removing a circular section of the nasal bones exposes the parietal cartilage of the septum, which is excised with a scalpel to expose the right and left nasal cavities. This opening into the nasal cavities provides access for the caudal and dorsal septal incisions.

The dorsal, ventral, and caudal septal incisions can be made using a guarded chisel, a cartilage scissor, or obstetrical wire (Bemis 1916, Tulleners & Raker 1983). Hemorrhage associated with removal of the nasal septum using obstetrical wire is less than that encountered when using a chisel or scissor because when using obstetrical wire, three of the four incisions required to remove the septum can be made simultaneously. This allows the septum to be removed rapidly, so that the nasal cavity can be packed to stop excessive hemorrhage. Using obstetrical wire also eliminates trauma to the adjacent turbinates, which can occur when the septum is removed using a guarded chisel (Tulleners & Raker 1983).

To remove the septum using obstetrical wire, three separate wires are placed around the septum to make the ventral, dorsal, and caudal septal incisions. Placement of the caudal and ventral wires requires insertion of the surgeon's hand into the horse's mouth, and so for convenience, gas anesthesia can be administered through an endotracheal tube placed through a tracheostomy.

To situate the ventral wire, the ends of a length of obstetrical wire are inserted into the nasopharynx on either side of the septum through the ventral nasal meatus so that the ends of the wire can be grasped over the caudal edge of the soft palate and exteriorized through the mouth. If the horse is too small to insert a hand into the caudal aspect of the mouth, the ends of the wire can be grasped, using endoscopic guidance, with a long forceps or with a wire snare inserted through the biopsy chamber of the endoscope. To prevent damaging the nasal mucosa with the ends of the wire, each end of the wire should be sheathed in a male dog urinary catheter. The two ends of the wire loop protruding from the mouth are tied together, the knot is covered with tape to protect the mucosa, and the knotted loop of wire is pushed back into the mouth and over the back edge of the soft palate. The knot is exteriorized by pulling on the wire emerging from one of the external nares. Tension on the wire secures the loop at the caudoventral aspect of the septum at the junction of the hard and soft palates (Fig. 25.10).

To situate the wire for the dorsal septal incision, the sheathed ends of a second length of obstetrical wire are inserted on either side of the septum at the trephine site and directed rostrally through the dorsal meatus of each nasal cavity so that the ends emerge at the external nares. Tension on the ends of the loop secures the loop at the caudodorsal aspect of the septum.
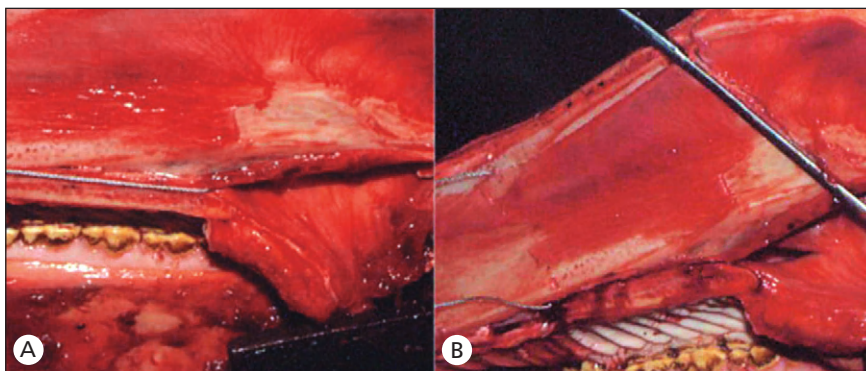
Fig. 25.10. Nasal septum resection. (A) Sagittal section of a head showing the loop at the caudoventral aspect of the septum at the junction of the hard and soft palates. (B) Sagittal section of a head showing the jaws of an intestinal forceps placed into the nasopharynx at a 60° angle to the nasal bones. The wire loop is situated caudal to the jaws of the forceps for the caudal septal incision.

To situate the wire for the caudal septal incision, the sheathed ends of a third length of obstetrical wire are inserted on either side of the septum at the trephine site and directed caudoventrally into the nasopharynx so that the ends of the wire can be grasped with fingers at the caudal edge of the soft palate and exteriorized through the mouth. The two ends of the wire loop exteriorized through the mouth are tied together, the knot is covered with tape to protect the mucosa, and the knotted loop of wire is pushed back into the mouth and over the back edge of the soft palate into the nasopharynx. By pulling on one strand of wire emerging from the trephine hole, the knot is exteriorized.

The caudal incision should be made at a 60° angle to the nasal bones (towards the nasopharynx), and to achieve this angle, the blades of a straight Doyen intestinal forceps are inserted through the trephine hole, one jaw on either side of the septum, and directed caudoventrally into the nasopharynx until the jaws of the forceps contact the soft palate. As the loop of wire for the caudal incision is pulled from the mouth into the nasopharynx, the loop comes to rest caudal to the jaws of the forceps (Fig. 25.10).

The caudal, ventral, and dorsal septal incisions are made simultaneously by the surgeon and two assistants with the three loops of wire. When the dorsal and ventral incisions approach the alar cartilages, the rostral aspect of the septum is incised, with a scalpel, to connect these two incisions. The rostral incision should be curved rostrally and should be at least 3 cm from the rostral limit of the septum so that support for the alar cartilages and external nares is maintained. The septum is then removed through either naris using a heavy Vulsellum forceps, and the nasal cavity is packed tightly with rolled gauze.

The nostrils may be sutured closed to retain the packing, and the cutaneous–periosteal flap is replaced and secured with skin staples. The endotracheal tube is replaced with a tracheostomy tube either before or after the horse has recovered from anesthesia. Gauze packing is removed after 24–48 h. Because horses are obligate nasal breathers, dislodgement or occlusion of the tracheotomy tube before the packing is removed causes the horse to asphyxiate. The nasal cavity typically develops a profuse, foul-smelling discharge after several days. Mucosal surfaces heal within 4–6 weeks, and the horse can resume work 8 weeks after surgery.

Prognosis

Longer term complications associated with nasal septum resection include excessive formation of granulation at either the rostral or caudal aspect of the cut edge of the septum, persistent respiratory noise, collapse of the nasal bones, and exercise intolerance (Tulleners & Raker 1983). The incised edge of the septum thickens as it heals; therefore, failure to angle the caudal incision toward
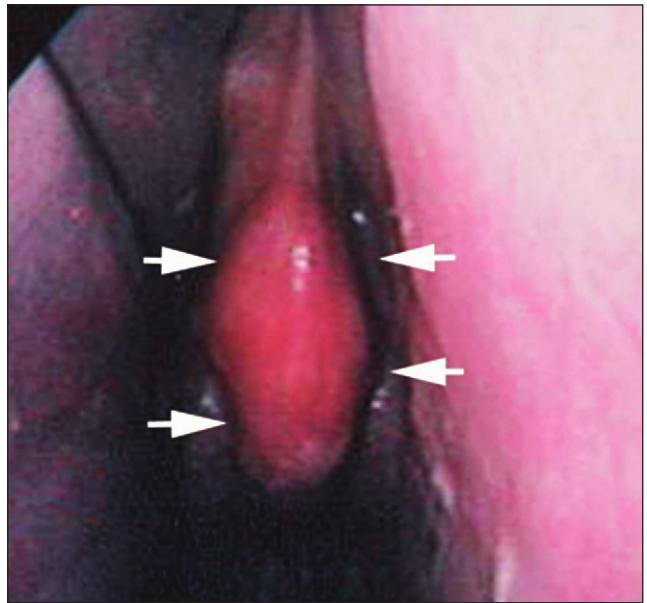
Fig. 25.11. Endoscopic view of the healed, caudal aspect of the incised edge of the septum. The thickened septal stump lies in the nasopharynx, rather than between the turbinates and so causes no nasal obstruction.

the nasopharynx can result in obstruction of the nasal cavity by the healed edge of the caudal portion of the septum. By incising the caudal aspect of the septum at a 60° angle to the nasal bones, the thickened septal stump resides in the nasopharynx, rather than between the conchae (Fig. 25.11).

Most horses continue to make an abnormal respiratory noise at exercise after the nasal septum is resected (Tulleners & Raker 1983), but full respiratory capacity is restored. Collapse of the bridge of the nose near the nasal diverticula may occur, especially when surgery is performed on horses less than 6 months old. Respiratory impairment resulting from collapse of the nose can sometimes be alleviated by resection of the alar folds (see section on Alar Fold Stenosis, p. 373).

Mycotic Nasal Infection

Mycotic infection of the nasal mucosa of horses is caused most commonly by the normally saprophytic fungus *Aspergillus fumigatus* (Greet 1981, McGorum et al 1992, Tremaine et al 1999) and less commonly by *Pseudallescheria boydii*, a saprophytic fungus that has been isolated from soil, sewage, and poultry and cattle manure (Brearley et al 1986, McGorum et al 1992, Davis et al 2000). These fungi are generally regarded as secondary invaders of damaged tissue, so the mechanism by which they infect the mucosa of the nasal cavities is not clear, except in cases where

mucosal damage was incurred by accidental trauma, by expanding growths, or by sinonasal surgery (Tremaine & Dixon 2001a). Horses confined to a stable containing moldy hay or straw are most likely to develop mycotic nasal plaques (Greet 1981, McGorum et al 1992, Tremaine et al 1999).

Signalment

There is no breed or sex predisposition for development of mycotic nasal plaques (Tremaine & Dixon 2001a), but the disease occurs most commonly in horses that are stabled on hay and straw, especially following sinonasal surgery (McGorum et al 1992). The condition is more commonly reported in Europe than in North America.

Clinical signs

Mycotic nasal plaques characteristically cause a chronic, malodorous, unilateral nasal discharge that may be blood-tinged, mucoid, purulent, or mucopurulent (Greet 1981, Brearley et al 1986, McGorum et al 1992, Davis et al 2000). Uncommonly, they may cause gross epistaxis. Affected horses often have an ipsilateral submandibular lymphadenopathy. Rhinoscopy reveals mycotic plaques, which can vary in size and color and which may be associated with an accumulation of a thick, tenacious, yellow discharge (McGorum et al 1992). Extensive destruction of the conchae is sometimes observed during rhinoscopy (McGorum et al 1992, Tremaine & Dixon 2001a).

Diagnosis

Diagnosis of mycotic nasal plaques is based on clinical signs, endoscopic identification of mycotic plaques (Fig. 25.12), cytologic examination of exudate or sections of plaques, histologic examination of lesions, and culture of a heavy and pure growth of potentially pathogenic fungi (Greet 1981, McGorum et al 1992). Failure to identify a mycotic plaque does not eliminate mycotic nasal plaques as a cause of clinical signs (Greet 1981). Histologic examination of lesions and cytologic examination of exudate from horses infected with *Aspergillus* spp. or *Pseudallescheria boydii* reveals fungal hyphae and conidiophores (McGorum et al 1992, Davis et al 2000). Results of fungal culture of exudate or microscopic examination of plaques should be interpreted cautiously because fungal spores and hyphae are commonly found in the respiratory tract of normal horses. Cytologic examination and culture of plaques are more reliable than cytologic examination and culture of nasal discharge. Serologic evaluation of horses affected with nasal mycosis does not reveal antibodies to *Aspergillus fumigatus* serotypes (McGorum et al 1992), but immunohistochemistry performed on infected tissue may confirm the identity of *Aspergillus fumigatus* as the causative agent (des Lions et al 2000).
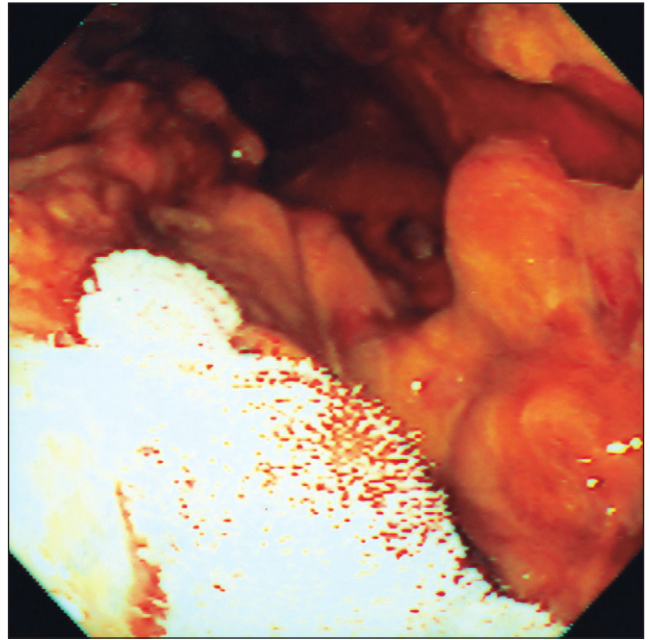
Fig. 25.12. Endoscopic image of nasal cavity of a horse that has a fistula into its maxillary sinus. This shows an extensive mycotic plaque caused by *Aspergillus* infection.

Treatment

Treatment of horses affected with nasal mycosis includes correcting the predisposing causes, removing the mycotic plaques and associated diseased tissue, and administering an antimycotic drug topically to the affected area (Greet 1981, McGorum et al 1992, Tremaine et al 1999). Large mycotic plaques and diseased tissue are removed transendoscopically (Davis et al 2000, Tremaine & Dixon 2001b), and exposed tissue is lavaged transendoscopically or through a catheter inserted into the ipsilateral paranasal sinuses, with a concentrated solution of an antimycotic drug, once or twice daily, for 1–2 weeks (McGorum et al 1992). Antifungal drugs locally effective against *Aspergillus* spp. include itraconazole, fluconazole, enilconazole, miconazole, ketoconazole, natamycin, and clotrimazole. Ancillary treatment is usually unnecessary but could include systemic administration of sodium iodide (67 mg/kg, intravenous, once daily) for 2–5 days and then oral administration of organic iodide (ethylenediamine dihydroiodide) (40 mg/kg, once daily) indefinitely (Scott & Miller 2003).

Fungal Granulomas

Fungal diseases incriminated in the development of granulomas in the nasal cavities of horses include rhinosporidiosis, conidiobolomycosis, cryptococcosis, and coccidioidomycosis.

Although fungal granulomas of the nasal cavity are generally uncommonly observed, they are encountered with some frequency in certain geographic regions.

Rhinosporidiosis is a chronic fungal infection of people, cattle, horses, mules, and other species caused by *Rhinosporidium seeberi* and characterized by nodular or polypoid growths on the nasal, vaginal, ocular, or oral mucosa (Smith 1961, Myers et al 1964, Londero et al 1977, Gillespie 1981). The disease does not become generalized and does not ordinarily endanger life. Only the nasal form has been reported to occur in horses. *Rhinosporidium seeberi* seems to favor temperate regions, and there appears to be positive correlation between the amount of contact of the horse with stagnant water and the frequency of occurrence of the infection. The few reported cases of the disease in horses in the USA have been located in southeastern states, perhaps because of the warm, wet climate of this region (Smith 1961, Myers et al 1964). The source of infection and method of spread have not been determined, but the disease is probably not contagious.

Conidiobolomycosis of horses, also known as rhinophycomycosis or entomorphomycosis, is a pyogranulomatous disease of the upper respiratory tract caused by infection of the mucosa and submucosa by the fungus *Conidiobolus coronatus*. Conidiobolomycosis is part of the pyogranulomatous disease complex known as equine phycomycosis (Bridges 1972, Murray et al 1978, Miller & Campbell 1982, Miller et al 1983, Owens et al 1985, Alfaro & Mendoza 1990, Campbell 1990). The other diseases of the phycomycosis complex are pythiosis, caused by invasion of a protistal organism, *Pythium insidiosum*, and basidiobolomycosis, caused by invasion of the fungus *Basidiobolus haptosporus* (Murray et al 1978, Miller & Campbell 1982, Miller et al 1983, Owens et al 1985, Campbell 1990). Pythiosis and basidiobolomycosis occur in tropical and subtropical regions and primarily affect the skin and subcutis, whereas conidiobolomycosis can also occur in more temperate climates and is found exclusively in the upper respiratory tract (Miller & Campbell 1982).

Conidiobolus coronatus is found in soil and decaying organic material and in insects (Miller 1983). Infection by the fungus most likely occurs while the horse is grazing. Mucosal damage, perhaps damage caused by bacterial or viral infection of the respiratory epithelium, may provide entry for the infective fungal conidia (Hanselka 1977), but whether or not the epithelium must be damaged for the organism to enter is not known. Infection does not seem to be associated with immunodeficiency (Steiger & Williams 2000).

Cryptococcosis is a granulomatous fungal disease, usually of the skin, meninges and brain, or respiratory system, caused by the yeast-like organism *Cryptococcus neoformans* (Gillespie 1981, Corrier et al 1984). The most frequently reported site of infection in horses is the nasal cavity. *Cryptococcus neoformans* is a ubiquitous saprophyte com-

Fig. 25.13. A sessile lesion of rhinosporidiosis (arrow) on the nasal septum at the right external naris.

monly found in soil that is enriched by bird droppings (Ainsworth & Austwick 1959, Gillespie 1981). Respiratory infection is acquired by the inhalation of contaminated dust. Transmission from one host to another has not been clearly demonstrated (Ainsworth & Austwick 1959, Corrier et al 1984).

Coccidioidomycosis is a granulomatous disease caused by infection with the fungus *Coccidioides immitis*. Other names for the disease include valley fever, San Joaquin fever, and desert fever (Zontine 1958). The disease in horses may be manifested as a nasal granuloma or by generalized debilitation (Zontine 1958, Hodgin et al 1984). The disease is endemic in arid and semiarid regions in North, Central, and South American countries (Ainsworth & Austwick 1959, Gillespie 1981). The usual mode of infection is by inhalation of the organism. The disease is not transmitted from animal to animal.

Clinical signs

Clinical signs of fungal granuloma include stertorous breathing, dyspnea caused by restricted airflow, halitosis, sneezing, dysphagia, epistaxis, and sanguinous, mucoid, or mucopurulent discharge from the affected nasal cavity. Granulomas can sometimes be seen at the external nares, but rhinoscopy may be necessary to observe the lesions.

With rhinosporidiosis, pedunculated or sessile polypoid, pinkish-tan nodules, usually less than 3 cm in diameter, may be visible on the nasal mucosa, usually close to the external nares (Fig. 25.13) (Smith 1961, Myers et al
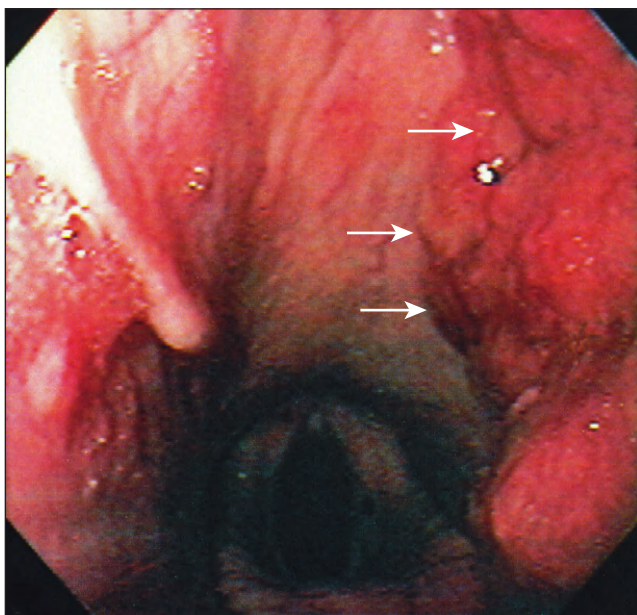
Fig. 25.14. Endoscopic view of the nasopharynx of a horse affected with conidiobolomycosis. The granulomatous lesion on the left wall of the nasopharynx (arrows) extended into the left nasal cavity.

1964, Londero et al 1977, Gillespie 1981), and during rhinoscopy, smaller, nodular granulomatous lesions may be found scattered throughout the nasal cavities (Londero et al 1977). The nodules bleed easily and are stippled with 1-mm diameter, white or yellow dots (Myers et al 1964, Londero et al 1977, Gillespie 1981, Allison et al 1986).

Conidiobolus coronatus causes small growths, 1–3 cm in diameter, within the nasal cavity and slightly larger growths, 1–5 cm in diameter, on the external nares (Pascoe 1981). Lesions can sometimes be seen to contain small granules, 2–5 mm in diameter (French et al 1985). Lesions caused by *Conidiobolus coronatus* do not appear to be as pruritic as lesions caused by pythiosis (Hutchins & Johnston 1972). Lesions of cryptococcosis or coccidiodomycosis may appear as a glistening, gelatinous mass during rhinoscopy (Fig. 25.14) (Roberts et al 1981, Corrier et al 1984, Hodgin et al 1984).

Pathology

Granulomas caused by rhinosporidiosis are covered by a thin, shiny epithelial membrane and contain white or yellow dots, which represent aggregates of sporangia (Smith 1961, Myers et al 1964, Londero et al 1977, Gillespie 1981). Microscopic examination of granulomas reveals hyperplastic epithelium covering highly vascular fibrous connective tissue, which is heavily infiltrated with inflammatory cells, especially neutrophils and eosinophils. Numerous sporangia in various stages of development,

measuring up to 400 μm in diameter, and each containing numerous endospores measuring 5–10 μm in diameter, are dispersed throughout the nodule.

Microscopic examination of granulomas of conidiobolomycosis reveals granulation tissue infiltrated by numerous eosinophils, neutrophils, macrophages, plasma cells, lymphocytes, giant cells, and hyphae surrounding necrotic masses (Hutchins & Johnston 1972, French et al 1985, Zamos et al 1996). Special stains, such as Gomori's silver stain, may be required to identify hyphae.

The granuloma caused by *Cryptococcus neoformans* has a yellow to yellowish-gray appearance, and cut sections of the mass contain many cysts filled with a gelatinous material (Watt 1970, Corrier et al 1984). Microscopic examination of sections of lesions caused by *Cryptococcus neoformans* reveals an inflammatory response consisting of a mixed population of inflammatory cells and periodic acid Schiff-positive, thickly encapsulated yeast bodies, 5–30 μm in diameter (Watt 1970, Corrier et al 1984).

The granulomatous nasal lesions caused by *Coccidioides immitis* are smooth with a glistening surface (Hodgin et al 1984). Microscopic examination of the lesions reveals a pyogranulomatous reaction containing spherical bodies, or spherules, 20–50 μm in diameter, often surrounded by focal accumulations of inflammatory cells (DeMartini & Riddle 1969, Hodgin et al 1984). The spherules have a double-contoured cell wall and can be found extracellularly and within giant cells. Spherules resemble an oocyst of a coccidium, and it is from this resemblance that the fungus derives its name (Gillespie 1981). Large spherules contain endospores, 2–5 μm in diameter.

Diagnosis

Fungal granulomas must be differentiated from ethmoidal hematomas, neoplasia, and nasal granulomas caused by periapical infection of a maxillary premolar. Diagnosis of fungal granuloma and identification of the causative organism are usually based on clinical signs, gross and histologic appearance of the lesions, and identification of the causative organism, either within lesions or by culture.

Granulomas caused by *Rhinosporidium seeberi* and *Coccidioides immitis* could be confused during cytologic or histologic examination of infected tissue because both organisms have a somewhat similar appearance in tissue (Allison et al 1986). *Rhinosporidium seeberi* cannot be cultured on conventional, artificial culture media (Ainsworth & Austwick 1959, Gillespie 1981).

Diagnosis of conidiobolomycosis can be confirmed by identifying characteristic hyphae of the causative organism during histologic examination of a lesion. *Conidiobolus coronatus* can be cultured with ease on ordinary media, but samples from suspected lesions are usually sent to laboratories with personnel experienced in isolating and identifying organisms causing phycomycosis (Newton & Ross

1993). Conidiobolomycosis can also be identified by polymerase chain reaction (Grooters & Gee 2002) or by an immunodiffusion test, using serum from the affected horse (Kaufman et al 1990, Steiger and Williams 2000).

Diagnosis of cryptococcosis can be confirmed by identifying *Cryptococcus neoformans* during cytologic examination of nasal exudate, by serology, or by culture (Watt 1970, Pearson et al 1983, Corrier et al 1984). Examination of nasal exudate stained with periodic acid–Schiff or India ink reveals the characteristic thickly encapsulated budding yeast bodies. Culture of *Cryptococcus neoformans* requires 3–5 days (Corrier et al 1984). The latex agglutination test has been used to diagnose cryptococcosis in people and cats (Medleau et al 1990) and would likely be useful in diagnosing the condition in horses.

Treatment

Treatment for rhinosporidiosis is by excision of the lesions (Smith 1961, Myers et al 1964, Hodgin et al 1984). Surgical margins should be wide to avoid recurrence. Freezing the entire lesion or the base of the lesion after excision may be effective (authors' experience). Therapeutic results have been obtained in dogs, when surgical excision was not possible, by administering dapsone (diaminodiphenylsulfone), a drug used for treatment of leprosy (Allison et al 1986). Therapy with dapsone can, however, result in hemolytic anemia, agranulocytosis, and methemoglobinemia. The use of dapsone for treatment of affected horses has not been reported. Experience in human patients affected with rhinosporidiosis is that the infection is locally persistent, and that repeated surgical removal of recurring polyps is often required (Ainsworth & Austwick 1959).

Treatment of horses affected by conidiobolomycosis includes excision of lesions and parenteral and topical administration of antifungal drugs. Complete excision of nasal and nasopharyngeal lesions of conidiobolomycosis may be difficult because lesions within these locations may be surgically inaccessible. Although horses with pythiosis have been treated successfully with a vaccine made from *Pythium insidiosum* (Miller 1981), immunotherapy as a treatment for horses with conidiobolomycosis does not seem to be effective (authors' experience, Taintor et al 2004).

Horses affected with conidiobolomycosis have been treated successfully with orally administered fluconazole (5 mg/kg, q 12 h, for 6 weeks) (Taintor et al 2004), but parenteral treatment of affected horses with the similar antifungal drugs, ketoconazole or itraconazole, is less likely to be successful because these drugs are absorbed poorly from the horse's gastrointestinal tract (Korenek et al 1994). Affected horses have also been treated successfully with parenteral and intralesional administration of amphotericin B (0.2–1.0 mg/kg, intravenous, in 1 liter 5% dextrose over 20–30 min every other day or 10–20 mg/lesion)

(Hanselka 1977, French et al 1985, Zamos et al 1996, Taintor et al 2003). Parenteral administration of amphotericin B is expensive, and prolonged use can result in nephrotoxicosis and thrombophlebitis.

Ancillary treatment of horses affected with conidiobolomycosis includes systemic administration of sodium iodide (67 mg/kg, intravenous, once daily) for 2–5 days and then oral administration of organic iodide (ethylenediamine dihydroiodide) (40 mg/kg, orally, once daily) indefinitely (Scott & Miller 2003). The mechanism of action of iodide against *Conidiobolus coronatus* is not known.

Treatment of horses with a nasal granuloma caused by *Cryptococcus neoformans* is usually unsuccessful (Corrier et al 1984). The disease is difficult to resolve because the gelatinous capsule that surrounds the yeast masks the attached opsonic antibody, protecting the organisms from phagocytosis. Therapy has included excision of the granuloma, cryotherapy, and parenteral treatment of the affected horse with sodium iodide and an antibiotic, most commonly amphotericin B (see earlier discussion on treatment of horses with conidiobolomycosis for dosages). The difficulty in resolving infection and the potential threat to human health may justify euthanasia of infected horses. However, there is no clear evidence that cryptococcosis is transmissible from one host to another or from animals to human beings (Roberts et al 1981).

The treatment of horses affected with coccidioidomycosis is by antifungal therapy (see earlier discussion on treatment of horses with conidiobolomycosis for drugs and dosages) in conjunction with surgical removal of lesions. Long-term, oral administration of itraconazole (2.6 mg/kg, q 12 h) was apparently effective in the treatment of a horse affected with coccidioidomycosis (Foley & Legendre 1992), even though this drug has been shown to be absorbed poorly from the horse's gastrointestinal tract (Korenek et al 1994).

Nasal Amyloidosis

Amyloidosis is a group of diseases characterized by the deposition of a homogeneous, extracellular proteinaceous substance, amyloid, in tissue (Husby 1988). The two major types of amyloid are amyloid AA, which is derived from a serum α-globulin, a normal acute-phase protein produced by the liver, and amyloid AL, which consists of monoclonal immunoglobulin light chains and fragments of light chains. Amyloid AA may be deposited in various tissues when its concentration in serum is chronically elevated by inflammation or antigenic stimulation (Husby 1988, Mould et al 1990).

Nasal amyloidosis is a disease peculiar to horses. Lesions are reported to be composed of amyloid AL, but the cause of deposition of amyloid AL in the nasal cavity of horses is idiopathic and is usually not associated with any

Fig. 25.15. A nodular mass composed of amyloid at the right external naris.

underlying disease (Shaw et al 1987, van Andel et al 1988, Mould et al 1990, Kasper et al 1994, Robertson & Rooney 1997). An inhaled toxicant or antigenic stimulus may be the inciting cause (Kasper et al 1994).

Signalment

Too few horses affected with nasal amyloidosis have been reported to conclude that the disease has an age, breed, or sex predilection.

Clinical signs

Clinical signs of nasal amyloidosis include the presence of multiple, nodular, mucosa-covered masses at the external nares (Fig. 25.15). The nasal septum and alar folds may be thickened. During endoscopic examination of the upper portion of the respiratory tract, the lesions may be seen extending into the nasopharynx (Shaw et al 1987, Kasper et al 1994). The masses are often ulcerated and bleed easily after gentle, digital manipulation. A common clinical sign of horses with amyloid deposits in the nasal cavities is epistaxis. Other signs include respiratory obstruction, abnormal respiratory noise, and decreased athletic performance.

Pathology

On cut section, the lesions have a pale yellow, waxy appearance (Shaw et al 1987, Kasper et al 1994). Microscopically, the nasal mucosa is intact. The submucosa contains an acellular, amorphous, homogeneous, pale, eosinophilic material (i.e. amyloid) and a few histiocytes and multi-

nucleated giant cells. Amyloid is deposited within and around blood vessels, the basement membrane of glands, and within the connective tissue (Smith et al 1972, Robertson & Rooney 1997). A specific stain for amyloid is Congo red, which stains the amyloid orange-red (Shaw et al 1987, Kasper et al 1994).

Diagnosis

Nasal amyloidosis must be differentiated from nasal neoplasia and nasal fungal granulomas. Diagnosis is based on the gross and histologic appearance of the lesion.

Treatment

Medical therapy appears to be ineffective, consequently removal of the masses (or affected structures, such as the alar folds or nasal septum) is the only treatment (Shaw et al 1987, Kasper et al 1994, Hawkins et al 1995). Removal of lesions of nasal amyloidosis apparently effects cure, and the masses are unlikely to recur (Shaw et al 1987, Husby 1988, Kasper et al 1994). Masses may be surgically inaccessible or so extensive, however, that complete removal is not possible.

Neoplasia

The most common nasal neoplasm of horses is the carcinoma (Schuh 1986), and the three most common intranasal carcinomas are the adenocarcinoma, squamous cell carcinoma, and undifferentiated carcinoma. Because of the variety of tissue found in the nasal cavities, other

neoplasms, such as fibroma, myxoma, chondroma, osteo-sarcoma, fibrosarcoma, neurofibroma, hemangiosarcoma, and lymphoma, may also occur, although less commonly.

Prevalence

Sinonasal neoplasia of horses is encountered uncommonly, compared to sinonasal neoplasia of other species (Head & Dixon 1999). Horses with sinonasal neoplasia represent only about 0.2% of all referred horses (Dixon & Head 1999), and comprise only 8–19% of all horses with sino-nasal disease (Boulton 1985, Tremaine & Dixon 2001a).

Signalment

Old horses are more at risk for nasal neoplasia in general and are especially at risk for neoplasia of epithelial origin (Madewell et al 1976, Dixon & Head 1999). Although fibro-osseous nasal tumors can be found in horses of any age, they are found most frequently in young horses.

Clinical signs

Clinical signs of nasal neoplasia typically become apparent slowly and insidiously and can include stertorous respi-ration, reduced airflow from the affected nasal cavity, and unilateral nasal discharge, which can be purulent, muco-purulent, sanguineous, or serosanguineous (Dixon & Head 1999). Because horses with nasal neoplasia commonly have mucopurulent nasal discharge, primary bacterial rhinitis or paranasal sinusitis is often mistakenly diagnosed.

Other signs include ipsilateral enlargement of the sub-mandibular lymph nodes, epiphora, and, with growth of the tumor, distortion of the nasal or maxillary bones. Ipsilateral enlargement of submandibular lymph nodes is usually caused by reactive lymphadenopathy associated with local infection and tumor necrosis, rather than by neoplastic involvement. Distortion of the nasal cavity by the neoplasm may cause the nasal septum to deviate into the contralateral nasal cavity. Affected horses may exhibit signs of systemic disease, including lethargy, anorexia, and weight-loss. The neoplasm may be visible at the external naris, but usually endoscopy is required to observe the mass. Some nasal squamous cell carcinomas develop in the hard palate and then invade the nasal cavity (and paranasal sinuses); therefore, oral examination of horses suspected of having sinonasal neoplasia may reveal a prolif-erating lesion on the hard palate (Dixon & Head 1999).

Pathology

Squamous cell carcinoma, the most common nasal neoplasm, is classified by its cytologic features and degree of keratinization as being either well-differentiated, moderately differentiated, or poorly differentiated (Schuh
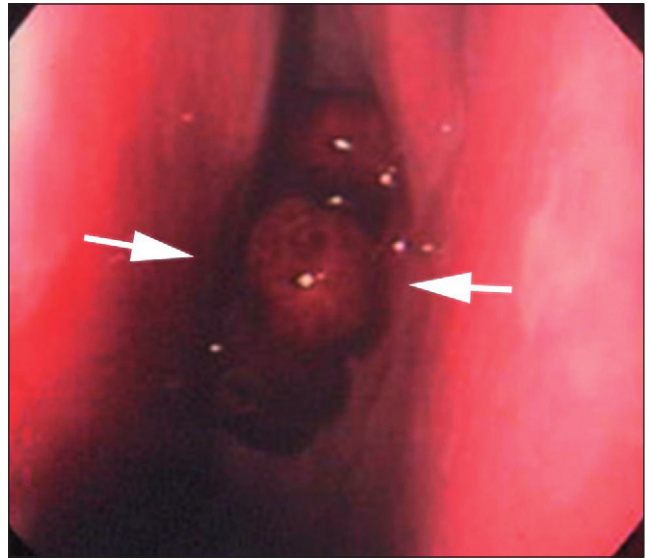
Fig. 25.16. Endoscopic view of the left nasal cavity of a horse. Because of its location, the carcinoma within the ethmoidal labyrinth could be mistaken during examination for a progressive ethmoidal hematoma.

1986). Histologic grading of nasal carcinomas is of little value, though, because grading is subjective and results vary with the site of sampling, and because the correlation between the degree of differentiation and the tendency to metastasize is poor (Schuh 1986, Head & Dixon 1999). The malignant nature of most intranasal carcinomas is reflected by their local invasiveness; however, despite their locally aggressive nature most sinonasal tumors are slow to metastasize to regional lymph nodes (Head & Dixon 1999). Metastasis usually only occurs late in the course of the disease.

Differential diagnosis

Nasal neoplasia must be differentiated from granulomas caused by periapical infection of a maxillary premolar, nasal amyloidosis, and nasal mycotic granulomas. A nasal neoplasm may occasionally bear some resemblance to a progressive ethmoidal hematoma (Fig. 25.16).

Diagnosis

Definitive diagnosis of nasal neoplasia is based on results of cytologic and histologic examination of biopsy specimens. Cytologic examination of a fine-needle aspirate from a nasal mass may be helpful in obtaining a diagnosis, but care should be taken not to misinterpret dysplastic cells found in severely inflamed mucosa as being neoplastic. Biopsy samples should be obtained from deep within the tumor to avoid sampling the overlying surface epithelium (Scarratt & Crisman 1998, Head & Dixon 1999).

Despite the use of radiography and endoscopy, the site of origin of most sinonasal tumors cannot be determined (Dixon & Head 1999). Computed tomography and magnetic resonance imaging offer more information about the origin and extent of neoplasia than does plain radiography and may aid the planning of an effective strategy for radiotherapy.

Treatment

Most horses affected with neoplasia of the nasal cavity are eventually euthanased because success of treatment is poor. Nasal neoplasms are relatively inaccessible, and because they are invasive, their margins are often poorly defined. The advanced stage of disease when recognized and the anatomical complexity and vascularity of the area involved make complete surgical excision or cryotherapy difficult or impossible. If immediate euthanasia is not a satisfactory option, the therapeutic objective is usually to palliate clinical signs. For example, horses experiencing difficult breathing as a result of occlusion of the nasal cavities by the neoplasm can be treated by temporary tracheostomy or by creating a permanent tracheal fistula.

Horses with sinonasal carcinoma have been treated successfully using fractionated, cobalt-60 radiotherapy, but multiple treatments with the horse anesthetized are necessary, and the equipment required is not readily available (Walker et al 1998). Even this form of therapy should be considered palliative, not curative.

Prognosis

Treatment of affected horses is usually not successful, and the disease is eventually fatal. The life expectancy of an affected horse depends on the type of neoplasm and the owner's tolerance of the clinical signs. The course of disease is usually protracted.

Nasal Polyps

A polyp is an uncommonly reported, smooth, mucosa-covered growth on a mucosal surface caused by a hyperplastic response of the mucosa or associated lymphoid tissue, usually to inflammatory or allergic stimulation (Smith et al 1972, Head & Dixon 1999).

Clinical signs

Polyps vary in size and may entirely fill the nasal cavity in which they are located. They may be solitary or multiple, but most are pedunculated (Smith et al 1972, Head & Dixon 1999). Clinical signs usually develop insidiously and may include unilateral nasal discharge, stertorous

Fig. 25.17. A polyp is visible at the left external naris of this horse. Reproduced with the permission of Dr Mark Crabill.

breathing, and dyspnea (Nickels 1993, Watt & Beck 1997). A polyp may be visible at the external nares (Fig. 25.17), but rhinoscopy may be required to observe the mass.

Pathology

Polyps are covered by nasal mucosa and are composed of fibrous and myxomatous tissue that contains numerous capillaries and is infiltrated by leukocytes, chiefly neutrophils and lymphocytes (Smith et al 1972). The histologic appearance of polyps indicates an inflammatory etiology and is similar to that of granulation tissue found in wounds.

Diagnosis

Diagnosis is based on clinical signs and gross and histologic appearance of the lesion, but distinguishing between a benign neoplasm and an inflammatory polyp may be difficult. Radiography of the skull may aid in determining the extent and origin of the polyp.

Treatment

Treatment of affected horses is to remove the polyp. Pedunculated polyps can be removed with a snare using endoscopic guidance (Watt & Beck 1997). Both pedunculated and sessile polyps can be removed transendoscopically using electrocautery or a laser. Large polyps in the caudal portion of the nasal cavity can be accessed through an osteoplastic nasal flap. The prognosis for resolution is good with removal of the lesion.
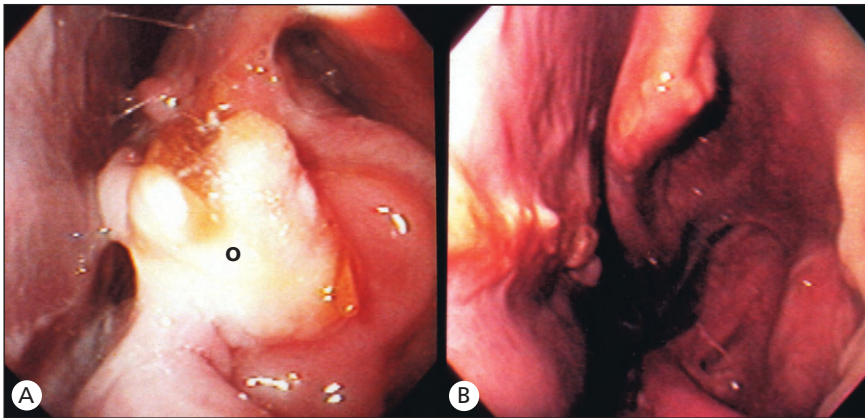
Fig. 25.18. (A) An osteoma (o) within the left nasal cavity of a horse. (B) An endoscopic view of the nasal cavity after the osteoma was excised.

Osteomas

Osteomas are smooth, solitary osseous growths protruding from the surface of a bone, typically a bone formed by intramembranous ossification (Spjut et al 1971, Pool 1978, Aegerter & Kirkpatrick 1979). They are regarded by some pathologists to be hamartomas and as such, are present at or soon after birth and represent a benign, disordered overgrowth of mature bone.

Signalment

Although female horses can be affected by sinonasal osteoma (Steinman et al 2002), in nearly all reports, horses affected with sinonasal osteoma have been male (Freeman et al 1990, DelPiero et al 1997). The cause of the preponderance of osteoma occurrence in male horses is not known, but the same predilection of osteomas for men is also reported (Spjut et al 1971).

Clinical signs

Most osteomas are probably present at birth, although years may elapse before clinical signs are recognized (Head and Dixon 1999). Clinical signs of nasally located osteomas (Fig. 25.18) are those produced by any expansile, space-occupying lesion in the nasal cavity and include mucopurulent nasal discharge, epiphora, restricted airflow, and facial distortion (Schumacher et al 1988).

Pathology

Osteomas may be sessile or pedunculated and are expansile rather than infiltrative. An osteoma may demonstrate slow but progressive growth and then may cease growth and remain quiescent for years (Pool 1978). They apparently do not undergo malignant transformation. Histologically, osteomas are composed of a central core of cancellous bone surrounded by a peripheral layer of dense compact bone (Pool 1978, Atallah & Jay 1981). The proportions of these two types of bone vary according to the rate of growth of the osteoma. Well-developed Haversian canal systems can be identified as the osteoma remodels (Head & Dixon 1999).

Diagnosis

Diagnosis is based on physical examination of the horse, endoscopic examination of the nasal cavities, and radiographic examination of the skull. Diagnosis can be confirmed by histological examination of a biopsy specimen, but because of their hardness, most osteomas are difficult to biopsy. An osteoma causing no clinical signs may be discovered incidentally during radiographic examination of the skull performed for reasons other than clinical signs caused by the osteoma (Scrutchfield et al 1994).

Treatment and prognosis

Removal of an osteoma from the nasal cavity may require creation of a nasal flap and therefore may be more difficult than removal of an osteoma from the paranasal sinuses (authors' experience). If removed completely, osteomas do not recur. Incomplete removal does not appear to stimulate growth, but the probability of growth of the remnants remains. If the osteoma was not completely removed, the horse should be monitored periodically for regrowth of the osteoma by radiographic and endoscopic examination (Pool 1978, Atallah & Jay 1981).

Osteodystrophia Fibrosa

Osteodystrophia fibrosa is a nutrition-induced, skeletal disease characterized by deposition of non-mineralized, fibrous tissue in affected bones (Joyce et al 1971). This disease causes thickening of the mandible, maxilla, conchae, and other facial bones, resulting in bilaterally symmetrical

enlargement of the head. Other names for the disease include miller's disease, bighead, and bran disease.

Osteodystrophia fibrosa is a manifestation of nutritional secondary hyperparathyroidism caused by diets that are low in calcium or by diets with three or more times as much phosphorus as calcium, regardless of whether the calcium content is deficient (Joyce et al 1971). The disease was previously caused by diets high in bran (hence the terms, miller's disease and bran disease), but now it occurs most commonly in horses eating large amounts of certain tropical grasses (Clarke et al 1996). Excessive dietary phosphorus, by unknown mechanisms, inhibits calcium absorption and causes hypocalcemia (Clarke et al 1996). Sustained dietary intake of excessive phosphorus results in decreased concentration of ionic calcium in the serum and subsequent stimulation of secretion of parathormone (Joyce et al 1971). The abnormally high concentration of parathormone causes bone resorption and deposition of fibrous tissue, preferentially in the skull.

Prevalence

Osteodystrophia fibrosa of horses is now an uncommon disease in Western countries because of improved dietary knowledge, but it occurs in subtropical climates where certain plants predispose horses to this dietary disorder (Clarke et al 1996). Young, growing horses are most commonly affected.

Clinical signs

The most prominent feature of the affected horses is bilaterally symmetrical, firm, pyramidal enlargement of the facial bones immediately dorsal and rostral to the facial crests and thickening of the horizontal rami of the mandible (Fig. 25.19) (Clarke et al 1996). Gross thickening of the maxillary bones, conchae, and hard palate by supraperiosteal deposition of fibro-osseous tissue occludes the nasal cavities, and severely affected horses may become dyspneic. Severely affected horses may also have loosening of the teeth and difficulty in masticating, which may lead to cachexia. In advanced cases of osteodystrophia fibrosa, other skeletal areas may be affected, and these lesions may predispose the horse to lameness as a result of fractures, avulsion of ligaments, and limb deformity (Clarke et al 1996).

Pathology

The maxillary, ventral conchal, and conchofrontal sinuses of horses affected by osteodystrophia fibrosa are filled with firm, slightly spongy tissue (Clarke et al 1996). During histologic examination of affected tissue, dense, highly cellular fibrous tissue arranged in whorls and streams is seen replacing cortical bone. Osseous trabeculae surrounded
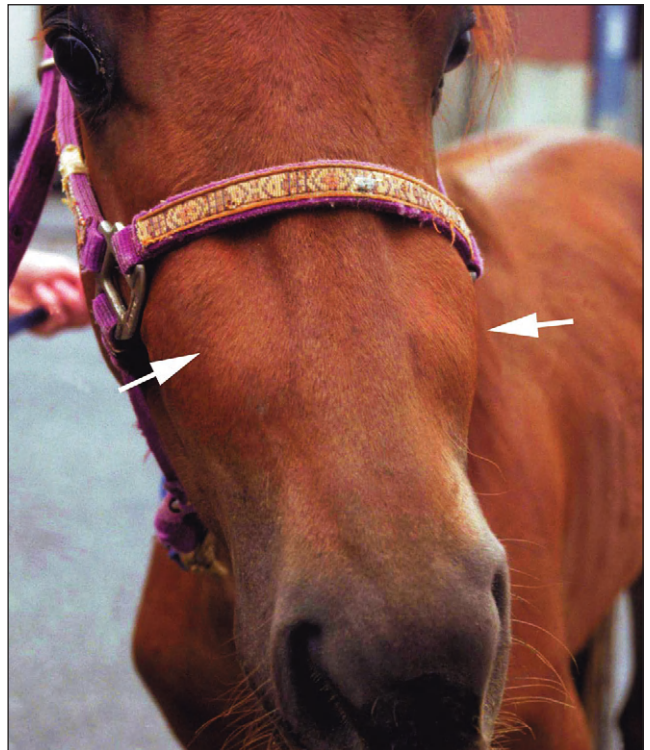
Fig. 25.19. A prominent feature of this horse affected with osteodystrophia fibrosa is the bilaterally symmetrical enlargement of the facial bones immediately dorsal and rostral to the facial crests.

by numerous, large, multinucleate osteoclasts are found within the fibrous tissue, which may have patchy cystic degeneration (Head & Dixon 1999). The parathyroid glands may show features of hypertrophy and hyperplasia (DelPiero et al 1997).

Diagnosis

Diagnosis of osteodystrophia is based on observed characteristic skeletal changes (Joyce et al 1971). Radiographic examination may reveal loss of trabeculation of long bones and increased radiolucency of all bones. Diagnosis of the condition can sometimes be assisted by detecting a low concentration of calcium and a high concentration of phosphorus in the urine. Concentrations of these minerals in the serum are usually normal because of the compensatory activity of the parathyroid glands (Joyce et al 1971).

Treatment and prognosis

Horses affected with osteodystrophia fibrosa should be treated by correcting the dietary calcium : phosphorus ratio to between 1.5 and 1. Although dietary management may partially resolve some of the skeletal lesions, severely affected horses may fail to show significant improvement

in respiratory capacity (Joyce et al 1971, Clarke et al 1996). Resection of deformed, occluding nasal conchae through trephination holes has been described (Berge & Westhues 1966), but permanent tracheostomy may be necessary to restore respiratory capacity. Facial deformity is not likely to resolve.

REFERENCES

Aegerter E, Kirkpatrick JA Jr 1979 Orthopedic Diseases, 3rd edn. WB Saunders, Philadelphia, pp.1–5

Ainsworth GC, Austwick PKC 1959 Fungal Diseases of Animals. Commonwealth Agricultural Bureau, Farnham Royal, pp.44–45

Alfaro AA, Mendoza L. 1990 Four cases of equine bone lesions caused by *Pythium insidiosum*. Equine Veterinary Journal 22: 295–297

Allison N, Willard MD, Bentinck-Smith J, Davis K 1986 Nasal rhinosporidiosis in two dogs. Journal of the American Veterinary Medical Association 188: 869–871

Atallah N, Jay MM 1981 Osteomas of the paranasal sinuses. Journal of Laryngology and Otology 95: 291–304

Ayars G 2000 Nonallergic rhinitis. Immunology and Allergy Clinics of North America 20: 283–302

Aylor MK, Campbell ML, Goring RL, Hillidge CJ 1984 Congenital bilateral choanal atresia in a Standardbred foal. Equine Veterinary Journal 16: 396–398

Baker GJ 1999 Abnormalities of development and eruption. In: Baker FJ, Easley JR (editors) Equine Dentistry, 1st edn. WB Saunders, Philadelphia, pp.49–59

Barakzai SZ, Dixon PM 2005 Sliding muco-periosteal hard palate flap for treatment of a persistent oro-nasal fistula. Equine Veterinary Education 17: 287–291

Beard W 1996 Upper respiratory causes of exercise intolerance. Veterinary Clinics of North America; Equine Practice 12: 435–453

Bemis H 1916 Removal of the nasal septum. Journal of the American Veterinary Medical Association 2: 397–399

Berge E, Westhues M 1966 Veterinary Operative Surgery. Williams and Wilkins, Baltimore, pp.140–142

Boles C 1979 Abnormalities of the upper respiratory tract. Veterinary Clinics of North America; Equine Practice 1: 89–111

Boulton CH 1985 Equine nasal cavity and paranasal cavity disease: a review of 85 cases. Journal of Equine Veterinary Science 5: 268–275

Brearley JC, McCandlish IA, Sullivan M, Dawson CO 1986 Nasal granuloma caused by *Pseudallescheria boydii*. Equine Veterinary Journal 18: 151–153

Bridges CH 1972 Mycotic diseases. In: Catcott EJ, Smithcors JF (editors) Equine Medicine and Surgery, 2nd edn. American Veterinary Publications, Wheaton, IL, pp.119–135

Campbell CK 1990 Pythiosis. Equine Veterinary Journal 22: 227–228

Clarke CJ, Roeder PL, Dixon PM 1996 Nasal obstruction caused by nutritional osteodystrophia fibrosa in a group of Ethiopian horses. Veterinary Record 138: 568–570

Corrier DE, Wilson SR, Scrutchfield WL 1984 Equine cryptococcal rhinitis. Compendium on Continuing Education for the Practicing Veterinarian 6: S556–S558

Crouch GM, Morgan SJ 1983 Bilateral congenital choanal atresia in a foal. Compendium on Continuing Education for the Practicing Veterinarian 5: 206–211

Davis PR, Meyer GA, Hanson RR, Stringfellow JS 2000 *Pseudallescheria boydii* infection of the nasal cavity of a horse. Journal of the American Veterinary Medical Association 217: 707–709

DelPiero F, Wilkins PA, Langsetmo I, Fubini SL 1997 An unusual cause of unilateral facial deformity in a yearling horse. Equine Practice 19: 8–11

DeMartini JC, Riddle WE 1969 Disseminated coccidioidomycosis in two horses and a pony. Journal of the American Veterinary Medical Association 155: 149–156

des Lions JA, Guillot J, Legrand E, Bisseaud O, Jensen HE 2000 Aspergillosis involving the frontal sinus in a horse. Equine Veterinary Education 12: 248–250

Dixon PM, Head KW 1999 Equine nasal and paranasal sinus tumours: part 2: a contribution of 28 case reports. Veterinary Journal 157: 279–294

Foerner JJ 1967 The diagnosis and correction of false nostril noises. Proceedings of the American Association of Equine Practitioners 13: 315–327

Foley JP, Legendre AM 1992 Treatment of coccidioidomycosis osteomyelitis with itraconazole in a horse. Journal of Veterinary Internal Medicine 6: 333–334

Frankeny RL 2003 Intralesional administration of formalin for treatment of epidermal inclusion cysts in five horses. Journal of the American Veterinary Medical Association 223: 221–222

Freeman DE, Orsini PG, Ross MW, Madison JB 1990 A large frontonasal bone flap for sinus surgery in the horse. Veterinary Surgery 19: 122–130

French DD, Haynes PF, Miller RI 1985 Surgical and medical management of rhinophycomycosis (conidiobolomycosis) in a horse. Journal of the American Veterinary Medical Association 186: 1105–1107

Gillespie JH 1981 Hagan and Bruner's Infectious Diseases of Domestic Animals, 7th edn. Cornell University Press, Ithaca, NY, pp.372–376

Gordon LR 1978 Cytology and histology of epidermal inclusion cysts in the horse. Journal of Equine Medicine and Surgery 2: 370–372

Goring RL, Campbell M, Hillidge CJ 1984 Surgical correction of congenital bilateral choanal atresia in a foal. Veterinary Surgery 13: 211–216

Greet TRC 1981 Nasal aspergillosis in three horses. Veterinary Record 109: 487–489

Greet TRC 1992 Outcome of treatment in 23 horses with progressive ethmoidal hematoma. Equine Veterinary Journal 24: 468–471

Grooters A, Gee MK 2002 Development of a nested polymerase chain reaction assay for the detection and identification of *Pythium insidiosum*. Journal of Veterinary Internal Medicine 16: 147–152

Hanselka DV 1977 Equine nasal phycomycosis. Veterinary Medicine/Small Animal Clinician 72: 251–253

Hawkins JF, Tulleners EP, Evans LH, Orsini JA 1995 Alar fold resection in horses: 24 cases (1979–1992). Journal of the American Veterinary Medical Association 206: 1913–1916

Haynes PF 1984 Surgery of the equine respiratory tract. In: Jennings PB (editor) The Practice of Large Animal Surgery, Vol. 1. WB Saunders, Philadelphia, pp.388–487

Head KW, Dixon PM 1999 Equine nasal and paranasal sinus tumours. Part 1: review of the literature and tumour classification. Veterinary Journal 157: 261–278

Hengerer AS, Stome M 1982 Choanal atresia: a new embryologic theory and its influence on surgical management. Laryngoscope 92: 913–921

Hodgin EC, Conaway DH, Ortenburger AI 1984 Recurrence of obstructive nasal coccidioidal granuloma in a horse. Journal of the American Veterinary Medical Association 184: 339–340

Hogan PM, Embertson RM, Hunt RJ 1995 Unilateral choanal atresia in a foal. Journal of the American Veterinary Medical Association 207: 471–473

Husby G 1988 Equine amyloidosis. Equine Veterinary Journal 20: 235–238

Hutchins DR, Johnston KG 1972 Phycomycosis in the horse. Australian Veterinary Journal 48: 269–278

Joyce J, Pierce K, Romane W, Baker JM 1971 Clinical study of nutritional secondary hyperparathyroidism in horses. Journal of the American Veterinary Medical Association 158: 2033–2042

Kasper CA, Fretz PB, Allen AL, Clark EG 1994 Nasal amyloidosis: a case report and review. Equine Practice 16: 25–30

Kaufman L, Mendoza L, Standard PG 1990 Immunodiffusion test for serodiagnosing subcutaneous zygomycosis. Journal of Clinical Microbiology 28: 1887–1890

Kelly DF, Watson WJ 1976 Epidermoid cyst of the brain in the horse. Equine Veterinary Journal 8: 110–112

Korenek NL, Legendre AM, Andrews FM 1994 Treatment of mycotic rhinitis with itraconazole in three horses. Journal of Veterinary Internal Medicine 8: 224–227

Lane JG 1998 Disorders of the ear, nose and throat. In: Mair T, Love S, Schumacher J, Watson E (editors) Equine Medicine Surgery and Reproduction. WB Saunders, Philadelphia, pp.81–117

Lane JG, Mair TS 1987 Observations on headshaking in the horse. Equine Veterinary Journal 19: 331–336

Londero AT, Santos MN, Freitas CJ 1977 Animal rhino-sporidiosis in Brazil. Report of three additional cases. Mycopathologia 60: 171–173

Madewell BR, Priester WA, Gillette EL, Snyder SP 1976 Neoplasms of the nasal passages and paranasal sinuses in domesticated animals as reported by 13 veterinary colleges. American Journal of Veterinary Research 37: 851–865

Maniglia A, Goodwin W 1981 Congenital choanal atresia. Otolaryngologic Clinics of North America 14: 167

McGorum BC, Dixon PM 1990 Vasomotor rhinitis with headshaking in a pony. Equine Veterinary Journal 22: 220–222

McGorum BC, Dixon PM, Lawson GHK 1992 A review of ten cases of mycotic rhinitis. Equine Veterinary Education 4: 8–12

McKellar GM, Collins AP 1993 The surgical correction of a deviated anterior maxilla in a horse. Australian Veterinary Journal 70: 112–114

Medleau L, Greene CE, Rakich M 1990 Evaluation of keto-conazole and itraconazole for treatment of disseminated cryptococcosis in cats. American Journal of Veterinary Research 51: 1454–1458

Miller RI 1981 Treatment of equine phycomycosis by immunotherapy and surgery. Australian Veterinary Journal 57: 377–382

Miller RI 1983 Equine phycomycosis. Compendium on Continuing Education for the Practicing Veterinarian 5: 472–478

Miller RI, Campbell RS 1982 Clinical observations on equine phycomycosis. Australian Veterinary Journal 58: 221–226

Miller RI, Wold D, Lindsay WA, Beadle RE, McClure JJ, McClure JR, McCoy DJ 1983 Complications associated with immunotherapy of equine phycomycosis. Journal of the American Veterinary Medical Association 182: 1227–1229

Milne EM, Woodman MP, Doxey DL 1994 Use of clinical measurements to predict the outcome in chronic cases of grass sickness (equine dysautonomia). Veterinary Record 134: 438–440

Mould JR, Munroe GA, Eckersall PD, Conner JG, McNeil PE 1990 Conjunctival and nasal amyloidosis in a horse. Equine Veterinary Journal Supplement 10: 8–11

Murray DR, Ladds PW, Johnson RH, Pott BW 1978 Metastatic phycomycosis in a horse. Journal of the American Veterinary Medical Association 172: 834–836

Myers DD, Simon J, Case MT 1964 Rhinosporidiosis in a horse. Journal of the American Veterinary Medical Association 145: 345–347

Newton JC, Ross PS 1993 Equine pythiosis: an overview of immunotherapy. Compendium on Continuing Education for the Practicing Veterinarian 15: 491–493

Nickels FA 1993 Diseases of the nasal cavity. Veterinary Clinics of North America; Equine Practice 9: 111–121

Orsini PG, Ross MW, Hamir AN 1992 Levator nasolabialis muscle transposition to prevent an orosinus fistula after tooth extraction in horses. Veterinary Surgery 21: 150–156

Owens WR, Miller RI, Haynes PF, Snider TG 1985 Phycomycosis caused by *Basidiobolus haptosporus* in two horses. Journal of the American Veterinary Medical Association 186: 703–705

Pascoe RR 1981 Equine Dermatoses. University of Sydney, Sydney, p.29

Pearson EG, Watrous BJ, Schmitz JA, Sonn RJ 1983 Crypto-coccal pneumonia in a horse. Journal of the American Veterinary Medical Association 183: 577–579

Pool RR 1978 Tumors of bone and cartilage. In: Moulton JE (editor) Tumors of Domestic Animals, 2nd edn. University of California Press, Berkeley, CA, pp.99–103

Puchol JL, Herran R, Durall L, Lopez J, Diaz-Bertrana C 2004 Use of distraction osteogenesis for the correction of deviated nasal septum and premaxilla in a horse. Journal of the American Veterinary Medical Association 224: 1147–1150

Roberts MC, Sutton RH, Lovell DK 1981 A protracted case of cryptococcal nasal granuloma in a stallion. Australian Veterinary Journal 57: 287–291

Robertson JL, Rooney JR 1997 The pathology of the equine respiratory system. In: Rantanen N, Hauser ML (editors) Proceedings of the 2nd International Dubai Equine Symposium. Mathew Rantanen Design, Wildomar, CA, pp.189–239

Scarratt WK, Crisman MV 1998 Neoplasia of the respiratory tract. Veterinary Clinics of North America; Equine Practice 14: 451–473

Schuh JC 1986 Squamous cell carcinoma of the oral, pharyngeal, and nasal mucosa in the horse. Veterinary Pathology 23: 205–207

Schumacher J, Smith BL, Morgan SJ 1988 An osteoma of the paranasal sinuses of a horse. Journal of the American Veterinary Medical Association 192: 1449–1450

Schumacher J, Moll HD, Schumacher J, Crabill M 1997 A simple method to remove an epidermal inclusion cyst from the false nostril of horses. Equine Practice 19: 11–13

Schummer A, Nickel R, Sack WO 1979 The Viscera of the Domestic Mammals, 2nd edn. Paul Parey, Berlin & Hamburg, p.304

Scott DW, Miller WHJ 2003 Equine Dermatology. Elsevier Science, St Louis, MO, pp.311–312

Scrutchfield WL, Schumacher J, Walker M, Crabill M 1994 Removal of an osteoma from the paranasal sinuses of a standing horse. Equine Practice 16: 24–28.

Servantie J, Sautet JY 1986 Hamartoma of the nasal septum in a yearling. Equine Practice 8: 11–14

Shaw DP, Gunson DE, Evans LH 1987 Nasal amyloidosis in 4 horses. Veterinary Pathology 24: 183–185

Silverman S, Merten DF, Anderson JH, Hendrickx AG 1977 Radiographic diagnosis of choanal atresia induced prenatally with triamcinolone in the baboon (*Papio cynocephalus*). Journal of Medical Primatology 6: 284–297

Sisson S, Grossman JD 1953 The Anatomy of Domestic Animals, 4th edn. WB Saunders, Philadelphia, pp.517–563

Smith HA 1961 Rhinosporidiosis in Texas horse. Southwest Veterinarian 15: 22–24

Smith HA, Jones TC, Hunt RD 1972 Veterinary Pathology, 4th edn. Lea & Febiger, Philadelphia, pp.261, 325, 1336

Spjut HJ, Dorfman HD, Fechner RE, Ackerman, LV 1971 Tumors of Bone and Cartilage. Armed Forces Institute of Pathology, Washington, DC, pp.17–119

Sprinkle FP, Crowe MW, Swerczek TW 1984 Choanal atresia in foals. Modern Veterinary Practice 65: 306

Steiger RR, Williams MA 2000 Granulomatous tracheitis caused by *Conidiobolus coronatus* in a horse. Journal of Veterinary Internal Medicine 14: 311–314

Steinman A, Sutton GA, Lichawski D, Johnston DE 2002 Osteoma of paranasal sinuses in a horse with inspiratory dyspnoea. Australian Veterinary Journal 80: 140–142

Stilson AE Jr, Herring DS, Robertson JT 1985 Contribution of the nasal septum to the radiographic anatomy of the equine nasal cavity. Journal of the American Veterinary Medical Association 186: 590–592

Taintor J, Schumacher J, Newton J 2003 Conidiobolomycosis in horses. Compendium on Continuing Education for the Practicing Veterinarian 25: 872–876

Taintor J, Crowe C, Hancock S, Schumacher J, Livesey L 2004 Treatment of conidiobolomycosis with fluconazole in two pregnant mares. Journal of Veterinary Internal Medicine 18: 363–364

Torre F 2000 Use of a stainless steel mesh combined with resection of the alar fold for correction of unilateral paralysis of the nostril in a Standardbred filly. Equine Veterinary Education 12: 234–236

Torre F 2003 Use of an autogenous cartilage graft from the auricular cartilage in the treatment of unilateral paralysis of the nostril in a racing Standardbred. Equine Veterinary Education 15: 10–14

Tremaine WH, Clarke CJ, Dixon PM 1999 Histopathological findings in equine sinonasal disorders. Equine Veterinary Journal 31: 296–303

Tremaine WH, Dixon PM 2001a A long-term study of 277 cases of equine sinonasal disease. Part 1: details of horses, historical, clinical and ancillary diagnostic findings. Equine Veterinary Journal 33: 274–282

Tremaine WH, Dixon PM 2001b A long-term study of 277 cases of equine sinonasal disease. Part 2: Treatments and results of treatments. Equine Veterinary Journal 33: 283–289

Tulleners EP, Raker CW 1983 Nasal septum resection in the horse. Veterinary Surgery 12: 41–47

Valdez H, McMullan WC, Hobson HP, Hanselka DV 1978 Surgical correction of deviated nasal septum and premaxilla in a colt. Journal of the American Veterinary Medical Association 173: 1001–1004

van Andel AC, Gruys E, Kroneman J, Veerkamp J 1988 Amyloid in the horse: a report of nine cases. Equine Veterinary Journal 20: 277–285

Vandeplassche M, Simoens P, Bouters R, De Vos N, Verschooten F 1984 Aetiology and pathogenesis of congenital torticollis and head scoliosis in the equine foetus. Equine Veterinary Journal 16: 419–424

Vasey JR, McKinnon AO, Axon JE, Hazard GH, Lescun TB 1995 Nasal flutter as a cause of poor performance in race horses – a review of 22 cases (1981–1995). Veterinary Surgery 24: 444

Walker MA, Schumacher J, Schmitz DG, et al 1998 Cobalt 60 radiotherapy for treatment of squamous cell carcinoma of the nasal cavity and paranasal sinuses in three horses. Journal of the American Veterinary Medical Association 212: 848–851

Watt BC, Beck BE 1997 Removal of a nasal polyp in a standing horse. Canadian Veterinary Journal 38: 108–109

Watt DA 1970 A case of cryptococcal granuloma in the nasal cavity of a horse. Australian Veterinary Journal 46: 493–495

Zamos DT, Schumacher J, Loy JK 1996 Nasopharyngeal conidiobolomycosis in a horse. Journal of the American Veterinary Medical Association 208: 100–101

Zontine WJ 1958 Coccidioidomycosis in the horse; a case report. Journal of the American Veterinary Medical Association 132: 490–492

26 Disorders of the Paranasal Sinuses

Henry Tremaine and David E Freeman

Introduction

Inflammation of the equine paranasal sinuses is a relatively uncommon disease that may be caused by primary bacterial or mycotic infections (Mason 1975a), or can be secondary to dental disease (van der Velden & Verzijlenberg 1984, Scott 1987, Tremaine & Dixon 2001a), facial trauma, sinus cysts, progressive ethmoid hematoma or sinonasal neoplasia (Mansmann & Wheat 1973, Gibbs & Lane 1987, Tremaine & Dixon 2001a). Equine sinusitis is usually unilateral but bilateral disease has been reported (Coumbe et al 1987, Lane 1993, Tremaine & Dixon 2001a). There is apparently no breed, age or gender predisposition to sinusitis. Clinical signs of any type of sinusitis usually include unilateral purulent nasal discharge, ipsilateral submandibular lymph node enlargement, and epiphora. Less common signs include facial swelling, exophthalmos, abnormal respiratory noises, head shaking, and exercise intolerance (Lane 1993, Tremaine & Dixon 2001a).

Primary Sinus Empyema (Primary Sinusitis)

Primary sinusitis is the result of obstruction of the normal nasomaxillary drainage with resulting accumulation of mucus in the sinus, which later becomes infected. Some cases occur following upper respiratory tract infections that cause inflammation, increase mucus production within the sinuses, and decrease drainage of secretions from the sinuses into the nasal cavity via the anatomically narrow nasomaxillary ostia. The nasal discharge in primary sinusitis is traditionally stated to be purulent and odorless (Mason 1975a), but malodorous nasal discharges can occur with primary sinusitis (Tremaine & Dixon 2001a), especially in association with inspissation of purulent material in the ventral conchal sinuses (Schumacher et al 1987).

Culture of exudates from primary sinusitis cases often yields a mixed bacterial growth that is of unclear etiologic significance. Isolated bacteria include *Streptococcus equi* var. *zooepidemicus* (Schumacher et al 1987, Ruggles et al 1993), *Corynebacterium* spp., (Schumacher & Crossland 1994), *Staphylococcus* spp. (Mason 1975a, Schumacher et al 1987, Tremaine & Dixon 2001a), *Pseudomonas aerugi-*

nosa, *Bacteroides* spp., *Peptostreptococcus* spp. (Ruggles et al 1993, Tremaine & Dixon 2001a), *Streptococcus equi* var. *equi* (Mansmann & Wheat 1973), and *Escherichia coli* (Mason 1975a, Schumacher et al 1987), although as noted, the etiologic importance of these isolates is often unclear.

Nasal endoscopy of horses with sinusitis usually reveals purulent exudate in the caudal nasal cavity draining from the nasomaxillary ostia of the rostral and/or caudal maxillary sinuses ("drainage angle") (Fig. 26.1). Marked accumulation of exudate in the ventral conchal sinus can result in swelling of the ventral concha, which may eventually prevent passage of the endoscope up the affected nasal cavity. Displacement of the nasal septum can occur in cases with gross distension of this sinus. Straight lateral radiographs of horses with primary sinusitis frequently reveal multiple fluid lines in some of the paranasal sinuses. Oblique radiographs are necessary to separate the left and right rows of maxillary cheek teeth for radiographic
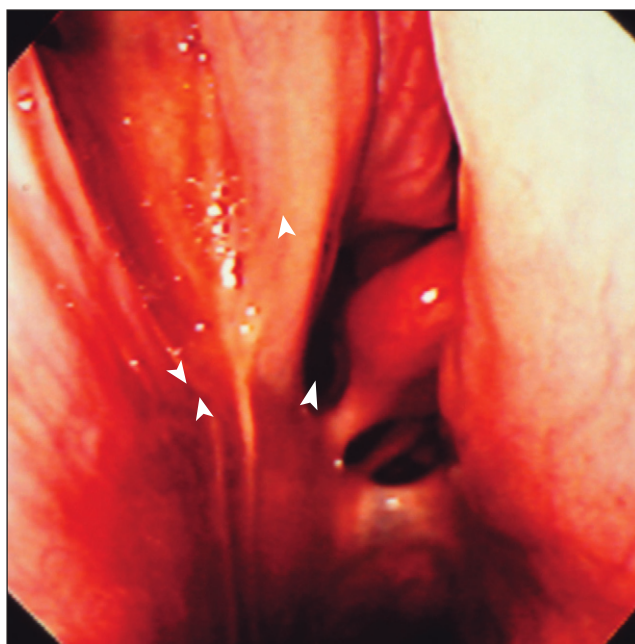
Fig. 26.1. Endoscopic view of the caudal aspect of the middle meatus ("drainage angle") in a horse with sinusitis down which purulent exudate from the maxillary sinuses is draining through the nasomaxillary ostia (arrowheads).
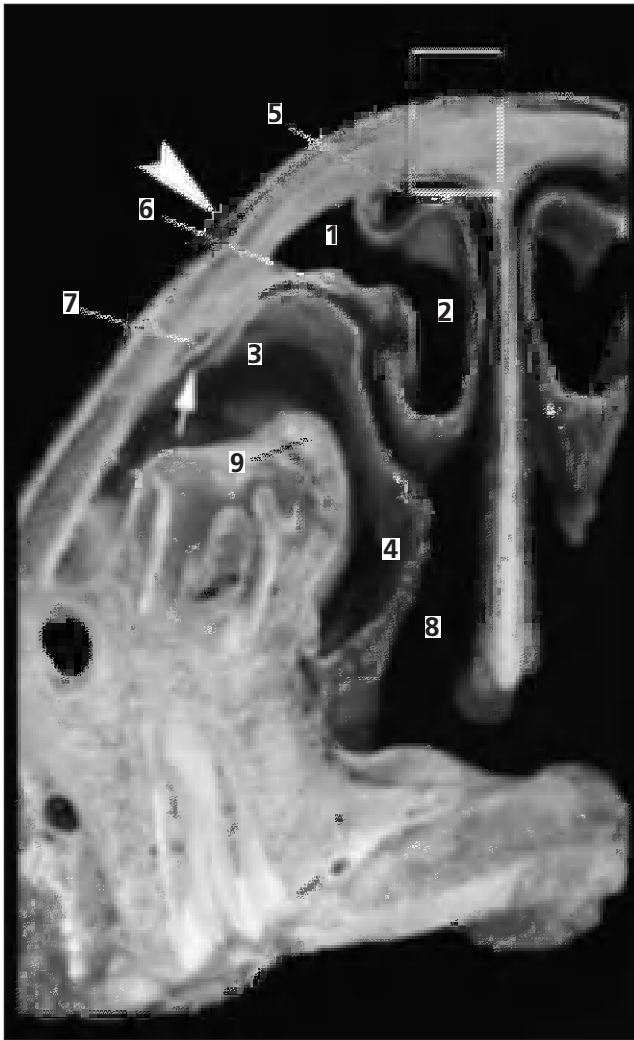
Fig. 26.2. Front view of a transverse section of the right paranasal sinuses and nasal passage through tooth 109 at the level of the most rostral end of a frontonasal bone flap. 1 = frontal sinus; 2 = dorsal conchal sinus; 3 = rostral maxillary sinus; 4 = ventral conchal sinus; 5 = dorsal meatus; 6 = middle meatus; 7 = nasolacrimal duct; 8 = ventral meatus; 9 = infraorbital nerve in the infraorbital canal. Arrow points to opening from the rostral maxillary sinus into the middle meatus. Rectangle is the point of fracture for a frontonasal bone flap and includes the point of separation from the underlying reflection of the dorsal nasal concha. The arrowhead is the lateral edge of the bone flap. Note the reserve dental crown occupies a large portion of the sinus cavities in this young horse and along with the infraorbital canal limits access to the sinuses.

Fig. 26.3. Transverse section of the skull of an aged horse at the level of the fourth cheek tooth (109, 209) showing the voluminous rostral maxillary sinus (RMS) and the ventral nasal meatus (VM).

examination of the dental apical areas. Dorsoventral radiographs are particularly useful for demonstrating distension of, and exudate within, the ventral conchal sinus (see Chapter 10).

Acute cases of primary sinusitis may spontaneously resolve or may respond to antimicrobial drug administration, with the organisms commonly isolated frequently being sensitive to penicillin. Chronic cases of primary

sinusitis (of > 2 months duration) frequently have gross thickening of the sinus mucosa, which can further restrict normal nasomaxillary drainage and such cases may only show a transient improvement to antibiotic treatment (Tremaine & Dixon 2001a). Treatment by sinus irrigation may be performed in these cases, via a sutured irrigation tube or Foley catheter placed via a trephine opening into the frontal or caudal maxillary sinuses (for lavage of the frontal and caudal maxillary sinuses), or into the rostral maxillary sinus (for lavage of the rostral maxillary and ventral conchal sinuses). Such cases may respond to lavage with 5–10 liters of water, saline or dilute disinfectants such as 0.05% povidine-iodine solution, once to twice daily for 5–10 days.

Cases with gross thickening of the sinus mucosa, and in particular cases with accumulations of inspissated pus in the sinus, may require surgical debridement and possibly sinonasal fistulation to improve drainage. An outline of sinus anatomy and surgical approaches is presented in Figs 26.2–26.4. The frontal, maxillary, and ventral conchal sinus are all most easily approached via a large nasofrontal bone-flap osteotomy (Freeman et al 1990) (Figs 26.4 and 26.5) where the bone is preserved or a smaller osteotomy where the bone is discarded (Figs 26.6–26.9). Even when radiographs or computed tomographic images demonstrate that the inflammation mainly involves the maxillary sinuses, a frontonasal flap is the preferred approach for a number of reasons (Freeman et al 1990). When the lesion is in the maxillary sinus, the frontal approach is far enough from it to allow creation of the flap without disturbing the lesion (e.g. sinus cyst), and yet close enough to allow its easy removal. It also provides a sufficiently clear view of the sinus interior to allow complete examination.

The incisions necessary for this type of flap do not involve muscles or large blood vessels, and the size and position of the flap can be designed to suit the lesion, even
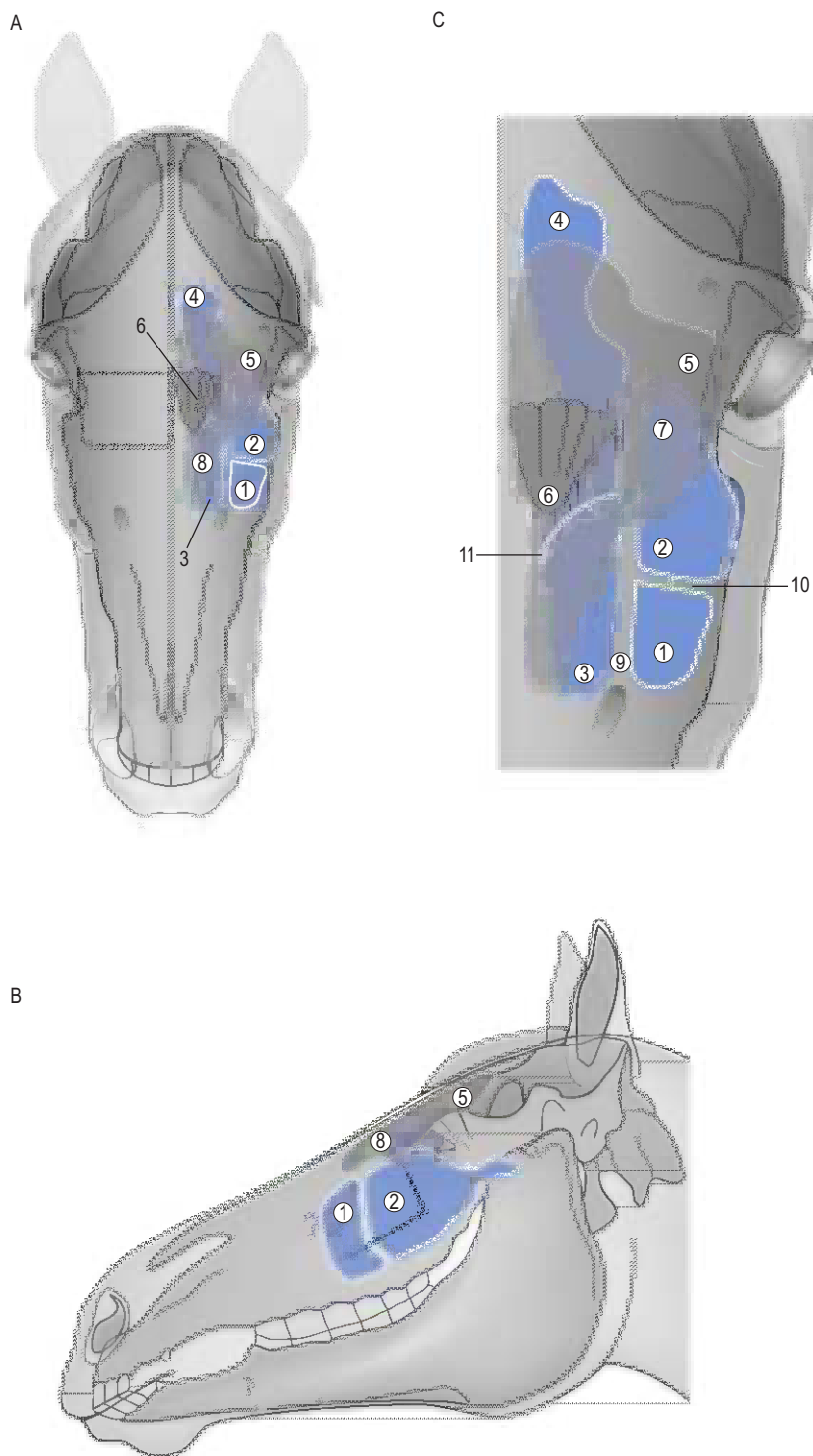
Fig. 26.4. Approaches to the sinuses through a frontonasal bone flap (broken line in A) and maxillary bone flap (broken line in B), and (C) expanded dorsal view of sinuses. 1 = rostral maxillary sinus; 2 = caudal maxillary sinus; 3 = ventral conchal sinus; 4 = sphenopalatine sinus; 5 = frontal sinus; 6 = ethmoidal labyrinth; 7 = frontomaxillary opening; 8 = dorsal conchal sinus (5 and 8 combine to form the conchofrontal sinus); 9 = infraorbital canal; 10 = bony maxillary septum; 11 = caudal bulla of ventral conchal sinus. Reproduced from Freeman 2003, with permission.
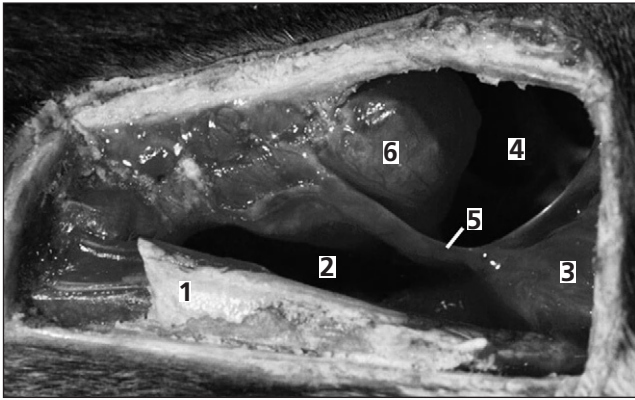
Fig. 26.5. Interior of the right conchofrontal sinus as viewed through a frontonasal bone flap in a cadaver specimen. For demonstration purposes, the entire flap has been removed. The rostral part of the head is to the left and the lateral margin is uppermost. 1 = reflection of dorsal nasal concha which has retained some of the bony attachment to the underside of the flap; 2 = dorsal conchal sinus; 3 = ethmoid labyrinth; 4 = caudal maxillary sinus; 5 = medial edge of the frontomaxillary opening; 6 = caudal bulla of the ventral conchal sinus. Reproduced from Freeman et al 1990, with permission.

Fig. 26.6. A curvilinear incision has been made through the skin and periosteum which have then been reflected back, to enable a right-sided nasofrontal bone osteotomy to be made in a standing sedated horse.

allowing access to the nasal passage if necessary (Freeman et al 1990). If the bone flap is constructed so that it is hinged on the dorsal midline, it will lie out of the surgeon's way when fully opened. The frontonasal flap can also be used for repulsion of cheek teeth, but access to 109 and 209 (the fourth maxillary cheek teeth) is limited using this approach. Alternatively, a caudal maxillary osteotomy may be used in older (>10 years) horses (Fig. 26.10), but the reserve crowns of the maxillary cheek teeth limit the access to the sinuses via this approach in younger animals. A maxillary approach to the rostral maxillary sinus gives even more restricted access to the sinus lumen because of the position of the reserve crowns of the third and fourth maxillary cheek teeth (Triadan 08s and 09s).

Bone flap osteotomies may be created under general anesthesia or in the standing sedated horse (Scrutchfield et al 1994, Quinn et al 2004). After making a rectangular or curved incision through the skin and periosteum, the bone flap is created with an oscillating saw, chisel or Gigli wire; the larger, three-sided bone flap may then be hinged back on its (fourth) uncut side, to fracture the bone, whilst retaining the flap's intact skin, subcutaneous tissue and periosteal attachments. Alternatively, an axial-based curvilinear incision may be made and the skin and perioosteum can be reflected. The osteotomy can be created using a 5-cm diameter trephine with the disc of bone being discarded (Figs 26.6–26.8). The skin and periosteum are closed over the osteotomy ensuring that a 5–10-mm shelf of bone is present peripheral to the osteotomy on which the periosteum can be laid, to help prevent dehiscence. Although sequestration of the flap has been cited as a risk

of retention of sinus osteotomy flaps, published reports do not confirm this to be a frequent occurrence, especially with larger flaps. Alternatively, despite the loss of a 5-cm disc of bone, albeit over a flat surface, the cosmetic results after discarding the flap are usually acceptable (Quinn et al 2004).

At sinusotomy, inspissated pus and grossly thickened mucosa are removed and the sinus can then be irrigated postoperatively (Fig, 26.9). If sinonasal drainage appears to be compromised, it may be improved by creation of a fistula through the dorsomedial wall of the ventral concha into the nasal cavity. Even when performed on the less vascular, dorsal aspect of the medial conchal wall, this fistulation will usually be accompanied by profuse hemorrhage. To control hemorrhage after such fistulation a 3-inch (7.6-cm) elasticated stockinet can be introduced into the sinus via the nasal cavity (Fig. 26.11). To place this packing, an assistant passes a Chambers' mare catheter up the nasal passage until it can be digitally directed into the sinus by the surgeon. A length of umbilical tape is tied to the end of the catheter in the sinus and this end is drawn out of the nostril while the other remains within the sinus. Then saline-soaked gauze

Fig. 26.7. A large (5-cm) diameter trephine is being used to create a large bone flap into the left frontal sinus in this horse, enabling surgical access to the dorsal conchal, frontal and caudal maxillary sinuses. The bone flap is discarded and the flap later closed by apposing the skin and periosteum.
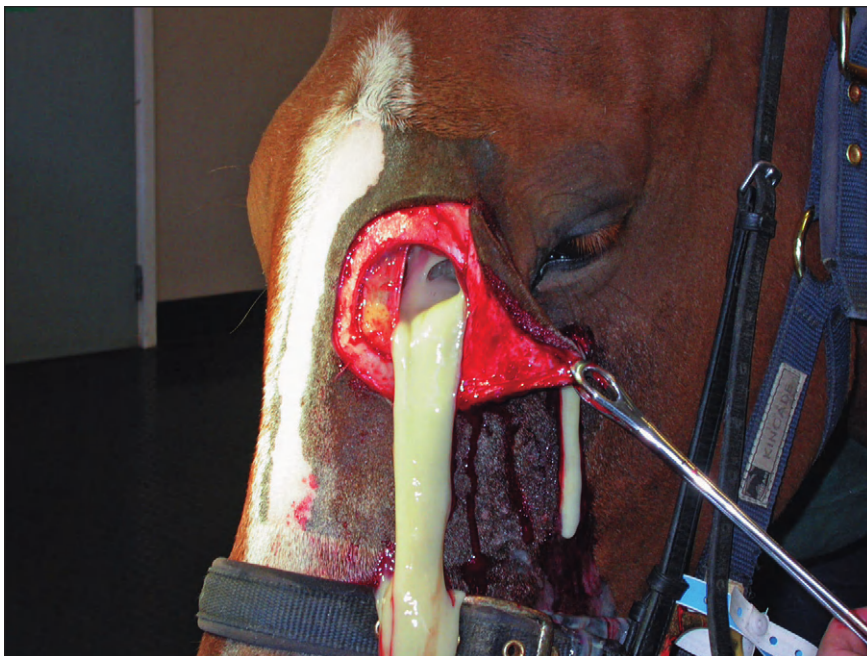
Fig. 26.8. Copious quantities of purulent exudate flowing from a nasofrontal bone flap osteotomy in a horse with chronic sinus empyema.

Fig. 26.9. The skin flap and periosteum are supported by a rim of frontal bone and are apposed using interrupted sutures (arrowheads). A maxillary trephine opening has then been made to allow post-operative irrigation of the maxillary sinuses through a Foley catheter.
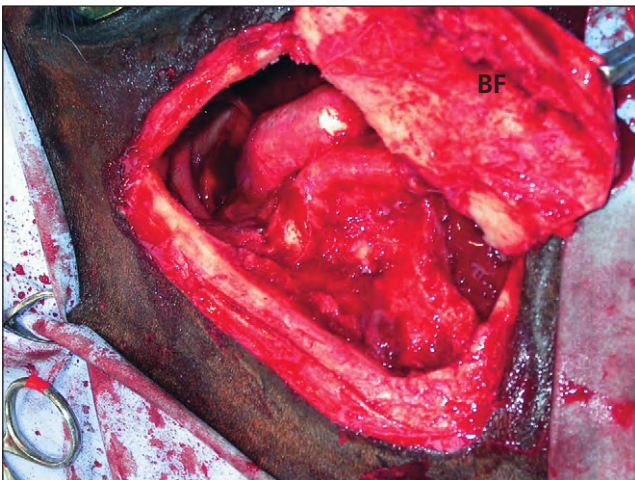
Fig. 26.10. A large maxillary bone flap (BF) has been created in this horse using an oscillating bone saw. This approach gives exposure to the caudal and rostral maxillary sinuses. The ventral conchal sinus is variably accessible dorsal to the infraorbital canal. This horse has extensive, inflamed soft tissue swelling within its caudal maxillary sinus.
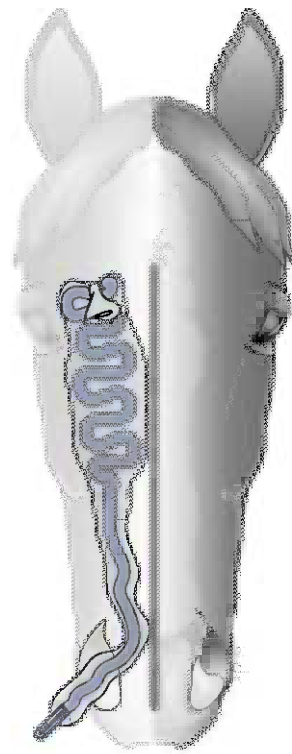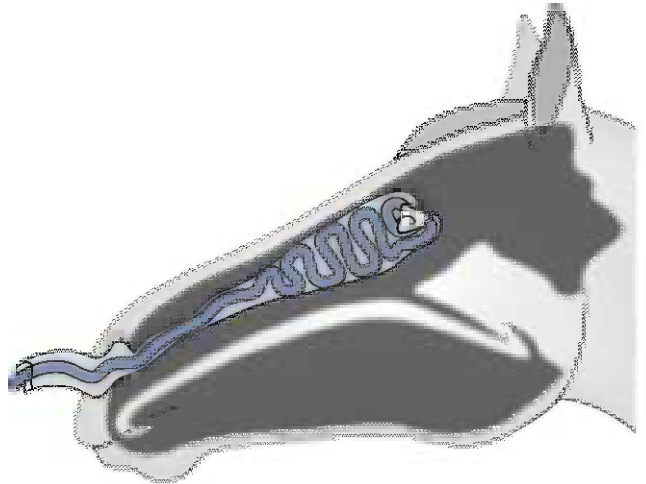
Fig. 26.11. Diagram outlining the postsurgical packing of a paranasal sinus to reduce hemorrhage following sinonasal fistulation.

bandage is placed within the "sock" of stockinet in accordion-fashion until the sinuses are packed.

The umbilical tape is tied around the redundant portion of stockinet, and the gauze within it, and used to draw them through the nostrils. The free end of stockinet, and gauze within it, are sutured to the roof of the false nostril with a heavy mattress suture over a butterfly of gauze sponge, and any excess packing is trimmed flush with the nostril. Alternatively, packing can be brought out through a trephine hole in adjacent intact bone. The purpose of the stockinet "sock" is to prevent migration of the packing into the pharynx, where it can be swallowed. It has been suggested that the upright position of the head when the procedure is performed in the standing horse results in less bleeding, although profuse hemorrhage can accompany fistulation of the venous conchal sinuses in standing horses. The necessity and efficacy of this sinonasal fistulation has been questioned (J. Schumacher, personal communication) and it is possible that sinonasal fistulation could alter mucociliary clearance and diminish intrasinus retention of endogenous (possibly bactericidal) nitric oxide.

The bone flap is replaced *in situ* (if retained) and may be secured with one or two wire sutures inserted into pre-placed drill holes in the flap and adjacent bone, although this may be unnecessary. The periosteum is closed with absorbable sutures and the skin is closed with staples or non-absorbable sutures. A lavage cannula or Foley catheter sutured into a separate trephine opening in the frontal sinus or caudal maxillary sinus allows postoperative irrigation of the sinuses. The prognosis for resolution of chronic sinusitis, including cases involving the ventral conchal sinus after surgical debridement, and where necessary, creation of sinonasal drainage is excellent (Tremaine et al 2001b, Quinn et al 2004).

Dental Sinusitis

Sinusitis commonly occurs with apical infections of the caudal maxillary cheek teeth (Triadan upper 08s–11s) (Mason 1975a, van der Velden & Verzijlenberg 1984, Lane 1993) and such dental infections caused 53% of sinusitis cases in one study (Tremaine & Dixon 2001a). Dental sinusitis occurs most frequently in horses aged 4–7 years (Dixon et al 2000b). Maxillary cheek teeth apical infections commonly occur following anachoresis (blood-borne infections of apices) (Dacre 2004) but also occur secondarily to idiopathic dental fractures (lateral slab or saggital), or with severe diastemata, and sometimes in conjunction with supernumerary cheek teeth (Dixon et al 1999, 2000a, Dacre 2004). Nasal discharge is frequently fetid when associated with dental secondary sinusitis, and also with intranasal tracts and granulomas resulting from infection of the first or second (or occasionally third) maxillary cheek tooth (Triadan 106–108, 206–208) (Lane 1994). Anaerobes including *Bacteroides fragilis*,

Fig. 26.12. Computed tomography transverse image of skull of a young horse at the level of the rostral maxillary sinuses, showing unilateral distortion of the overlying maxillary and nasal bones caused by an expansive soft tissue density mass within the sinus. Reproduced with the permission of Dr Wolfgang Henninger, University of Veterinary Medicine, Vienna.

B. melaninogenicus, *B. oralis* and *Fusobacterium mortiferum* have been cultured from nasal discharge with such infections (Mackintosh & Colles 1987), but their precise etiologic role remains unclear.

Radiography is an insensitive technique for detection of dental infections, especially in younger horses, because the radiographic changes associated with anatomical development of cheek teeth apices (i.e. blunt apices, absence of roots, wide periodontal spaces and absence of lamina dura denta in this region) are similar to the radiographic signs of early apical infection (see Chapter 10). In such cases, the presence of apical infection can sometimes be confirmed by gamma scintigraphy, which is more sensitive than radiography in selected cases, particularly in the early stages of the disease (Weller et al 2001) (see Chapter 12). Computed tomography and magnetic resonance imaging are also increasingly used to obtain highly detailed images of structures within the equine head and thus make an early and accurate diagnosis of apical infections (Tiejte at al 1998, Morrow et al 2000, Henninger et al 2003) (Fig. 26.12).

Sinusitis secondary to maxillary dental apical infections usually necessitates removal of the affected cheek tooth before resolution of the sinusitis will occur. Because of difficulty with the extraction of cheek teeth and the major long-term consequences following such extractions, this procedure should never be undertaken lightly. Definite

diagnosis of dental involvement in sinusitis using radiography, scintigraphy or computed tomography is essential before embarking on tooth removal. Anecdotal reports suggesting that endodontic therapy of infected pulp *per os*, effectively sealing the oral cavity from the sinus, will result in resolution of the sinus (T. Johnson, personal communication) have not been critically evaluated.

Infected cheek teeth may be removed via oral extraction, repulsion or via a lateral buccotomy. The latter technique can be used for the rostral three maxillary cheek teeth but not for the caudal maxillary cheek teeth. Extraction *per os* is associated with considerably reduced complications compared to repulsion, and additionally, may be accomplished in the standing horse (Tremaine 2004b, Dixon et al 2005). Dental extractions involving the maxillary cheek teeth that cannot be achieved by oral extraction (e.g. badly fractured or carious cheek teeth) can be performed under general anesthesia via a bone-flap osteotomy or via trephine opening. Intraoperative imaging to ensure accurate alignment of the punch with the affected tooth before repulsing the tooth is advised, to avoid iatrogenic damage to adjacent structures.

If dental extraction is performed *per os* in horses with dental sinusitis, lavage of the affected paranasal sinuses should also be performed post extraction. Intraoperative radiographs should be taken after dental removal (especially by repulsion) to attempt to identify the possible presence of intraalveolar bone or dental fracture fragments that are likely to sequestrate. Following oral extraction the alveolus can be temporarily packed with an antibiotic-soaked swab (Dixon et al 2005), but following repulsion a more robust alveolar packing is required, such as an acrylic plug attached to adjacent cheek teeth, to prevent the development of an oromaxillary fistula. Unsuccessful treatment of sinusitis can be attributed to oromaxillary fistula, persistent alveolar osteitis, abscesses within the overlying sinus, failure to remove all the infected tooth and infected or loose alveolar bone, and failure to treat obligate anaerobes with appropriate antibiotics such as metronidazole (De Moor & Verschooten 1982, Mackintosh & Colles 1987). The presence of small alveolar sequestra, which are not identifiable on postoperative radiographs, are an occasional cause for persistent clinical signs of sinusitis. These apparently develop later as the result of damage to alveoli by the repulsion process. The long-term prognosis for both primary and dental sinusitis cases is good (Tremaine & Dixon 2001b).

Mycotic Sinusitis

Equine sinonasal diseases associated with fungal infection are rare in the horse in the UK. Greet (1981) first described three cases of mycotic rhinitis in horses caused by *Aspergillus fumigatus*, and subsequent reports are sparse. Of ten cases of sinonasal mycosis described by McGorum

et al (1992), *Aspergillus fumigatus* was cultured from six, *Pseudallescheria boydii* from one, and *Penicillium* spp. from a single case. *Pseudallescheria boydii*, an opportunistic saprophyte, has also been isolated from a frontal sinus lesion (Johnson et al 1975).

Aspergillus fumigatus is ubiquitous in dead vegetation including hay and straw. The mechanism of infection of the nasal chambers or paranasal sinuses of horses by normally saprophytic fungi is not clear, but previous trauma from surgery or nasogastric tube passage may be a factor in some cases (Watt 1970, Greet 1981, Tremaine & Dixon 2001b).

Mycotic sinonasal infections caused by other fungal organisms are common in warm humid climates. These have involved infection with *Cryptococcus neoformans* (Watt 1970, Corrier et al 1984), *Coccidioides immitis* (DeMartini & Riddle 1969, Hodgkin et al 1984), *Rhinosporidium seeberi* (Myers et al 1964), *Conidiobolus coronatus* (*Entomophthora coronata*) (Bridges et al 1962, Hanselka 1977, Zamos et al 1996), *Conidiobolus lamprauges* (Humber et al 1989) and *Hyphomyces destruens* (Hutchins & Johnston 1972). Such mycotic granulomas are characterized by the presence of necrotic foci or "kunkers" within proliferative granulation tissue. Nasal infections by these lesions are described in detail in Chapter 25.

Sinus mycosis has also been reported secondary to other intrasinus lesions such as progressive ethmoidal hematoma and can also occur following sinus surgery for other diseases such as progressive ethmoidal hematoma, sinus cysts or following head trauma (McGorum & Dixon 1992, Tremaine & Dixon 2001a).

Mycotic sinus infections commonly cause a unilateral nasal discharge, which may vary from mucopurulent, purulent to sanguineous, and is frequently malodorous (McGorum et al 1992, Tremaine & Dixon 2001a).

The treatment of superficial mycotic lesions with antimycotic drugs including nystatin (Campbell & Peyton 1984), enilconazole or natamycin (McGorum et al 1992) by topical application directly or via an endoscope carries a good prognosis although recurrence is possible. Surgical removal of large intrasinus fungal granulomas or plaques or of any underlying cause such as sequestra, cysts or progressive ethmoidal hematoma lesions, followed by sinus irrigation with a topical antifungal such as natamycin or miconazole, usually results in rapid resolution of the lesions.

Halicephalobus gingivalis Infection

Halicephalobus gingivalis is a saprophytic nematode found in decaying humus and infection through an unknown route can involve the sinuses, central nervous system, and, to a lesser extent, the kidney in certain geographical regions (Pearce at al 2001). Infection of the sinuses produces a mass of gray–yellow fibrous tissue that obliterates the sinuses and their walls, loosens teeth and distorts sinus

architecture. Infection can be unilateral or bilateral, can involve both the upper and lower jaws, and can spread from there to the kidneys and cerebellum (Freeman 1991a).

Predominant clinical signs of *H. gingivalis* infection are facial distortion with firm swellings in the maxilla, unilateral or bilateral nasal discharge, marked dyspnea and stridor, difficulty in eating, and weight loss (Pearce et al 2001). The condition can be confused with squamous cell carcinoma but the female rhabditiform nematodes and their larvae and eggs can be seen in clusters or scattered throughout a biopsy specimen. Surgical debulking, intra-operative lavage with ivermectin, and subsequent oral ivermectin was successful in one horse with a periorbital granuloma (Freeman 1991a). However, the response to ivermectin is not always favorable and the prognosis appears to be poor, especially because of risk of spread to other organs.

Sinus Cysts

Sinus cysts are expansive fluid-filled space-occupying lesions which develop within the sinuses (Leyland & Baker 1975, Dixon 1985, Lane et al 1987) of young to old horses. Congenital intrasinus cysts have also been reported (Sanders-Shamis & Robertson 1987, Beard et al 1990). Equine sinus cysts most commonly occur in the maxillary sinuses but they can also occur in the other sinuses.

The etiology of these lesions is unclear and no breed or sex predisposition has been identified. It has been suggested that they are developmental in origin (Beard et al 1990), or associated with dental tissues (Boulton 1985), but little evidence for this theory has been found, although one case described by Dixon (1985) was attached to dental alveoli. A common etiology between these lesions and ethmoid hematomas has been suggested (Lane et al 1987) as both lesions histologically contain areas of hemorrhage and hemosiderophages, but little factual evidence for this association has been found (Tremaine et al 1999). Sinus cysts are frequently associated with a nasal discharge and facial swelling (Fig. 26.13). The nasal discharge varies from mucoid, mucopurulent to purulent, and is thought to be the result of sinus infection secondary to obstruction of normal sinonasal drainage. A consistent clinical feature caused by the expansive nature of sinus cysts is distortion of the frontal, maxillary, and conchal bones (Lane et al 1987, Caron 1991, Freeman 1991b, Tremaine & Dixon 2001a). This may result in gross facial swelling and exophthalmos as a result of thinning of the overlying maxillary or frontal bones, and nasal obstruction as a result of the expansion of the lesion within the sinuses and conchae. Horses are affected unilaterally in almost all cases, but expansion of a frontal sinus cyst with lysis of the intersinus septum and expansion into the contralateral frontal sinus, resulting in bilateral clinical signs, can occur (H. Tremaine, personal observations). Large maxillary

sinus cysts can expand into the nasal cavity, causing compression of the nasal septum and bilateral nasal air-flow obstruction.

Diagnosis of sinus cysts is assisted by endoscopy, which may reveal distortion of nasal conchae. Radiographic features of sinus cysts include the presence of a rounded, expansive, soft tissue density lesion in the frontal or maxillary sinuses. Distortion and thinning of the surrounding bones may be evident as the lesion increases in size, and secondary distortion of adjacent dental apices within the sinuses may be present. The contents of the cysts frequently appear radiographically as a homogeneous soft tissue density shadow. The radiodense capsule may contain spicules of mineralized tissue (Fig. 26.14) and extralesional fluid lines may be present if secondary sinus empyema is present (Tremaine & Dixon 2001a). Centesis of the lesion via needle aspiration (e.g. using a 16-gauge needle inserted into areas of thinned, swollen bone) or via a sinusotomy is diagnostic, yielding a viscous, usually sterile, translucent yellow fluid which is odorless and may contain some leukocytes (Dixon 1985, Lane et al 1987, Tremaine & Dixon 2001a, Beard & Hardy 2003). Treatment of the

Fig. 26.13. The large swelling of the left side of this 8-year-old horse's rostral maxillary area (arrows) is the result of bone remodeling in response to an expanding cyst within the maxillary sinuses.
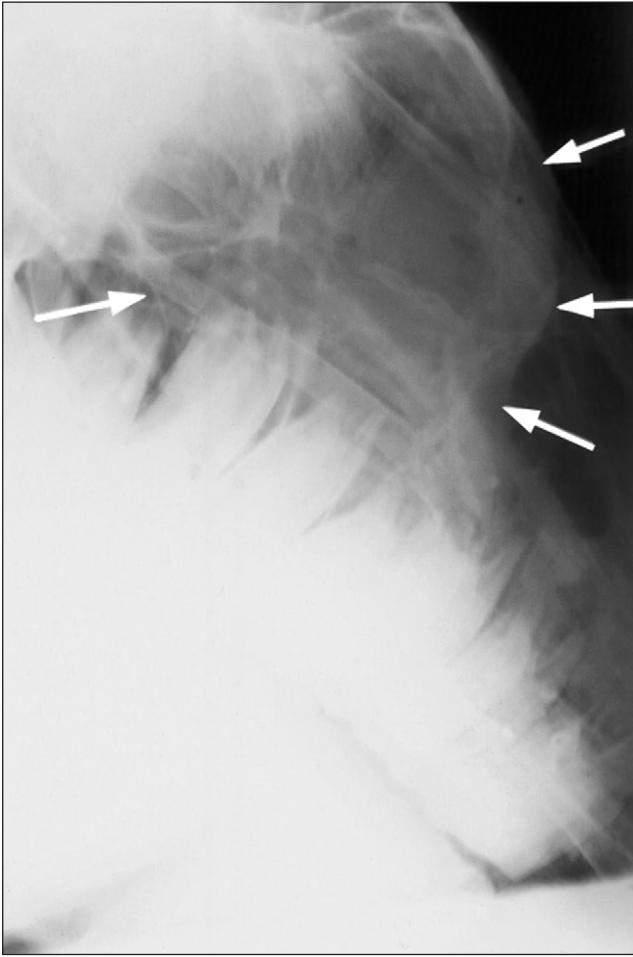
Fig. 26.14. Radiograph showing distortion of the sinuses as the result of a sinus cyst with an increased soft tissue density radio-opacity (arrows) throughout the sinus.

lesion by surgical drainage may be effective in some cases (O'Connor 1930, Dixon 1985, Lane et al 1987) but total removal of the lesion via a nasofrontal or maxillary osteotomy approach, under general anesthesia or standing chemical restraint, is the treatment of choice (Fig. 26.15) (Dixon 1985, Lane et al 1987, Tremaine & Dixon 2001b).

Histologic examination of sinus cysts has revealed extensive resorption and remodeling of the bones surrounding the cyst, replacement of the normal bony septa within the sinus by fibrous tissue, and replacement of the loose intrasinus connective tissue with bony spicules (Tremaine et al 1999). The cysts themselves are lined by ciliated columnar respiratory epithelium with focal areas of ulceration, areas of submucosal calcification and of subepithelial hemorrhage, and chronic inflammation may be present (Lane et al 1987, Tremaine et al 1999).

Progressive Ethmoidal Hematoma

Progressive ethmoidal hematomas are observed most commonly in the nasal cavity arising from the ethmo-turbinates. Less commonly, lesions arise in the frontal or maxillary sinuses. The etiology, clinical signs, and treatment of these lesions are discussed in Chapter 27. Cases with clinical signs typical of progressive ethmoidal hematoma (i.e. low-grade chronic, unilateral epistaxis) and with endoscopic evidence of drainage of small volumes of blood from the sinonasal drainage areas and which do not reveal a lesion in the nasal cavities should be subjected to careful examination of the sinuses by radiography, sinoscopy or sinusotomy (Fig. 26.16).
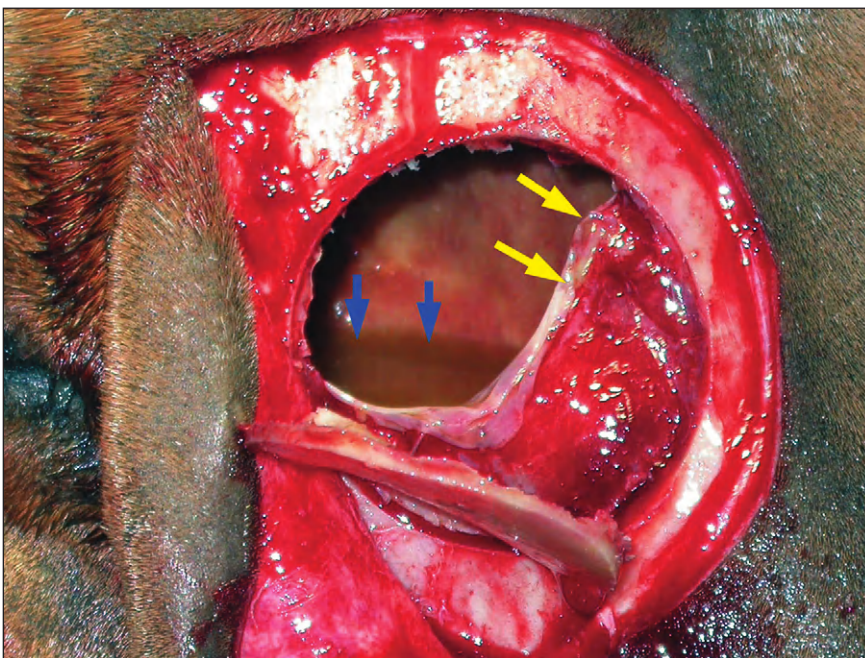
Fig. 26.15. Frontal sinus bone flap osteotomy showing a partially removed sinus cyst wall (yellow arrows) with a residual pool of honey-colored exudates (blue arrow) typical of this type of lesion.

Sinus Neoplasia

Neoplasia of the nasal and paranasal sinuses is a relatively rare condition in the horse (Cotchin 1956, Madewell et al 1976, Sundbergh et al 1977, Priester & Mackay 1980) and there are only a few multiple case studies of equine sinus neoplasia (Cotchin 1967, Madewell et al 1976, Stunzi & Hauser 1976, Hilbert et al 1988, Dixon & Head 1999).

Although the sinuses are lined by ciliated respiratory mucosa, squamous cell carcinomas are probably the most common sinus neoplasia (Head & Dixon 1999). These lesions are usually direct extensions of lesions originating in the oral cavity (usually the lateral aspects of the hard palate) or from metaplastic epithelium within the sinuses themselves (Reynolds et al 1979, Hill et al 1989, Head & Dixon 1999). They display rapid local expansion and induce considerable necrosis of adjacent tissue.

Other tumor types recorded with paranasal sinus involvement include spindle cell sarcoma, mastocytomas, hemangiosarcoma, angiosarcoma and lymphosarcoma (Lane 1985, Adams et al 1988, Richardson et al 1994, Malikides et al 1996, Dixon & Head 1999).

A group of fibro-osseous lesions, often of overlapping histologic classification, have been reported in the paranasal sinuses of horses. These include osteomas, which have been found in the frontal and maxillary sinuses (Gorlin et al 1963, Schumacher et al 1988, Dixon & Head 1999), osteochondromas (Adair et al 1994), fibromas (Barber et al 1983) and fibrosarcomas (Hultgren et al 1987, Dixon & Head 1999). Tumors of dental tissue origin with involvement of the maxillary sinuses have been reported, although such neoplasms more frequently affect the mandibular or rostral maxillary cheek teeth (Pirie & Dixon 1993) and such lesions, although more common in older animals, have been described in foals (Roberts et al 1978).

Clinical signs associated with neoplasia are similar to those of other expansive lesions affecting the paranasal sinuses and include nasal discharge (purulent or muco-purulent, occasionally hemorrhagic), facial swelling (Fig. 26.17), epiphora, and nasal obstruction. However, as a consequence of the large space into which sinus lesions can expand, facial swelling and other signs may be absent until an advanced stage. Head shaking, exophthalmos, and
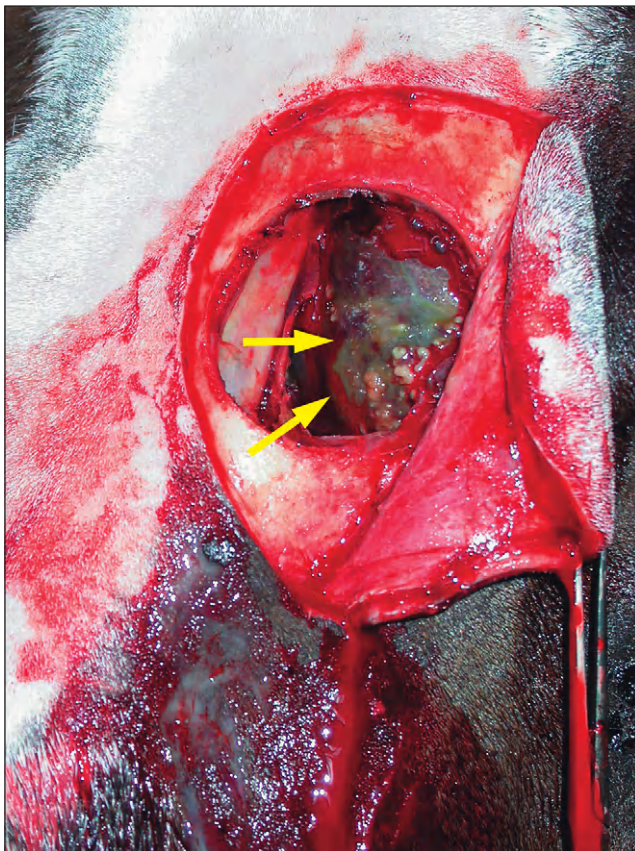
Fig. 26.16. Nasofrontal sinus bone flap surgery showing an intrasinus progressive ethmoidal hematoma (arrows), which was not detectable on nasal endoscopy and which is covered by inspissated pus.

Fig. 26.17. This pony has a rapidly expanding maxillary tumor, which caused loosening and secondary apical infections of the adjacent maxillary cheek teeth.

epistaxis are less commonly observed (Hill et al 1989, Tremaine & Dixon 2001a).

The diagnosis of intrasinus neoplasia requires, as for other sinus lesions, clinical and oral examination, radiography, sinoscopy, and possibly scintigraphy and computed tomography. Wherever possible, histopathology of biopsy specimens should be performed to confirm the diagnosis and help establish a prognosis.

Surgical resection of benign lesions, such as osteomas, via a nasofrontal flap, may carry a good long-term prognosis (Schumacher et al 1988, Head & Dixon 1999, Tremaine & Dixon 2001b). However, the aggressive nature and the complex anatomical location of most sinonasal tumors usually prevent complete resection and consequently, a poor prognosis is present following surgical treatment of these lesions (Dixon & Head 1999). Exceptions include osteomas which are usually amenable to treatment because they are benign (some may not even be true neoplasms but hamartomas), grow slowly, have pedunculated or sessile attachments over a small base, and tend to form well-circumscribed lesions rather than infiltrate (Freeman 1991b).

Beta-radiotherapy with cobalt-60 has been attempted with limited success for soft tissue sinus neoplasms. In one report, the results of aggressive radiotherapy of advanced squamous cell carcinomas in three horses was encouraging, because radiation-induced complications were mild, and survival duration and quality of life were good (Walker et al 1998).

Traumatic Injuries of the Paranasal Sinuses

Fractures involving the premaxilla are common in foals (Hardy 1991) and depression fractures of the frontal and maxillary sinuses have been commonly reported in adult horses (Sullins & Turner 1982, Tremaine & Dixon 2001a). Traumatic hemorrhage into the sinuses may lead to a profuse short-term epistaxis, which is often followed by an unexpectedly prolonged (>4 weeks) intermittent low-grade epistaxis. Open sinus fractures frequently lead to secondary sinusitis (Dixon 1993a), and the presence of intrasinus sequestra may result in chronic suppuration with persistent sinusitis (Lane 1993). Repair of these fractures is possible by elevating the depressed bone flap (Fig. 26.18) and, if it is unstable once elevated, immobilizing it in the reduced position with stainless steel wires. To facilitate elevation of the fracture fragments, holes can be drilled in adjacent undamaged bone and a periosteal elevator, Steinmann pins, or Langenbeck retractors can be passed through these to pry up depressed fragments. If the elevated fragments wedge firmly together in their normal position and form a stable union it may be unnecessary to wire them (Turner 1979), but large

Fig. 26.18. This figure shows a horse with a large depressed maxillary fracture (arrow on fixed side of depressed fracture) undergoing surgical repair of this injury. Fractured bones such as this can be elevated and stabilized with wire with a good cosmetic outcome.

fragments should be wired to stable adjacent bone. The fracture fragments can also be exposed through a large curvilinear skin flap, especially if an open fracture is present and intrasinus access is required. Blood clots and loose bone fragments are removed and the sinus cavity is flushed liberally with saline. All small fragments without full periosteal attachments should also be removed. Following repair of the bone and skin wounds, the head should be bandaged so as to cover the wound, if possible, and the horse should be recovered from general anesthesia either with assistance or wearing a padded headguard. Healing after repair of sinus injuries is usually excellent, particularly if the skin remains intact (Tremaine 2004b) although suture exostoses may remain.

In horses with long-standing, healed depression fractures, fluorocarbon polymer and carbon fiber can be used to restore the facial contour (Valdez & Rook 1981), or the healed maligned areas can be cut with a bone saw, elevated and then wired into a more anatomically normal position. However, a better cosmetic appearance can be obtained by primary open reduction of such large depressed fractures shortly after injury, rather than by facial reconstruction later. If severe or open sinus fractures are not treated, complications such as sinusitis, sequestra formation, facial deformity, abnormal bone growth in young horses, and nasal obstruction can be expected.

Nasofrontal Suture Exostoses

Swellings of the nasofrontal region of the head as a result of periostitis of the suture lines between the nasal and frontal bones, and more rarely the nasal, lacrimal,

and malar bones have been described (Gibbs & Lane 1987, Speirs 1992, Trotter 1993, Tremaine & Dixon 2001a). They occur in many breeds but the incidence appears to be particularly high in thoroughbreds and thoroughbred crosses (Dixon 1991). Although most are possibly traumatic in origin, including following sinonasal surgery, especially after a large nasofrontal osteotomy, the exact etiology of such lesions remains unknown in other cases. Affected horses present with bilateral, firm, non-painful swellings, rostral to the eye, accompanied by epiphora in some cases. Differentiation from facial fractures and sinusitis is usually possible clinically and radiologically. Radiographs frequently demonstrate proliferative periosteal changes of the widened and incompletely closed suture line. The swellings usually remodel and regress gradually without treatment, but in some cases continued instability has resulted in progressive increases in the size of these swellings.

Miscellaneous Sinus Disorders

Frontal sinus eversion is probably a congenital defect that forms a hard, slow-growing protuberance over, and communicating with, the frontal sinus (Martin & McIlwraith 1981). The bony protuberance can be removed through a large elliptical incision and the resulting defect in the frontal bone can be repaired with synthetic polypropylene mesh (Marlex) and skin.

Osteodystrophia fibrosa or secondary nutritional hyperparathyroidism can develop in horses on a high phosphorus diet, such as bran, or on some tropical grasses (Clarke et al 1996) and can be attributed to relative calcium deficiency (Freeman 1991b). It is rare under modern management conditions. Conchal necrosis (De Moor & Verschooten 1982) may be caused by advanced mycotic rhinitis (Tremaine & Dixon 2001b) that usually responds to removal of the affected concha by intranasal curettage and lavage.

The reserve tooth crowns of young (2- to 4-year-old) Welsh and miniature ponies and other smaller pony breeds can project a considerable distance into the nasal and sinus cavities and cause firm, painless, bilateral swellings in the maxillary bones that should not be confused with injuries or disease. Facial lumps or "horns" can be seen in horses as symmetrical painless prominences of the nasal and frontal bones and possibly are caused by an embryologic fault.

REFERENCES

Adair HS, Duncan RB, Toal RL 1994 Solitary osteochondroma of the nasal bone in a horse. Cornell Veterinarian 84: 25–31

Adams R, Calderwood-Mays MB, Peyton LC 1988 Malignant lymphoma in three horses with ulcerative pharyngitis. Journal of the American Veterinary Medical Association 193: 674–676

Barber SM, Clark EG, Fretz PB 1983 Fibroblastic tumour of the premaxilla in two horses. Journal of the American Veterinary Medical Association 182: 700–702

Beard WL, Hardy J 2003 Diagnosis of conditions of the paranasal sinuses of the horse. Equine Veterinary Education 13: 265–273

Beard WL, Robertson JT, Leeth B 1990 Bilateral congenital cysts in the frontal sinus of a horse. Journal of the American Veterinary Medical Association 196: 453–454

Boulton CH 1985 Equine nasal cavity and paranasal sinus disease: a review of 85 cases. Journal of Equine Veterinary Science 5: 268–275

Bridges CH, Romane WM, Emmons CW 1962 Phycomycosis of horses caused by *Entomophthora coronata*. Journal of the American Veterinary Medical Association 140: 673–677

Campbell ML, Peyton LC 1984 Muscle flap closure of a frontocutaneous fistula in a horse. Veterinary Surgery 13: 185–188

Caron JP 1991 Diseases of the nasal cavity and paranasal sinuses. In: Colahan PT, Mayhew IG, Merritt AM, Moore JN (editors) Equine Medicine and Surgery. American Veterinary Publications, Goleta, CA, pp.386–397

Clarke CJ, Roeder PL, Dixon PM 1996 Nasal obstruction caused by nutritional osteodystrophia fibrosa in a group of Ethiopian horses. Veterinary Record 139: 568–570

Corrier DE, Wison SR, Scrutchfield WL 1984 Equine cryptococcal rhinitis. Compendium on Continuing Education for the Practicing Veterinarian 6: 556–558.

Cotchin E 1956 Neoplasms of the Domesticated Animals. Commonwealth Agricultural Bureaux, Buckingham, UK

Cotchin E 1967 Spontaneous neoplasms of the upper respiratory tract in animals In: Muir CS, Shanmugaratnam K (editors) Cancer of the Naso-Pharynx. Medical Examination Publishing Co, Flushing, NY, pp.203–259

Coumbe KM, Jones RD, Kenward JH 1987 Bilateral sinus empyema in a six year-old mare. Equine Veterinary Journal 19: 559–560

Dacre IT 2004 A pathological, histological and ultrastructural study of diseased equine cheek teeth. PhD thesis, University of Edinburgh

DeMartini JC, Riddle WE 1969 Disseminated coccidiomycosis in two horses and a pony. Journal of the American Veterinary Medical Association 155: 149–156

De Moor Von A, Verschooten F 1982 Empyem und Nekrose der Nasenmuscheln beim Pferd (Empyema and necrosis of the nasal conchae in a horse). Deutsche Tierarztliche Wochenschrift 89: 275–281

Dixon PM 1985 Equine maxillary cysts. Equine Practice 7: 25–33

Dixon PM 1991 Swellings of the head region in the horse. In Practice 13: 257–263

Dixon PM 1993a Nasal cavity. In: Equine Respiratory Endoscopy. Boehringer Ingelheim, Bracknell, pp.22–29

Dixon PM 1993b Ethmoturbinates. In: Equine Respiratory Endoscopy. Boehringer Ingelheim, Bracknell, pp.43–48

Dixon PM, Head KW 1999 Equine nasal and paranasal sinus tumours: part 2: A contribution of 28 case reports. Veterinary Journal 157: 279–294

Dixon PM, Tremaine WH, Pickles K et al 1999 Equine dental disease Part 2: A long term study of 400 cases: disorders of development and eruption and variations in position of the cheek teeth. Equine Veterinary Journal 31: 519–528

Dixon PM, Tremaine WH, Pickles K et al 2000a Equine dental disease Part 3: A long term study of 400 cases: disorders

of wear, traumatic damage and idiopathic fractures, tumours and miscellaneous disorders of the cheek teeth. Equine Veterinary Journal 32: 9–18

Dixon PM, Tremaine WH, Pickles K et al 2000b Equine dental disease Part 4: A long term study of 400 cases: apical infections of cheek teeth. Equine Veterinary Journal 32: 182–194

Dixon PM, Dacre I, Dacre K et al 2005 Standing oral extraction of cheek teeth in 100 horses (1998–2003). Equine Veterinary Journal 37: 105–112

Freeman DE 1991a Nasal passages. In: Beech J (editor) Equine Respiratory Disorders. Lea and Febiger, Philadelphia, pp.253–274

Freeman DE 1991b Paranasal sinuses In: Beech J (editor) Equine Respiratory Disorders. Lea and Febiger, Philadelphia, pp.275–305

Freeman DE 2003 Sinus disease. Veterinary Clinics of North America; Equine Practice 19: 209–243

Freeman DE, Orsini PG, Ross MW et al 1990 A large fronto-nasal flap for sinus surgery in the horse. Veterinary Surgery 19: 122–130

Gibbs C, Lane JG 1987 Radiographic examination of the nasal and paranasal sinus regions of the horse. Part 2: Radiological findings. Equine Veterinary Journal 19: 474–482

Gorlin RJ, Meskin LH, Brodey R 1963 Odontogenic tumours in man and animals, pathological classification and clinical behaviour – a review. Annals of the New York Academy of Science 108: 722–771

Greet TRC 1981 Nasal aspergillosis in three horses. Veterinary Record 109: 487–489

Hanselka DV 1977 Equine nasal phycomycosis. Veterinary Medicine/Small Animal Clinician 72: 251–253

Hardy J 1991 Upper respiratory obstruction in foals, weanlings, and yearlings. Veterinary Clinics of North America; Equine Practice 7: 105–122

Head KW, Dixon PM 1999 Equine nasal and paranasal sinus tumours. Part 1: review of the literature and tumour classification. Veterinary Journal 157: 261–278

Henninger W, Frame M, Willman M et al 2003 CT features of alveolitis and sinusitis in horses. Veterinary Radiology and Ultrasound 44: 269–276

Hilbert BJ, Little CB, Klein K et al 1988 Tumours of the paranasal sinuses in 16 horses. Australian Veterinary Journal 65: 86–88

Hill FWE, Moulton JE, Schiff PH 1989 Exophthalmos in a horse resulting from an adenocarcinoma of the paranasal sinus. Journal of South African Veterinary Medicine Association 60: 104–105

Hodgkin EC, Conaway DH, Ortenburger AI 1984 Recurrence of obstructive nasal coccidioidal granuloma in a horse. Journal of the American Veterinary Medical Association 184: 339–340

Hultgren BD, Schmotzer WB, Watrous BJ et al 1987 Nasal–maxillary fibrosarcoma in young horses: a light microscopic study. Veterinary Pathology 24: 194–196

Humber RA, Brown CC, Korngay RW 1989 Equine zygomycosis caused by *Conidiobolus lamprauges*. Journal of Clinical Microbiology 27: 573–576

Hutchins DR, Johnston KG 1972 Phycomycosis in the horse. Australian Veterinary Journal 48: 269–277

Johnson GR, Schiefer B, Pantekoek JFCA 1975 Maduromycosis in a horse in Western Canada. Canadian Veterinary Journal 16: 341–344

Lane JG 1985 Palatine lymphosarcoma in two horses. Equine Veterinary Journal 17: 465–467

Lane JG 1993 Management of sinus disorders, part 1. Equine Veterinary Education 5: 5–9

Lane JG 1994 A review of dental disorders of the horse, their treatment and possible fresh approaches to management. Equine Veterinary Education 6: 13–21

Lane JG, Longstaffe JA, Gibbs C 1987 Equine paranasal sinus cysts: a report of 15 cases. Equine Veterinary Journal 19: 534–544

Leyland A, Baker JR 1975 Lesions of the nasal and paranasal sinuses of the horse causing dyspnoea. British Veterinary Journal 131: 339–346

Mackintosh ME, Colles CM 1987 Anaerobic bacteria associated with abscesses in the horse and donkey. Equine Veterinary Journal 19: 360–362

Madewell BR, Priester WA, Gillete EL et al 1976 Neoplasms of the nasal passages and paranasal sinuses in domestic animals as reported by 13 veterinary colleges. American Journal of Veterinary Research 37: 851–856

Malikides N, Reppas G, Hodgson JL et al 1996 Mast cell tumours in the horse: four case reports. Equine Practice 18: 12–17

Mansmann RA, Wheat JD 1973 The diagnosis and treatment of equine upper respiratory diseases. In: Proceedings of the 18th Annual Convention of the American Association of Equine Practitioners. Lexington, KY, pp.388–487

Martin GS, McIlwraith CW 1981 Repair of a frontal sinus eversion in a horse. Veterinary Surgery 10: 149

Mason BJE 1975a Empyema of the equine paranasal sinuses. Journal of the American Veterinary Medical Association 167: 727–731

Mason BJE 1975b Spindle-cell sarcoma of the equine para-nasal sinuses and nasal chamber. Veterinary Record 96: 287–288

McGorum BC, Dixon PM, Lawson GHK 1992 A review of ten cases of mycotic rhinitis. Equine Veterinary Education 4: 8–12

Meschter CL, Allen D 1984 Lymphosarcoma within the nasal cavities of an 18 month old filly. Equine Veterinary Journal 16: 475–476

Morrow K, Park RD, Spurgeon, TL et al 2000 Computed tomography of the equine head. Veterinary Radiology and Ultrasound 41: 491–497

Myers DD, Simon J, Case MT 1964 Rhinosporidiosis in a horse. Journal of the American Veterinary Medical Association 145: 345–346

O'Connor JJ 1930 Operations. Dollar's Veterinary Surgery. Bailliere, Tindall and Cox, London, pp.222–231

Pearce SG, Bouré LP, Taylor JA et al 2001 Treatment of a granuloma caused by *Halicephalobus gingivalis* in a horse. Journal of the American Veterinary Medical Association 219: 1735–1738

Pirie RS, Dixon PM 1993 Mandibular tumours in the horse: a review of the literature and 7 case reports. Equine Veterinary Education 5: 287–294

Priester WA, Mackay FW 1980 The occurrence of tumors in domestic animals. National Cancer Institute Monograph 54: 59–99

Quinn G, Lane JG, Kidd JF 2005 Fronto-nasal flap surgery in standing horses. Equine Veterinary Journal 37: 138–142

Reed SM, Boles CL, Dade AW et al 1979 Localised equine nasal coccidiomycosis granuloma. Journal of Equine Medicine and Surgery 3: 119–123

Reynolds BL, Stedham MA, Lawrence JM et al 1979 Adeno-carcinoma of the frontal sinus with extension to the brain in a horse. Journal of the American Veterinary Medical Association 174: 734–736

Richardson JD, Lane JG, Nicholls PK 1994 Nasopharyngeal mast cell tumour in a horse. Veterinary Record 134: 238–240

Roberts MC, Groenendyk S, Kelly WR 1978 Ameloblastic odontoma in a foal. Equine Veterinary Journal 10: 91–93

Ruggles AJ, Ross MW, Freeman DE 1991 Endoscopic examination of normal paranasal sinuses in horses. Veterinary Surgery 20: 418–423

Ruggles AJ, Ross MW, Freeman DE 1993 Endoscopic examination and treatment of paranasal sinus disease in 16 horses. Veterinary Surgery 22: 508–514

Sanders-Shamis M, Robertson JT 1987 Congenital sinus cyst in a foal. Journal of the American Veterinary Medical Association 190: 1011–1012

Schumacher J, Crossland LE 1994 Removal of inspissated purulent exudate from the ventral conchal sinus of three standing horses. Journal of the American Veterinary Medical Association 205: 1312–1314

Schumacher J, Honnas C, Smith B 1987 Paranasal sinusitis complicated by inspissated exudate in the ventral conchal sinus. Veterinary Surgery 16: 373–377

Schumacher J, Smith BL, Morgan SJ 1988 Osteoma of paranasal sinuses of a horse. Journal of the American Veterinary Medical Association 192: 1449–1450

Scott EA 1987 Sinusitis. In: Robinson NE (editor) Current Therapy in Equine Medicine 2. WB Saunders, Philadelphia, pp.605–607

Scrutchfield WL, Schumacher J, Walker M et al 1994 Removal of an osteoma from the paranasal sinuses of a standing horse. Equine Practice 16: 24–27

Speirs VC 1992 Diseases of the paranasal sinuses. In: Robinson NE (editor) Current Therapy in Equine Medicine 3. WB Saunders, Philadelphia, pp.217–224

Stunzi H, Hauser B 1976 Tumours of the nasal cavity (International histological classification of tumours of domestic animals). Bulletin of the World Health Organisation 53: 257–263

Sullins KE, Turner AS 1982 Management of fractures of the equine mandible and premaxilla (Incisive bone). Compendium on Continuing Education for the Practicing Veterinarian 4: S480–S489

Sundbergh JP, Burustein T, Page EH et al 1977 Neoplasms of equidae. Journal of the American Veterinary Medical Association 170: 150–152

Taylor J 1955 The horse. The Head and Neck. Oliver & Boyd, London

Tietje S, Becker M, Bockenhoff G 1998 Computed tomographic evaluation of head diseases in the horse: 15 cases. Equine Veterinary Journal 28: 98–105

Tremaine WH 2004a The management of head fractures in horses. In Practice 26: 142–149

Tremaine WH 2004b Oral extraction of equine cheek teeth. Equine Veterinary Education 16: 151–159

Tremaine WH, Dixon PM 2001a A long-term study of 277 cases of equine sinonasal disease. Part 1: details of horses, historical, clinical and ancillary diagnostic findings. Equine Veterinary Journal 33: 274–282

Tremaine WH, Dixon PM 2001b A long-term study of 277 cases of equine sinonasal disease. Part 2: treatments and results of treatments. Equine Veterinary Journal 33: 283–289

Tremaine WH, Clarke CJ, Dixon PM 1999 Histopathological findings in equine sinonasal disorders. Equine Veterinary Journal 31: 296–303

Trotter GW 1993 Paranasal sinuses. Veterinary Clinics of North America; Equine Practice 9: 153–169

Tulleners EP, Raker C 1983 Nasal septum resection in the horse. Veterinary Surgery 12: 41–47

Turner AS 1979 Surgical management of depression fractures of the equine skull. Veterinary Surgery 8: 29

Valdez H, Rook JS 1981 Use of fluorocarbon polymer and carbon fiber for restoration of facial contour in a horse. Journal of the American Veterinary Medical Association 178: 249–251

van der Velden MA, Verzijlenberg K 1984 Chronic purulent maxillary sinusitis in horses. Tijdschrift voor Dergeneesekunde 109: 793–799

Walker MA, Schumacher J, Schmitz DG et al 1998 Cobalt 60 radiotherapy for treatment of squamous cell carcinoma of the nasal cavity and paranasal sinuses in three horses. Journal of the American Veterinary Medical Association 212: 848–851

Watt DA 1970 A case of cyptococcal granuloma in the nasal cavity of a horse. Australian Veterinary Journal 46: 493–494

Weller R, Livesey L, Maierl J et al 2001 Comparison of radiography and scintigraphy in the diagnosis of dental disorders in the horse. Equine Veterinary Journal 33: 49–58

Zamos DT, Schumacher J, Loy JK 1996 Nasopharyngeal conidiobolomycosis in a horse. Journal of the American Veterinary Medical Association 208: 100–101

27 | Progressive Ethmoidal Hematoma

Jim Schumacher and Padraic M Dixon

Progressive ethmoidal hematoma (PEH), first described by Cook & Littlewort (1974), is an idiopathic, slowly expanding, non-neoplastic mass found in the nasal cavity or paranasal sinuses of horses; it has been described as a hemorrhagic polyp (Head & Dixon 1999). The mass usually originates from the submucosa of one of the larger ethmoturbinates (i.e. endoturbinates 1–5) (Figs 27.1 and 27.2) (Cook & Littlewort 1974, Boles 1979, Specht et al 1990, Bell et al 1993a), and the most ventral aspect of the ethmoidal labyrinth, which protrudes into the caudal portion of the nasal cavity, is the most common site of a PEH (Bell et al 1993a, Head & Dixon 1999) (Figs 27.3 and 27.4). Less commonly, lesions arising from the sinusal (intrasinus) portion of the ethmoidal labyrinth grow into the paranasal sinuses, especially the maxillary or frontal sinuses. PEH lesions may occasionally arise from the submucosa of areas of the paranasal sinuses other than the ethmoidal labyrinth (Cook & Littlewort 1974, Sullivan et al 1984, Freeman et al 1990, Specht et al 1990, Greet 1992, Bell et al 1993a), but Cook & Littlewort (1974) surmised that only PEHs that arise from the ethmoidal labyrinth are capable of achieving a large size.

Recurrent hemorrhage and the development of a large fibrous skeleton can cause the PEH to expand along the lines of least resistance into the ipsilateral nasal passage (Fig. 27.4), nasopharynx or paranasal sinuses, inducing varying degrees of pressure (often minimal) on adjacent tissues. The cause of PEH is unknown, but the disease may originate from a congenital or acquired hemangiomatous lesion, which predisposes to repeated hemorrhage into the submucosa of the ethmoidal labyrinth (Platt 1975). The blood supply to the ethmoid region is complex with a dual arterial supply present in the ethmoid labyrinth (Bell et al 1995), resulting in a complex network of vessels. However, so far, the description of PEH as a primary vascular lesion remains speculative (Tremaine et al 1999).

Prevalence

The prevalence of PEH in the general equine population is unknown, but has been recorded as approximately one horse per 2,500 (0.04%) at referral hospitals (Boulton 1985, Bell et al 1993b), and horses with PEH represent about 8% of referral horses with disease of the nasal passages and paranasal sinuses (Tremaine & Dixon 2001b).

Signalment

PEH has been reported to occur in most breeds except standardbreds, with thoroughbred and Arabian horses most commonly affected (Bell et al 1993a). There is no apparent gender difference in prevalence of PEH (Bell et al 1993a); however, females may be more likely to be bilaterally affected than males (Rothaug & Tulleners 1999). Horses of any age may develop PEH, but horses less
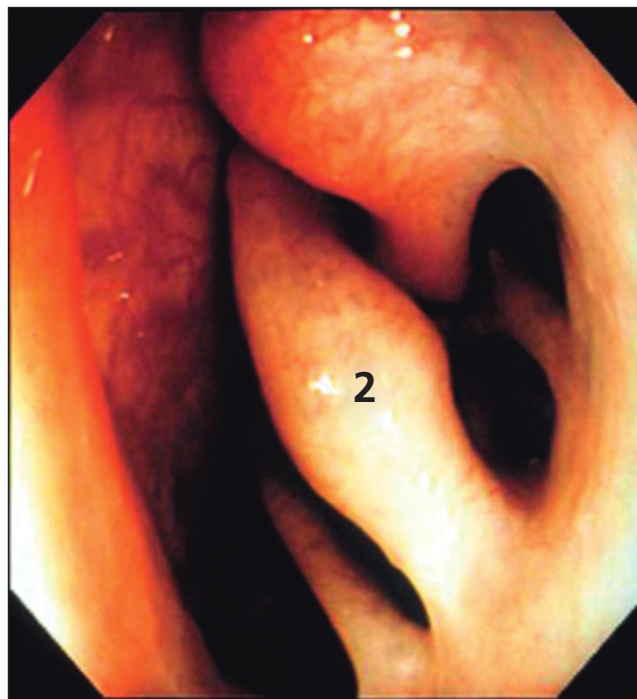
Fig. 27.1. Nasal endoscopic view of the normal equine ethmoturbinates. Note the varying size of the individual endoturbinates (ethmoturbinates), with endoturbinate number two (2) being largest. In other normal horses endoturbinate number two can be two to three times this diameter.
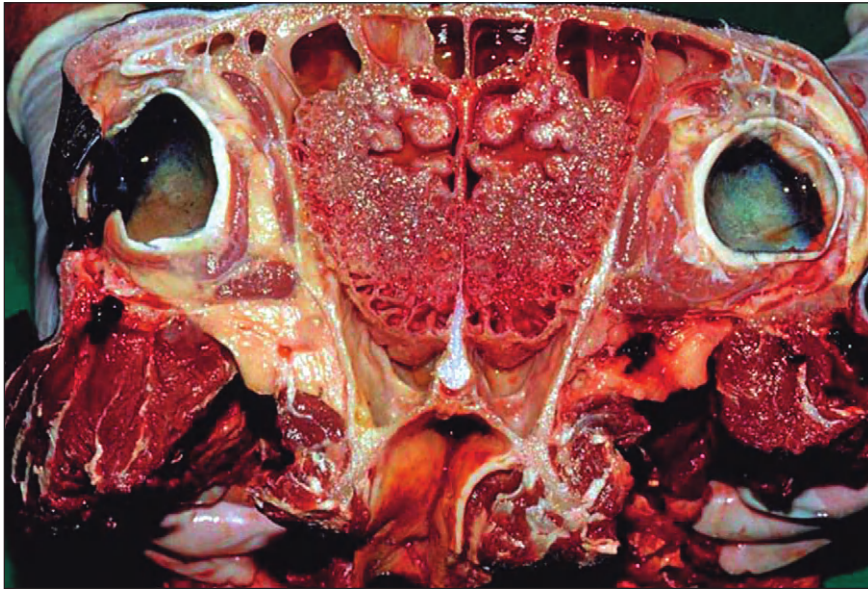
Fig. 27.2. Transverse section of a normal equine skull at the level of the ethmoturbinates showing the tightly coiled endoturbinates, vertical septum between left and right ethmoturbinates, and the overlying conchofrontal sinuses. Note the cavities (small sinuses) present within the larger, ventrally situated endoturbinates.
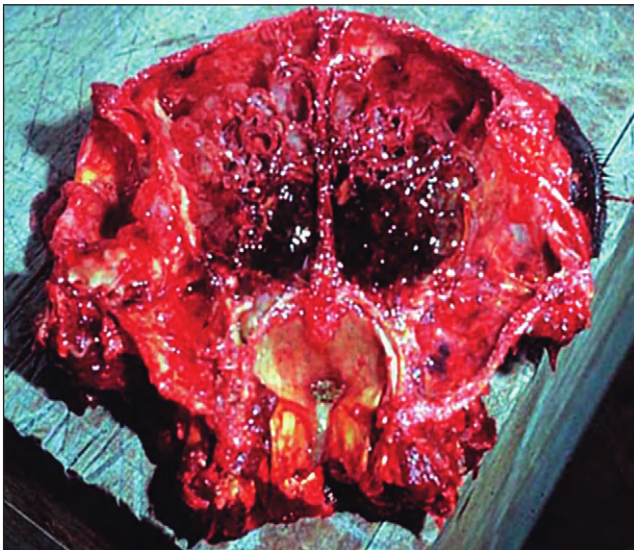
Fig. 27.3. Transverse section of a skull of a horse suffering from bilateral PEH. Most of the ethmoturbinates on the left side have been replaced by a large PEH lesion, whilst on the right side, the ventromedial aspect of the ethmoturbinates is similarly affected.
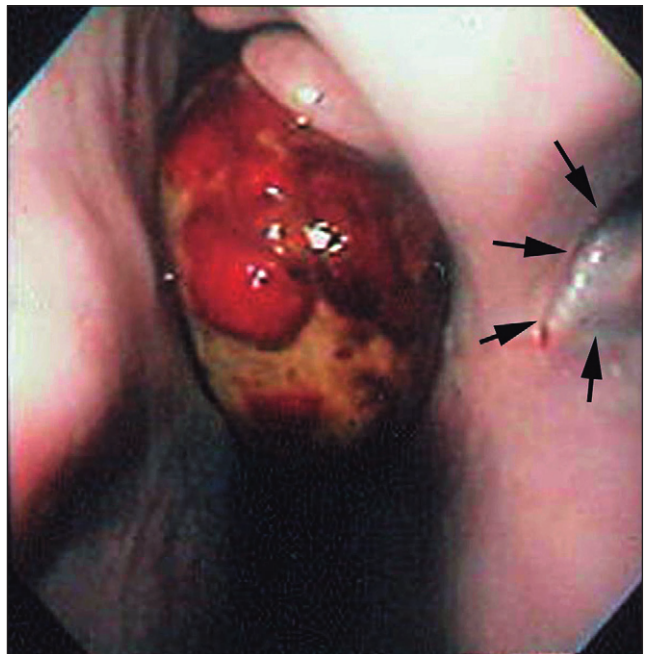
Fig. 27.4. A PEH viewed on endoscopy of the left nasal cavity. This PEH is multicolored with focal areas of bright red, indicating areas of recent hemorrhage. The brown–green areas are caused by hemosiderin within the PEH, at sites where older hemorrhages have occurred. The caudal aspect of the middle meatus ("drainage angle") is indicated by arrows.

than 3 years old are seldom affected although a case of neonatal PEH has been reported (Colbourne et al 1997). The average age of affected horses is about 10 years (Platt 1975, Bell et al 1993a, Schumacher et al 1997, Rothaug & Tulleners 1999, Tremaine & Dixon 2001a).

Clinical Signs

Small PEH lesions may be subclinical (Laing & Hutchins 1992, Schumacher et al 1997, Rothaug & Tulleners 1999), because the limited amount of hemorrhage from them can be accommodated by the normal, caudally directed mucociliary flow and then swallowed. The most common clinical sign caused by a PEH is a chronic, intermittent, low-volume, hemorrhagic or serohemorrhagic discharge from the affected, and sometimes the unaffected, nasal passage, that is caused by ulceration of the mucosa covering the lesion (Cook & Littlewort 1974, Greet 1992, Behrens et al 1993, Bell et al 1993a, Schumacher et al 1997, Tremaine & Dixon 2001a). The nasal discharge is usually spontaneous but rarely may be induced by exercise (Cook & Littlewort 1974, Behrens et al 1993). PEH is the most common cause of low-grade (perhaps a few drops per day), chronic (possibly of many months duration) unilateral epistaxis or mucohemorrhagic nasal discharge in horses (Boles 1979, Tremaine & Dixon 2001a). A lesion may rarely cause a purulent nasal discharge as a result of induced local damage to the adjacent tissue, or obstruction of paranasal sinus drainage.

A large PEH within the nasal cavity may reduce airflow through that nasal cavity and so result in stertorous breathing at exercise (Hanselka & Young 1975, Leyland & Baker 1975, Specht et al 1990, Laing & Hutchins 1992, Colbourne et al 1997, Schumacher et al 1997) and rarely, large PEH lesions may be visible at the nostril (Schumacher et al 1997, Frees et al 2001). A PEH originating on the nasal portion of the ethmoidal labyrinth usually expands rostrally into the nasal cavity, but rarely it may expand caudally into the nasopharynx, and even grow around the caudal edge of the nasal septum into the contralateral nasal cavity (Schumacher et al 1997) causing bilateral nasal obstruction that may cause stertorous breathing and even dyspnea at rest. As a lesion within the paranasal sinuses expands, it may push the medial wall of the dorsal and ventral conchal sinuses into the ipsilateral nasal cavity and, less commonly, in the case of a very large intrasinus PEH, it may cause facial deformity. Facial swelling is much less likely with PEH lesions than with sinus cysts or sinonasal neoplasia (Tremaine & Dixon 2001a).

Other reported clinical signs associated with PEH include ipsilateral submandibular lymphadenopathy, head shaking, head shyness, halitosis (as a result of local necrosis of PEH lesions or secondary infection), exophthalmos, episodes of choking and ptyalism (as the result of extension of PEH to the nasopharynx), and low-grade

anemia (Cook & Littlewort 1974, Specht et al 1990, Greet 1992, Laing & Hutchins 1992, Bell et al 1993a, Schumacher et al 1997, Tremaine & Dixon 2001a).

Differential Diagnosis

Other causes of equine epistaxis include mycosis of a guttural pouch, paranasal sinus or nasal cavity, facial and sinus trauma (e.g. maxillary or nasal bone fracture), and rupture of the longus capitus muscles, all of which usually cause more acute and severe hemorrhage (except sinonasal mycosis). Lower grade epistaxis can, however, occur with upper or lower respiratory tract neoplasia, infection of a nasolacrimal duct (dacryohemorrhea), exercise-induced pulmonary hemorrhage, pleuropneumonia, disseminated intravascular coagulation, thrombocytopenia, and nasal amyloidosis (Schumacher et al 1992, Bell et al 1993a). Masses located in a nasal cavity or the paranasal sinuses that may grossly resemble PEH include neoplasms, sinus cysts, polyps, and fungal granulomas, such as those caused by *Cryptococcus neoformans* (Bell et al 1993a, Tremaine & Dixon 2001b).

Diagnosis

PEH can be provisionally diagnosed by its endoscopic appearance of a smooth, red to green mass originating from the nasal portion of the ethmoidal labyrinth (Fig. 27.4) (Schumacher et al 1992). The ethmoidal labyrinth from which the PEH arises may be obscured by the bulk of the PEH. Rarely, the PEH may protrude caudally beneath the ventral border of the vomer bone into the contralateral nasal cavity obscuring the contralateral ethmoidal labyrinth, giving the false impression that the horse has a separate PEH in each nasal cavity. The PEH acquires its color from the hemoglobin underlying its mucosal lining and so may be of varying shades of red, green, gray, and yellow, often with focal differences in coloring, depending on the time of the most recent hemorrhage (which causes red lesions) that later turn to hemosiderin (green-colored) (Platt 1975, Head & Dixon 1999). White, fungal plaques, including those caused by secondary infection with *Aspergillus* spp., may occasionally cover the dorsal surface of the lesion, especially of PEHs within a paranasal sinus (authors' personal observation). Even if clinical signs have been unilateral, both nasal cavities should be examined endoscopically because about 15% of affected horses are affected bilaterally (Bell et al 1993a, Schumacher et al 1997, Rothaug & Tulleners 1999).

A PEH that originates from the sinusal portion of the ethmoidal labyrinth is not seen during nasal endoscopy, unless the PEH protrudes into the nasal cavity through the dorsal or ventral ethmoidal meati (Cook & Littlewort 1974) or through the nasomaxillary aperture (Behrens et al 1993). Hemorrhagic or serohemorrhagic discharge from a
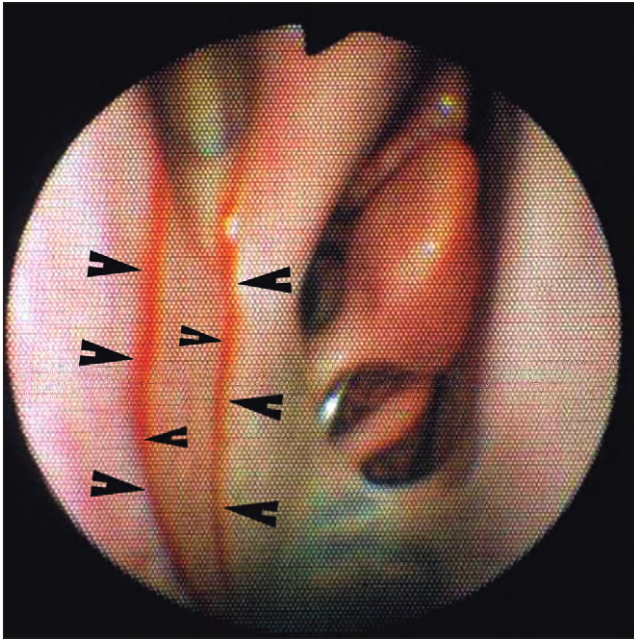
Fig. 27.5. Endoscopy of the caudal aspect of the right nasal cavity of a horse with an intrasinus PEH. Notice the normal morphology of these ethmoturbinates. Two streaks of blood (arrowheads) can be seen flowing from the "drainage angle" of the right maxillary sinus.
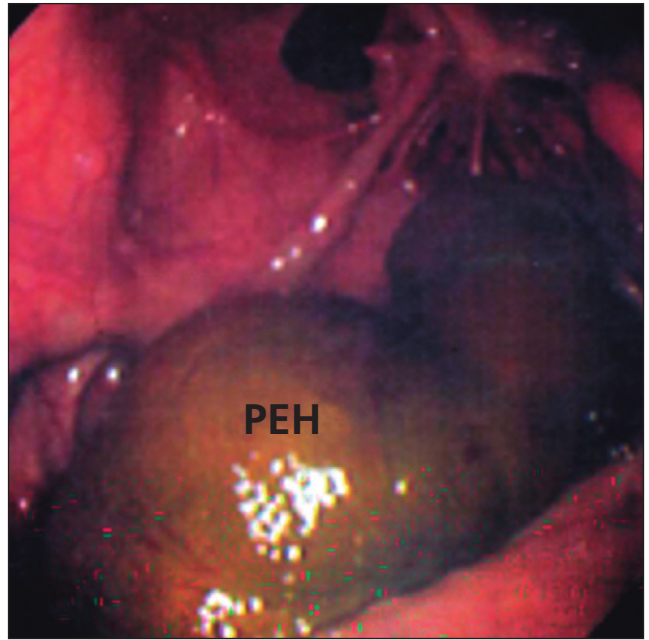
Fig. 27.6. View at sinoscopy of a maxillary sinus showing a large green-tinged PEH within its lumen, with minimal inflammation of the sinus mucosa.

PEH within the paranasal sinuses can sometimes be seen emerging from the ventral or dorsal ethmoidal meati (Cook & Littlewort 1974) or through the nasomaxillary aperture ("drainage angle") (Fig. 27.5) (Cook & Littlewort 1974, Etherington et al 1982, Tremaine & Dixon 2001b). A PEH originating within the paranasal sinuses can usually be observed by sinoscopy of the caudal maxillary or concho-frontal sinuses (Fig. 27.6) (see Chapter 18) (Tremaine & Dixon 2001b).

The extent of a PEH can be determined by endoscopic, radiographic or computed tomographic imaging (Greet 1992, Tietje et al 1996, Colbourne et al 1997). A PEH at the more common rostroventral ethmoidal labyrinth site is best imaged on lateral radiographic projections, centered on the medial canthus of the eye. The PEH appears as an abnormal opacity of soft tissue density, rostral to the ethmoidal labyrinth (Cook & Littlewort 1974, Schumacher et al 1997, Tremaine & Dixon 2001b). Superimposition of the eyes and ethmoidal labyrinths may prevent radiographic identification of small PEH lesions (Rothaug & Tulleners 1999, Tremaine & Dixon 2001b); in these cases, computed tomography can be useful (Tietje et al 1996, Colbourne et al 1997). Skull radiography after administration of a water-soluble, radiographic contrast medium into the paranasal sinuses may help delineate the margins of an intrasinus PEH (Behrens et al 1991), but sinoscopy could as readily be performed and would likely provide a more definitive diagnosis.

Gross and Histologic Appearance

While the history and clinical signs are sometimes pathognomonic, the endoscopic appearance of PEH lesions is usually diagnostic. Histological examination of the lesion confirms the diagnosis, or at least eliminates the presence of other similar appearing masses of the nasal cavity. A uterine biopsy forceps, directed to the lesion using endoscopic guidance, can be used to obtain adequate tissue for histological diagnosis because a transendoscopic biopsy instrument does not usually retrieve a sufficient sample size for diagnosis (Colbourne et al 1997; authors' personal observation). The biopsied lesion often collapses because of leakage of the semi-liquid contents, leaving only the remaining capsule.

Macroscopically, PEHs have a smooth surface, and as noted, range from purple–red to green–yellow in color. PEHs are usually covered by a flattened columnar or cuboidal ciliated epithelium, containing mucous glands, and occasionally by stratified squamous epithelium, which is often focally ulcerated with a localized underlying inflammatory response. The cut surface of a PEH is reddish-brown and, on section, the lesions often collapse because of their liquid contents. Deeper tissues contain areas of recent hemorrhage, with a variable inflammatory infiltrate of plasma cells, lymphocytes, and, less commonly, neutrophils (Tremaine et al 1999). Hemosiderophages and multi-nucleate giant cells are often numerous (Platt 1975, Tremaine et al 1999). The degree of fibroplasia can vary
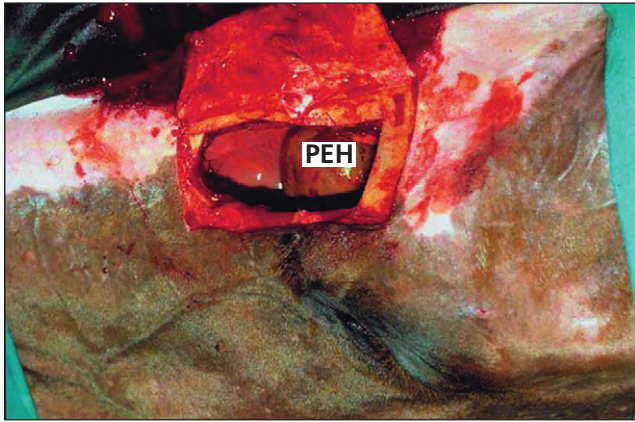
Fig. 27.7. View of the nasofrontal region of an anesthetized horse through a small nasofrontal flap revealing a PEH lying within the rostral aspect of the frontal sinus.
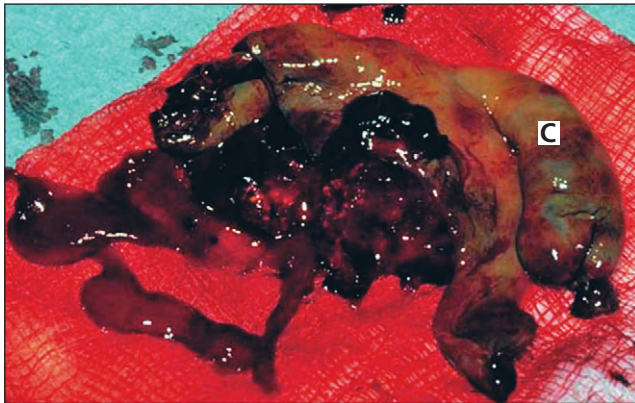
Fig. 27.8. This surgically excised PEH has collapsed, which is usual. The green–brown colored, smooth mucosal capsule (C) of the PEH lesion is identifiable, as is the internal stroma containing free blood and some dark colored, more organized stroma.

Fig. 27.9. This endoscopic image shows an intrasinus PEH with a needle-tipped transendoscopic catheter (C) deeply inserted into it to allow intralesional formalin therapy.

1992, Behrens et al 1993); ablating the lesion using a cryogen (Meagher 1986) or laser (Meagher 1990); surgically debulking the lesion and then ablating its base with a cryogen or laser (Tate 1991, 1996, Rothaug & Tulleners 1999); or injecting formaldehyde solution into the lesion (Schumacher et al 1997, Marriott et al 1999, Tremaine & Dixon 2001b). Successful removal of a PEH using a snare placed around the base of the lesion under endoscopic guidance has also been reported (Hanselka & Young 1975, Boulton 1985).

Surgical ablation

Surgical ablation was the main treatment for PEH lesions until a decade or so ago, when it was largely replaced by the less invasive treatments described below. A PEH arising from the sinusal portion of the ethmoidal labyrinth can be exposed and excised through a frontonasal osteoplastic (bone) flap (Figs 27.7–27.9) (Cook & Littlewort 1974, Freeman et al 1990, Specht et al 1990, Greet 1992, Behrens et al 1993, Tremaine & Dixon 2001b) created as described by Blackford et al (1985) or Freeman et al (1990), following which the floor of the dorsal conchal sinus is fenestrated (using a scissors or a rongeur) into the middle meatus of the nasal cavity and the PEH is then excised (Greet 1992). This surgery is usually performed with the horse anesthetized, and there is often marked blood loss. Intrasinus PEH lesions can similarly be excised through a frontonasal or maxillary osteoplastic flap with the horse anesthetized or sedated and standing (Schumacher et al 2000).

from loose connective tissue and fibrin containing few fibroblasts in some cases, to a dense fibrous connective tissue stroma in other cases (Tremaine et al 1999).

Treatment

There are various treatment options for PEH. Treatment of horses whose lesion does not protrude beyond the external lamina of the ethmoidal bone is often not necessary, because lesions this small often cause no clinical signs of disease (Schumacher et al 1997). However, if treatment is not performed, these horses should be monitored for clinical signs and endoscopically examined periodically because the lesion is considered to be progressive. Rarely, a PEH may disappear spontaneously (Laing & Hutchins 1992, authors' personal observation). Horses affected by PEH can be treated by excising the lesion (Cook & Littlewort 1974, Specht et al 1990, Greet 1992, Laing & Hutchins

Severe, intraoperative hemorrhage is the most common complication of excision of a PEH. Creating a frontonasal flap to expose the paranasal sinuses and sinusal portion of the ethmoidal labyrinth generally causes relatively little hemorrhage, but perforating the floor of the dorsal conchal sinus and excising the PEH are usually accompanied by severe hemorrhage. The use of suction may improve visibility. Methods of decreasing hemorrhage include the use of electrocautery and application of gauze sponges soaked in cold, physiological saline solution, with or without epinephrine, to the hemorrhaging tissue.

Removing a PEH with the horse standing and its head elevated may decrease the severity of hemorrhage by lowering the blood pressure at head level as compared to recumbency (Schumacher et al 2000) and also eliminates anesthetic risk. However, these advantages must be weighed against the problems that could be encountered during surgery if the horse hemorrhages severely while its head cannot be properly restrained. Horses selected for standing surgery of paranasal sinuses and nasal passage must be docile and must not resent manipulations of their head.

To prevent cardiovascular problems associated with severe hemorrhage and hypovolemia, the horse should receive a balanced electrolyte solution intravenously during surgery. Replacing blood during surgery is seldom necessary, but if the surgeon is inexperienced in this procedure, having at least 4–6 liters of compatible blood available for transfusion or having a compatible donor on standby may be prudent. Very rarely, an anemic horse should receive blood from a suitable donor during or before surgery.

A technique for controlling hemorrhage in anesthetized horses is to temporarily occlude both common carotid arteries in the mid-neck region (Wynn-Jones et al 1986, Freeman et al 1990). An umbilical tape ligature is placed loosely around each artery before sinusotomy, with the horse anesthetized (Wynn-Jones et al 1986, Lane 1993). The ligatures are tightened while the lesion is excised, to temporarily occlude the arteries. Both common carotid arteries can be occluded for at least 15 min without causing neurological deficits because the ventral spinal artery, which contributes a large amount of the blood supply to the circle of Willis, prevents cerebral ischemia during bilateral carotid occlusion (Wynn-Jones et al 1986, Lane 1993). Temporarily ligating the common carotid arteries prolongs surgical time, is sometimes ineffective in reducing severe hemorrhage (Greet 1992, Laing & Hutchins 1992, Tremaine & Dixon 2001b), and may result in damage to the adjacent recurrent laryngeal nerve (may result in permanent laryngeal dysfunction) or vagosympathetic trunk (may result in bradycardia and apnea) (Wynn-Jones et al 1986, Lane 1993).

Postoperatively, only the paranasal sinuses require packing (using rolled gauze, either dry or saturated with a dilute [e.g. 1 : 10,000] solution of epinephrine to control hemorrhage) if an intrasinus PEH has been removed. The end of the gauze is exited through a separate trephine opening created adjacent to the osteoplastic flap and the gauze is pulled out 24–48 h later and an indwelling lavage system is then inserted for 5–7 days of sinus lavage.

Both the nasal cavity and the paranasal sinuses are postoperatively packed to control hemorrhage if the PEH has been removed from the nasal portion of the ethmoidal labyrinth. They can be packed separately, using separate rolls of gauze, if a separate trephine hole is created (see above), or the sinuses and nasal cavity can be packed with a continuous roll of gauze. When using one continuous roll of gauze, one end of the gauze is introduced into the nasal cavity with a long grasping forceps and is then pulled into the paranasal sinuses through the created sinonasal fistula. The end of the gauze exiting the nasal cavity should be sutured to the nostril to prevent complete loss of the gauze in case the horse accidentally swallows the pack. To decrease the likelihood of the pack being swallowed, the nasopharynx can be inspected endoscopically through the contralateral nasal cavity to ensure that the pack has not been pushed into the nasopharynx. Inserting the gauze pack into an elastic, tubular stocking (Tubular Stockinette, Baxter Healthcare Corporation, Deerfield, IL, USA) inserted into the nasal cavity prevents the gauze from being swallowed (Greet 1992, Lane 1993) as described in detail in Chapter 26.

Although some surgeons prefer to remove the packing as late as 72–96 h after surgery (Freeman et al 1990), the packing can usually be removed safely at 24 h, because longer duration of packing can lead to local infection at the site of surgery (authors' personal observation). Repacking of the nasal cavity may occasionally be required if severe and prolonged hemorrhage is encountered immediately after removal of the packing. After packing removal, the paranasal sinuses can be lavaged to remove blood clots through a trephine opening created adjacent to the frontonasal flap for about 5 days post surgery. Gravity flow, rather than pressurized flow, should be used when lavaging the paranasal sinuses particularly in the first 24–48 h of lavage after creation of an osteoplastic flap to avoid forcing fluid into the subcutaneous tissue at the sinusotomy, which may increase the incidence of wound infection and dehiscence around the edges of the flap. One of us (PMD) uses an extremely dilute solution of povidone iodine to lavage the sinuses.

Fatal postoperative complications associated with surgical excision of a PEH include meningoencephalitis (Greet 1992, Rothaug & Tulleners 1999) and intracranial hemorrhage (Tremaine & Dixon 2001b). Meningoencephalitis can occur from damage to the cribriform plate or tracking of infection through the plate (Greet 1992). Less serious complications are infection and dehiscence of the cutaneous incision, usually at the rostral margin of the incision

(Freeman et al 1990, Greet 1992); sequestration of a section of the osseous portion of the frontonasal flap; suture periostitis; and opportunistic fungal infection at the surgical site (Greet 1992, Tremaine & Dixon 2001b).

Cryotherapy

A cryogen, such as liquid nitrogen, delivered as a spray or through a probe, can be administered nasally to ablate small PEHs after applying a topical anesthetic to the nasal cavity (Meagher 1990). Cryogenic ablation of a PEH results in minimal hemorrhage, and the cryogen can be administered with the horse sedated and standing, using endoscopic guidance. Only small lesions on the nasal portion of the ethmoidal labyrinth can be ablated in this manner, and treatment may be more palliative than curative.

Liquid nitrogen can also be applied, through a probe or as a spray, to the origin of a PEH after a PEH has been excised (Cook & Littlewort 1974, Meagher, 1990, Specht et al 1990). Control of hemorrhage, at least temporarily, is required because hemorrhage prevents the effective application of the cryogen (Meagher 1990). Conflicting reports exist as to the efficacy of this treatment. Meagher (1990) found that it decreased the incidence of recurrence while Specht et al (1990) found no such advantage. One major complication associated with this technique is that freezing the cribriform plate could result in brain damage, especially if the cribriform plate has been thinned or destroyed by pressure from the expanding PEH (Cook & Littlewort 1974, Meagher 1990).

Laser ablation

A PEH less than 5 cm in diameter that originates on the nasal portion of the ethmoidal labyrinth can be photoablated using a neodymium : yttrium–aluminum–garnet (Nd:YAG) laser transendoscopically with the horse standing, following topical local anesthesia of the nasal cavity (Meagher 1990, Tulleners 1992) as described in Chapter 39 –Laser Surgery of the Upper Respiratory Tract. Lesions are best photoablated at 60 watts using a non-contact technique (Tulleners 1992). Treatments should be at least 7 days apart to permit the devitalized tissue to slough. Horses with even small PEHs may require 12 to 14 applications of the laser, and because of the high cost of a Nd:YAG laser, this form of treatment is available only at large referral centers (Tate 1991, 1996, Rothaug & Tulleners 1999).

Laser ablation can be performed to the base of a PEH after the lesion has been excised through a frontonasal flap (Rothaug & Tulleners 1999). The origin of the PEH is destroyed in contact fashion at 18–20 watts. Laser-assisted excision of PEHs may result in fewer recurrences than surgical removal, because the PEH can be transected closer to its origin than is possible using surgical ablation alone (Tulleners 1996). A study by Rothaug and Tulleners (1999) found no difference in the incidence of recurrence with this technique compared to surgical excision alone.

Chemical ablation

A PEH can be ablated with a 4% aqueous solution of formaldehyde gas (10% formalin) (Schumacher et al 1997, Marriott et al 1999, Tremaine & Dixon 2001b). Formalin can be injected into a PEH located on the nasal portion of the ethmoidal labyrinth, in a standing sedated horse, using a transendoscopic polypropylene catheter. The end of the tubing can be cut diagonally to penetrate the PEH capsule, or the lesion can be injected using commercially available polypropylene tubing with a retractable, swaged-on needle. A small PEH can be injected by inserting the needle into the center of the mass; however, to inject a large lesion, the short needle plus a few centimeters of the catheter are inserted into the center of the lesion.

The PEH is injected with formalin until it expands and begins to leak solution. Horses often snort and become agitated by leakage of formalin solution over their nasal cavity; having some 50-ml syringes filled with lukewarm water allows the area to be immediately lavaged to relieve this discomfort. The lesion is injected at 3- to 4-week intervals until it is eliminated or becomes so small and deep within the ethmoidal recess that the lesion can no longer be injected (Schumacher et al 1997). Usually, only two to five treatments, performed at 3- to 4-week intervals, are required to eliminate a lesion, regardless of the lesion's size.

A lesion originating on the sinusal portion of the ethmoidal labyrinth can also be injected with formaldehyde solution, using endoscopic guidance or direct visualization, through a trephine hole in the caudal maxillary or conchofrontal sinus. Injecting solely the nasal portion of a PEH that resides both in the nasal cavity and the adjacent paranasal sinuses may cause both the sinusal and nasal portions of the lesion to resolve (Schumacher et al 1997).

No complications were associated with chemical ablation of PEHs of three horses in one study (Marriott et al 1999) or of six horses in another study (Tremaine and Dixon 2001b). In another report, however, one of 21 affected horses treated by chemical ablation developed laminitis within 24 h after each of three treatments (Schumacher et al 1997). The association between intralesional injection of formalin and the development of laminitis may have been coincidental because the horse had suffered periodically from laminitis before PEH treatment. In another report, one horse affected with a large PEH was euthanased when it developed severe CNS depressing and ataxia within minutes of the intralesional injection of formalin (Frees et al 2001). Neurological abnormalities developed because erosion and necrosis of the cribriform

plate by the PEH had resulted in an abnormal communication between the nasal cavity and the ventral cranial vault, allowing formalin to migrate to the frontal lobe of the brain.

At this recommended concentration, formalin desiccates and coagulates the PEH by hydrolyzing protein (Osol & Farrar 1947, Schumacher et al 1997). While complications have been associated with intravenous injection of formaldehyde solution, including restlessness, lacrimation, salivation, elevation of the tail, and tenesmus (Roberts 1943), these complications have not been reported after intralesional injection of PEH with formalin (Schumacher et al 1997, Marriott et al 1999, Tremaine & Dixon 2001b).

Ablating PEHs using formalin avoids general anesthesia; complications associated with surgical ablation, such as severe hemorrhage and infection of the cutaneous incision; problems associated with ablation by laser, such as availability of equipment; and problems associated with cryotherapy, such as availability of equipment and lesions being too large to be effectively frozen. Owners should be informed of the potential, but relatively rare, complications of this form of treatment, such as colic, laminitis, and severe neurological deficits.

Prognosis

Axial deviation of the medial wall of the dorsal and ventral conchal sinuses, caused by a PEH contained within the paranasal sinuses, usually resolves rapidly after the PEH has been removed. Even a deviated nasal septum may return to its normal position (Colbourne et al 1997, authors' personal observation). Facial distortion caused by an expanding intrasinus PEH is also likely to improve after PEH removal.

Regardless of the elected treatment method, the prognosis for long-term resolution of a PEH is guarded to poor. Incomplete excision may account for recurrence of PEH in some horses, or new PEHs may develop at a site adjacent to or distant from (e.g. contralateral ethmoidal complex) the original PEH (Rothaug & Tulleners 1999). Cook and Littlewort (1974) found that signs of disease reappeared in five of 12 horses (41.6%) at 3–44 months after surgery and believed it likely that the final proportion of lesions that recurred would have been higher if the time of follow-up had been extended. In a study by Specht et al (1990), PEH reappeared in four of nine horses (44.4%) after surgery (mean time of follow-up was 61 months). In a study by Greet (1992), three of 21 horses (i.e. 14.3%) with PEH that were treated by surgical ablation had reappearance of lesions 7–32 months after surgery. Resolution of disease was determined by owner questionnaire, and not all horses were examined endoscopically to confirm resolution of the lesion. Rothaug and Tulleners (1999) found the incidence of disease recurrence in seven horses affected bilaterally to be 43% and that of 12 horses affected

unilaterally to be 8% (median time of follow-up was 26 months; range 3–110 months). The studies by Rothaug and Tulleners (1999), Cook and Littlewort (1974) and Specht et al (1990) did not indicate whether or not horses that had no recurrence of clinical signs were examined endoscopically. Determining the treatment method that has the lowest incidence of recurrence, based on results of retrospective studies, is difficult because the long-term results obtained are difficult to compare.

Rothaug and Tulleners (1999) recommended endoscopic examination of both nasal cavities of affected horses every 3–6 months for at least 5 years after treatment to determine if the lesion has reappeared, but the length of time after which a lesion is unlikely to recur has not been determined. Recurrence has been observed as long as 3–4 years after surgical removal and the authors recommend bilateral nasal endoscopy at least annually for this period.

REFERENCES

Behrens E, Schumacher J, Morris E 1991 Contrast paranasal sinusography for evaluation of disease of the paranasal sinuses of five horses. Veterinary Radiology 32: 105–109

Behrens E, Schumacher J, Ford T 1993 Progressive ethmoidal hematomas in eight horses (1986–1990). Texas Veterinary Medical Journal 55: 16–18

Bell BTL, Baker GJ, Foreman JH 1993a Progressive ethmoid hematoma: background, clinical signs, and diagnosis. Compendium on Continuing Education for the Practicing Veterinarian 15:1101–1110

Bell BTL, Baker GJ, Foreman JH 1993b Progressive ethmoid hematoma: characteristics, cause, and treatment. Compendium on Continuing Education for the Practicing Veterinarian 15:1391–1398

Bell BTL, Baker GJ, Abbott LC et al 1995 The macroscopic vascular anatomy of the equine ethmoidal area. Anatomia Histologia Embryologia 24: 39–45

Blackford JD, Goble DO, Henry RW et al 1985 Triangulated flat technique for nasofrontal surgery. Veterinary Surgery 14: 287–294

Boles C 1979 Abnormalities of the upper respiratory tract. Veterinary Clinics of North America; Large Animal Practice 1: 89–111

Boulton CH 1985 Equine nasal cavity and paranasal sinus disease: a review of 85 cases. Journal of Equine Veterinary Science 5: 268–275

Colbourne CM, Rosenstein DS, Steficek BA et al 1997 Surgical treatment of progressive ethmoidal hematoma aided by computed tomography in a foal. Journal of the American Veterinary Medical Association 211: 335–338

Cook WR, Littlewort MC 1974 Progressive hematoma of the ethmoid region in the horse. Equine Veterinary Journal 6: 101–107

Etherington WG, Vasey JR, Horney FD 1982 Ethmoid hematoma of the equine. Canadian Veterinary Journal 8: 231

Freeman DE, Orsini PG, Ross MW et al 1990 A large fronto-nasal bone flap for sinus surgery in the horse. Veterinary Surgery 19: 122–130

Frees KE, Gaughan EM, Lillich JD et al 2001 Severe complication after administration of formalin for treatment of

progressive ethmoidal haematoma in a horse. Journal of the American Veterinary Medical Association 219: 950–952

Greet TRC 1992 Outcome of treatment in 23 horses with progressive ethmoidal haematoma. Equine Veterinary Journal 24: 468–471

Hanselka DV, Young MF 1975 Ethmoidal hematoma. Veterinary Medicine and Small Animal Clinician 70: 1289–1291

Head KW, Dixon PM 1999 Equine nasal and paranasal sinus tumours. Part 1: Review of the literature and tumour classification. Veterinary Journal 157: 261–278

Laing JA, Hutchins DR 1992 Progressive ethmoidal haematoma in horses. Australian Veterinary Journal 69: 57–58

Lane JG 1993 The management of sinus disorders of horses – Part 2. Equine Veterinary Education 5: 69–73

Leyland A, Baker JR 1975 Lesions of the nasal and paranasal sinuses of the horse causing dyspnoea. British Veterinary Journal 131: 339–346

Marriott MR, Dart AJ, Hodgson DR 1999 Treatment of progressive ethmoidal haematoma using intralesional injections of formalin in three horses. Australian Veterinary Journal 77: 371–373

Meagher DM 1986 The evaluation and surgical treatment of ethmoid hematomas in the horse. Veterinary Surgery 15: 128

Meagher DM 1990 Ethmoid hematoma. In White NA, Moore JN (editors) Current Practice of Equine Surgery. Lippincott, Philadelphia, pp.227–230

Osol A, Farrar GE 1947 The Dispensatory of the United States of America, 24th edn. Lippincott, Philadelphia, pp.483–486

Platt H 1975 Haemorrhagic nasal polyps of the horse. Journal of Pathology 115: 51–55

Roberts SJ 1943 The effects of various intravenous injections on the horse. American Journal of Veterinary Research 4: 226–239

Rothaug PG, Tulleners EP 1999 Neodymium:yttrium–aluminum–garnet laser-assisted excision of progressive ethmoid hematomas in horses: 20 cases (1986–1996) Journal of the American Veterinary Medical Association 214: 1037–1041

Schumacher J, Dean PM, Welch R 1992 Dacryohemorrhea as a cause of epistaxis from two horses. Journal of the American Veterinary Medical Association 200: 366–367

Schumacher J, Yarbrough T, Pascoe J et al 1997 Transendoscopic chemical ablation of progressive ethmoidal hematomas in standing horses. Veterinary Surgery 27: 175–181

Schumacher J, Dutton DM, Murphy DJ et al 2000 Paranasal sinus surgery in sedated, standing horses. Veterinary Surgery 29: 173–177

Specht TE, Colahan PT, Nixon AJ et al 1990 Ethmoidal hematoma in nine horses. Journal of the American Veterinary Medical Association 197: 613–616

Sullivan M, Burrel MH, McCandish IA 1984 Progressive haematoma of the maxillary sinus in a horse. Veterinary Record 114: 191–192

Tate LP 1991 Applications of lasers in equine upper respiratory surgery. Veterinary Clinics of North America; Equine Practice 7: 165–195

Tate LP 1996 Transendoscopic neodymium:yttrium–aluminum–garnet laser ablation of progressive ethmoid hematoma in the horse. American Society of Laser Medicine and Surgery Abstracts Supplement 8: 45

Tietje S, Becker M, Bockenhoff G 1996 Computed tomographic evaluation of head diseases in the horse: 15 cases. Equine Veterinary Journal 28: 98–105

Tremaine WH, Dixon PM 2001a A long-term study of 277 cases of equine sinonasal disease. Part 1: Details of horses, historical, clinical and ancillary diagnostic findings. Equine Veterinary Journal 33: 274–282

Tremaine WH, Dixon PM 2001b A long-term study of 277 cases of equine sinonasal disease. Part 2: Treatments and results of treatments. Equine Veterinary Journal 33: 283–289

Tremaine WH, Clarke CJ, Dixon PM 1999 Histopathological findings in equine sinonasal disorders. Equine Veterinary Journal 31: 296–303

Tulleners EP 1992 Nasal passages. In: Auer J (editor) Equine Surgery. Saunders, Philadelphia, pp.443–446

Tulleners EP 1996 Instrumentation and techniques in transendoscopic upper respiratory tract laser surgery. Veterinary Clinics of North America; Equine Practice 12: 373–395

Wynn-Jones G, Jones RS, Church S 1986 Temporary bilateral carotid artery occlusion as an aid to nasal surgery in the horse. Equine Veterinary Journal 18: 125–128

28

Disorders of the Guttural Pouches
(Auditory Tube Diverticuli)

G Barrie Edwards and Tim Greet

Anatomy

The guttural pouches, which are not present in any domesticated animals other than Equids, are paired ventral diverticuli of the eustachian tubes extending from the nasopharynx to the middle ear. Their location, configuration, and anatomical relationships contribute to the variety of clinical signs demonstrated when the pouches are involved in a disease process.

Each pouch is an air-filled space with a capacity of 300–500 ml in adults. The two pouches are in contact ventral to the longus capiti muscles, where the membranous portion of their medial wall separates them. The pouch is reflected around the dorsal border of the stylohyoid bone, which courses through its caudolateral aspect, dividing it into a small lateral compartment and a larger medial compartment. The medial compartment is two to three times the size of the lateral compartment and extends more caudally and ventrally. The lateral compartment is related to the ramus of the mandible laterally, and the ventral aspect of the medial compartment is laterally related to the mandibular and parotid salivary glands (Sisson 1975). The pouches are lined by ciliated epithelium, but non-ciliated microvillous and mucus-producing cells are also evident on electron microscopy (Pirie et al 1990). The lining of the pouch contains a large number of lymphocytic follicles, and its lumen contains a complex microflora of bacteria, fungi, and viruses. The walls of the guttural pouch are thin and intimately associated with many vital structures, including the pharynx, larynx, esophagus, parotid and mandibular salivary glands, and the retropharyngeal lymph nodes. The associated neurovascular anatomy is extensive and complex. The roof of the guttural pouch directly contacts a large area of the ventral surface of the skull from the occipital condyles to the mandibular articulation. In the center of this area are the foramen lacerum and the tympanic bulla, and structures that enter and leave the foramen lacerum must cross the pouch. A fold of mucous membrane extending from the roof of the pouch along the caudal aspect of the medial compartment contains the glossopharyngeal (IX), vagus (X), accessory (XI), and the hypoglossal (XII) nerves, as well as the pharyngeal branches of IX and X, the sympathetic trunk with the cranial cervical ganglion, and the internal carotid

artery (ICA; Fig. 28.1). The facial nerve (VII) passes for a short distance over the caudodorsal aspect of the lateral compartment.

The external carotid artery (ECA) lies along the wall of the lateral compartment and, after giving off the superficial temporal artery, continues as the maxillary artery along the roof of the guttural pouch. The lateral retropharyngeal lymph nodes contact the lateral wall of the medial compartment, and the medial retropharyngeal lymph nodes contact the medial compartment's ventral wall. Each pouch communicates with the pharynx through a slit-like opening on the pharyngeal wall, rostroventral to the pharyngeal recess. A thin plate of fibrocartilage is located in the mucosa of the medial aspect of the ostium. The pharyngeal opening into the pouch lies halfway up from the base of the pouch. Therefore, when a horse's head is in the upright position, the ostium only provides an overflow outlet for the escape of fluid, which may accumulate in the pouch. However, the ostia open during swallowing, allowing effective drainage when the horse is grazing. Dilatation of the ostia also results in an exchange of air
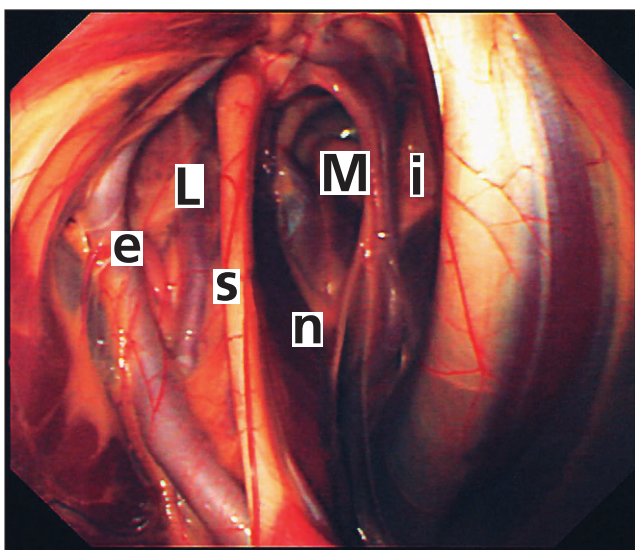
Fig. 28.1. Endoscopic view of the right guttural pouch. s = stylohyoid bone; L = lateral compartment; e = external maxillary artery; M = medial compartment; i = internal carotid artery; n = IX, X, XI cranial nerves.

during breathing, which exposes the pouches to airborne infectious agents. It has been shown experimentally that ventilation of the guttural pouch cools the blood in the ICA (Baptiste et al 2000), and the normal exchange of air during breathing may be a brain-cooling mechanism to dissipate the heat that is generated by muscular exertion.

The main disorders of the guttural pouches are tympany, mycosis, empyema, chondroids, diverticulitis, and neoplasia.

Diagnosis

Guttural pouch disease is suggested by a history of nasal discharge (mucopurulent material or blood), parotid area swelling, dyspnea, and evidence of cranial nerve and cranial sympathetic trunk dysfunction. Distension of the pouch may be characterized by external swelling medial to the parotid salivary gland and by nasopharyngeal compression. Most guttural pouch diseases do not, however, cause external swelling. Guttural pouch disease can be definitively diagnosed by endoscopic examination of the nasopharynx and pouch, by lavage of the pouch, and by radiographic examination of the parotid region.

Physical Examination

External palpation in the parotid area is helpful to detect swellings caused by tympany, empyema, abscessation of adjacent lymph glands, or neoplastic foci, particularly within the parotid lymph nodes. Occasionally, guttural pouch mycosis may produce painful foci deep to the base of the ear, when the head of the stylohyoid bone or the tympanohyoid articulation have become involved.

Endoscopy

Non-specific evidence of guttural pouch disease, such as nasopharyngeal collapse and the presence of blood or purulent material draining from the pharyngeal orifice, can be found on endoscopic examination of the nasopharynx. However, blood or pus from other respiratory sources can occasionally be aspirated into the guttural pouch ostia during swallowing and can appear to be draining from there. Endoscopy of the guttural pouch usually allows all the anatomical structures within the pouch to be examined, unless it is filled with blood, pus, or chondroids. Passage of an endoscope into each guttural pouch is simplified by using a wire leader or biopsy instrument introduced through the biopsy channel, which is invariably eccentrically located, to raise the cartilage flap before advancing the endoscope into the pouch. To achieve this, the endoscope must be introduced via the ventral meatus and directed caudodorsally to enter the pouch. If the biopsy channel of the endoscope is located at the 5 o'clock position, introduction of the endoscope into the right

guttural pouch is facilitated by counter-clockwise rotation of the endoscope once the lead wire has passed under the cartilaginous flap. Such rotation is not required when introducing the endoscope into the left guttural pouch.

Radiographic Examination

On an erect, lateral projection, centered one-third of the way down the caudal border of the vertical ramus of the mandible, the normal guttural pouches appear as dark triangular areas, extending rostrally and caudally to this border, with their apex located below the petrous temporal bone and their base lying dorsal to the larynx and nasopharynx. The stylohyoid bones can be seen running in a rostroventral to dorsocaudal direction through the pouches. The air that normally fills the guttural pouches provides contrast for good diagnostic radiographic images, enabling identification of gas–fluid interfaces or loss of air contrast as the result of the presence of inspissated pus, enlarged lymph nodes, and tumors (Cook 1973). The degree of nasopharyngeal compression caused by distension of the pouch can be radiographically evaluated.

Surgical Approaches to the Guttural Pouch

Historically, several surgical approaches have been used for the treatment of diseases affecting the guttural pouch. The ideal surgical approach should afford the best access, with minimal risk of damage to adjacent structures. The guttural pouches are difficult to approach surgically, because of the numerous important neural and vascular structures that surround them. All approaches, other than the "Whitehouse" approach, are usually performed with the horse anesthetized and placed in lateral recumbency with the affected pouch uppermost; for the "Whitehouse" approach, the horse is placed in dorsal recumbency; however, this latter approach can be performed with the horse standing.

Hyovertebrotomy

The hyovertebrotomy approach is the most commonly employed surgical approach to the guttural pouches. It is used primarily for identification of the carotid artery prior to intra-arterial balloon catheterization and/or the placement of surgical ligatures around the ICA or any other vessels affected by guttural pouch mycosis. Less commonly this approach is used to provide access to the dorsal aspect of the pouch, so allowing inspection of the pouch and possible removal of its contents. This approach is often performed to aid creation of ventral drainage from the pouch (see below). An approximately 10-cm long, curved incision is made parallel and cranioventrad to the wing of the atlas. Occasionally, a moderately sized

superficial vein is encountered here, but this vein can usually be reflected or ligated. The parotid fascia immediately below the skin in this region is very dense and must be incised with a scalpel or scissors. However, dissection deep to this level must be carried out using blunt instrumentation or digitally, to avoid injury to the many neural or vascular structures in this area. The dorsal aspect of the parotid salivary gland is usually retracted rostrally out of the surgical field using a strong, self-retaining retractor, such as a rib retractor, which is placed between the parotid gland and the wing of the atlas, or by using hand-held retractors. This ensures a clear view of the loose, fibrous-areolar tissue that surrounds the vessels and nerves deep in this site and also exposes the dorsal aspect of the guttural pouch.

If the intention is to identify the appropriate artery before inserting a balloon catheter and/or ligating it, the vessels should be carefully separated from the surrounding fascia and the adjacent nerves must be identified and preserved. The use of aneurysm needles can be very helpful in identifying the carotid arterial trifurcation, and in allowing separation of the arteries from surrounding tissue. Digital identification of an arterial pulse is useful but if there is any doubt about whether a structure is an artery or a nerve, the "needle test" can be employed with complete safety. This involves the gentle insertion of a 25-gauge needle into the structure in question. If inserted into a nerve, no blood will emerge, but if inserted into an artery, a small spurt of arterial blood will be obtained that can be readily controlled after needle removal.

If the guttural pouch is to be surgically entered using this approach, passing an endoscope into the pouch beforehand is helpful, because light from the lens of the endoscope is transmitted through the wall of the pouch, allowing its easy identification. This allows the lining, which is usually covered with the same loose areolar tissue as the nerves and vessels, to be grasped with rat-tooth forceps. The lumen is then carefully penetrated, and the incision is expanded digitally to minimize the risk of injury to adjacent neural or vascular structures. The pouch mucosa can be sutured or left to heal by second intention. If it is to be repaired, a continuous suture of absorbable suture material such as polyglactin can be used, taking great care to avoid damaging any nerve or blood vessel. The parotid fascia and subcutaneous tissues can readily be apposed using a continuous suture of the same material. The skin is usually closed with stainless steel staples.

Viborg's triangle

This anatomical landmark is formed by the intersection of the tendon of the sternocephalicus muscle, the caudal aspect of the mandible, and the linguofacial vein. It is named after the Danish veterinary surgeon Erik Viborg, and is the site of a surgical approach remembered by

countless generations of veterinary students. It is, however, the least used of surgical approaches because it provides limited access to the pouch, unless the pouch is grossly distended, such as may occur with marked tympany or empyema, and also because it requires dorsal reflection of the parotid salivary gland and its duct, which then interferes with access to the lateral compartment of the guttural pouch in many horses.

Modified Whitehouse approach

This approach is similar to that used for laryngoplasty and is performed by making a 7–10-cm skin incision ventral to the linguofacial vein. The skin incision is continued by blunt dissection deep to the vein to reveal the lateral aspect of the larynx and the related thyropharyngeal and cricopharyngeal muscles. As with the previously described approaches, pointing the tip of a flexible endoscope (previously inserted into the ipsilateral guttural pouch) toward the wall of the lateral compartment helps to identify the guttural pouch deep within the incision, thus allowing the wall of the medial compartment of the pouch to be safely penetrated.

The modified Whitehouse is the preferred approach for establishing optimal drainage of purulent material from the pouch, and allows removal of large or firm chondroids. It is the most direct approach and allows flexible, safe, surgical access to the contralateral pouch through the midline septum, which is best identified by trans-septal illumination from an endoscope inserted into the contralateral pouch.

The incision in the lateral wall of the medial compartment is usually left to heal by second intention. However, if the mucosa is to be closed, great caution must be observed to avoid the closely related, vital neural structures.

Whitehouse approach

This approach has been used both for ligating the ICA and for establishing drainage of purulent material, but in the authors' view, it is less than ideal for either purpose.

Using this approach, the horse is positioned in dorsal recumbency, and a 20–25-cm long ventral midline incision is made from the basihyoid bone in a caudad direction to the trachea. The sternohyoid muscles are separated, and the larynx and trachea are pushed to one side to allow ventral access to the medial compartment of either guttural pouch, taking great care to avoid the pharyngeal branch of the vagus nerve and the cranial laryngeal nerve, which are both in the surgical field.

If the intention is to ligate the ICA, it may be necessary to use long-handled instruments because the artery is situated very deep in the wound using this approach. Ligating the ICA in the roof of the medial compartment without damaging the closely associated cranial sympathetic nerve and causing Horner syndrome is almost

impossible. In the authors' view, arterial ligation is far more satisfactorily achieved via a hyovertebrotomy approach.

In general, direct surgical approaches into the guttural pouch should be avoided whenever possible, because of the high risk of life- or career-threatening iatrogenic nerve injury. Whenever a surgical approach is used, inserting an endoscope into the appropriate pouch to allow the wall to be identified by transmural illumination can make the procedure much safer, as previously noted. If an animal is dyspneic as a consequence of guttural pouch empyema, pouch drainage may be considered as an emergency procedure and can be performed in the standing patient. However, in such cases it may be preferable to provide relief by performing a temporary tracheotomy, before taking a more considered approach to guttural pouch drainage (e.g. by large volume lavage of the affected pouch via an indwelling catheter). Additionally, the dyspnea in some of these patients may be the result of ventral nasopharyngeal compression caused by grossly swollen lymph nodes, which may not be resolved by pouch drainage.

The hyovertebrotomy approach is the most commonly used guttural pouch surgical approach used to prevent fatal epistaxis in cases of guttural pouch mycosis. Access via a hyovertebrotomy incision is adequate for both proximal ligation of the affected artery and for insertion of a balloon catheter via a small arteriotomy incision. All arteries can be approached by that method but if the maxillary artery is involved an approach must also be made to the greater palatine artery just rostral to the premolar arcade to insert a balloon catheter, as later described.

Septal fenestration or ostial enlargement in cases of guttural pouch tympany can be performed via a modified Whitehouse (laryngoplasty type) approach. However, ostial enlargement can be carried out far more safely via chronic catheterization of the affected pouch, or by transendoscopic laser surgery. To summarize, surgical approaches to the guttural pouch by whatever method should be used only when no other alternative treatment is available.

Disorders of the Guttural Pouch

Guttural pouch tympany

History and clinical signs

This is a relatively uncommon condition typically affecting young foals, but horses up to 1-year-old can be affected. Although Arabian foals appear to be over-represented in the population affected by this disorder, the authors have encountered the condition in horses and ponies of many breeds. The condition may have a genetic component (Blazyczek et al 2004b). Fillies appear to be much more commonly affected than colts but the condition is seen in both sexes (Blazyczek et al 2004a).

Fig. 28.2. Guttural pouch tympany.

The presenting signs of the condition are variable but a marked, often fluctuating, air-filled swelling of the parotid and ventral laryngeal areas is the usual predominating feature (Fig. 28.2), and the term "bullfrog" is sometimes used as a colloquial description because of this appearance. The swelling is usually more marked on one side, because the condition is usually unilateral. However, the naturally deformable nature of the soft tissues of the pharynx often results in some swelling on the contralateral side, which can make identification of the affected side by clinical examination alone more difficult. In a small but significant proportion of affected horses, the swelling is bilaterally symmetrical because the condition is bilateral.

In addition to the above swelling, most affected foals make adventitious "snoring" noises, particularly when suckling. In bilateral cases, severe dyspnea may be the most dramatic feature. A variable degree of nasal return of milk is commonly noted but, as with the respiratory noise, the volume of return can vary with the degree of nasopharyngeal distension present at that time. In the most severely affected animals this can result in aspiration pneumonia.

The swelling often fluctuates during the course of 24 h, and some foals may appear to recover during the day while being turned out to grass with their dam; presumably the degree of tympany is reduced by the more frequent swallowing efforts associated with suckling and intermittent grazing. Another variable feature of guttural pouch
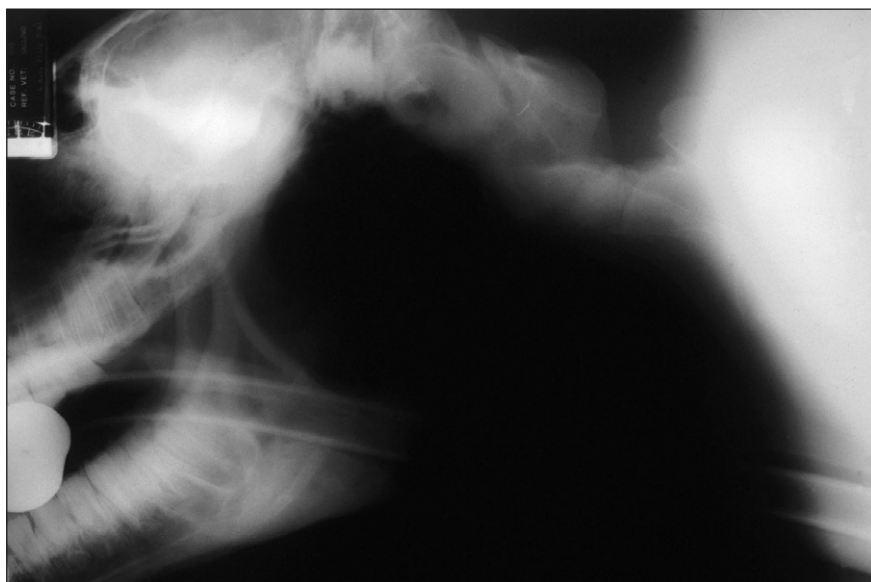
Fig. 28.3. Guttural pouch tympany. Lateral radiographic projection of the head showing a grossly distended, air-filled guttural pouch extending caudally to the ventral straight muscles of the neck.

tympany is the degree of retention of mucus within the affected pouch; in some cases, the guttural pouch becomes secondarily infected, producing an empyema.

The cause of guttural pouch tympany is unknown. Some authors believe a "ball-valve effect" of the pharyngeal ostium of the guttural pouch, perhaps related to the mucosal rim within the cartilaginous flap of the ostium, is responsible. However, the authors have never seen any anatomical deformity of this structure. Additionally, in unilateral cases, there is no apparent anatomical difference between the normal and affected sides, indicating a possible functional basis for this disorder. Interestingly, the authors have noted the condition occasionally developing in more mature animals. Whilst it is impossible to be sure whether the problem has gone unnoticed earlier in life, it seems highly improbable because the presenting signs are usually obvious.

Endoscopic and radiological features

The clinical signs of guttural pouch tympany are usually diagnostic; however, both endoscopic and radiological confirmation are valuable. An endoscopic view of the nasopharynx in affected foals usually reveals significant luminal obstruction caused by collapse of the nasopharyngeal roof, as a result of distension of the overlying pouch. The nasopharynx may be obviously asymmetric if the foal is unilaterally affected, but even in some unilaterally affected foals nasopharyngeal collapse is surprisingly symmetrical. Inserting the endoscope into the affected guttural pouch usually causes an instant and dramatic pouch deflation. As noted above, other than the occasional presence of mucus or purulent material within the pouch, no structural abnormalities are observed. Unless the pouch is filled with exudate, it will deflate in

a characteristic manner, confirming that the primary condition is one of tympany rather than empyema.

Standing lateral radiographic projections of affected horses show marked enlargement of the affected pouch (Fig. 28.3). On occasions, there may be a radiodense fluid shadow with a typical "fluid line" (much as in a case of paranasal sinusitis). The normal air shadow of the guttural pouches can extend as far caudad as the ventral tubercle of the first cervical vertebra (Cook 1973), but in cases of tympany this shadow is usually massively enlarged (Fig. 28.3). The radiological appearance of the guttural pouches produces a complex shadow because of superimposition of the smaller lateral and the larger medial compartment of each pouch. However, it is usually possible to distinguish between unilateral and bilateral tympany cases on radiological features alone.

Treatment

The aim of treatment is to alleviate guttural pouch distension by enlarging the pharyngeal opening of the guttural pouch, or if the foal is affected unilaterally, by creating communication between adjacent pouches through the septum. It is also possible to create drainage of air from the pouches directly into the nasopharynx, through the pharyngeal recess (as described later). Treatment is necessary, other than in the most mildly affected animals, to relieve airway obstruction and/or dysphagia.

In unilateral cases the distended pouch can be drained effectively by creating a defect in the common septum. Traditionally, this has been achieved using conventional surgery via any of the surgical approaches described above, but it is most easily achieved via a modified Whitehouse approach. The advent of surgical lasers has encouraged the use of a neodymium : yttrium–aluminum–garnet (Nd:YAG)

or diode laser to create the defect under endoscopic control (Tate et al 1995). Whilst requiring expensive equipment and specialist training, this method of treatment is usually effective. However, great care must be observed when firing the laser because there is the potential to injure vital structures in the contralateral pouch. One foal treated using Nd:YAG laser had effective drainage of the distended pouch but was found dead in a pool of blood approximately 7 days postoperatively (authors' personal observations). Clearly a blood vessel in the lateral compartment of the contralateral pouch had been damaged by the laser beam but it was only when the wall of the vessel sloughed, approximately 1 week later, that the vascular injury became apparent.

Another reported technique using a transendoscopic laser is to create a fistula in the pharyngeal recess to provide drainage to one or both pouches. The authors have no first hand experience of this technique but it is obvious that great caution should be observed to avoid damage to vital structures and it is desirable that a contact laser be used.

Without doubt, the simplest and safest approach to the treatment of foals affected with guttural pouch tympany is the prolonged catheterization of the pharyngeal ostium of the pouch. A Foley catheter inserted into the pouch can be retained by inflating the balloon with water. Retention of the catheter within the pouch for 4–6 weeks results in inflammation and remodeling of the overlying cartilaginous flap, leaving the ostium permanently open and the pouch deflated. This technique can be used in unilateral or bilateral cases. The obvious shortcoming of this simple and safe technique is the propensity for foals or their dams to remove the indwelling catheters. This can occur when the foal or its dam pulls on, or treads on the exposed end of the catheter. Such displacement can cause considerable owner frustration because the catheter can only be replaced by a veterinarian under endoscopic guidance. The other previously described techniques may be employed if this technique of prolonged catheterization fails.

Guttural pouch mycosis

Guttural pouch mycosis (GPM) is a fungal infection that invades the mucosa and, frequently, the closely associated various neurovascular structures. *Aspergillus* spp, particularly *Aspergillus fumigatus* and *A. nidulans* and *Candida* spp. are the most commonly incriminated organisms. There is no apparent age, sex, breed or geographic predisposition to this disease, although it is seen most frequently in stabled horses during the warmer months of the year and is seldom recorded in warmer climates. The lesion, which is usually, but not always, unilateral, is usually found at one of two characteristic sites. The majority of lesions develop on the roof of the medial compartment over the petrous temporal bone and the distal segment of the ICA, or much
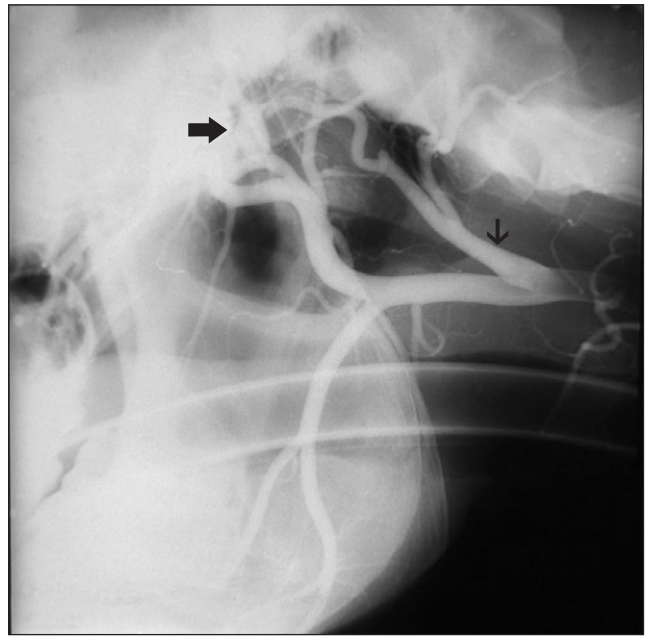
Fig. 28.4. Angiogram showing a large aneurysm (large arrow) of the internal carotid artery in the roof of the medial compartment. The internal carotid artery and the occipital artery have a common trunk (small arrow).

less frequently, on the lateral wall of the lateral compartment where the ECA and maxillary artery are located. Greet (1987) reported that all 35 cases in his series had lesions over the ICA while Freeman & Ross (1987) suggest that at least a third of horses with epistaxis caused by GPM bleed from fungal erosions of the ECA and its branches.

GPM is an opportunistic infection requiring appropriate environmental conditions or predisposing factors to colonize the mucous membrane of the pouch. Colles & Cook (1983) showed that 18 out of 25 horses with GPM had aneurysms of some of the arteries that ran through their guttural pouch. In some cases, angiography will show aneurysmal dilatation of the diseased arteries (Fig. 28.4), but aneurysm formation does not appear to precede or follow arterial invasion consistently and, therefore, is not necessarily involved in the pathogenesis of arterial infection.

Typically, the mycotic infection causes formation of a diphtheritic membrane that is closely attached to the underlying tissues and has an irregular surface elevated above the mucosal surface. The size of the lesion may vary from less than 1 cm in diameter to one which covers most of the medial and lateral compartments (Fig. 28.5). It may be brown, yellow, green, black, or white and is composed of necrotic tissue, cell debris, fungal mycelia, and a variety of bacteria. The fungal mycelia can be found throughout the depth of the diphtheritic membrane and also invading the adjacent tissues, including nerves and arteries (Fig. 28.6). The associated inflammation can also extend to underlying
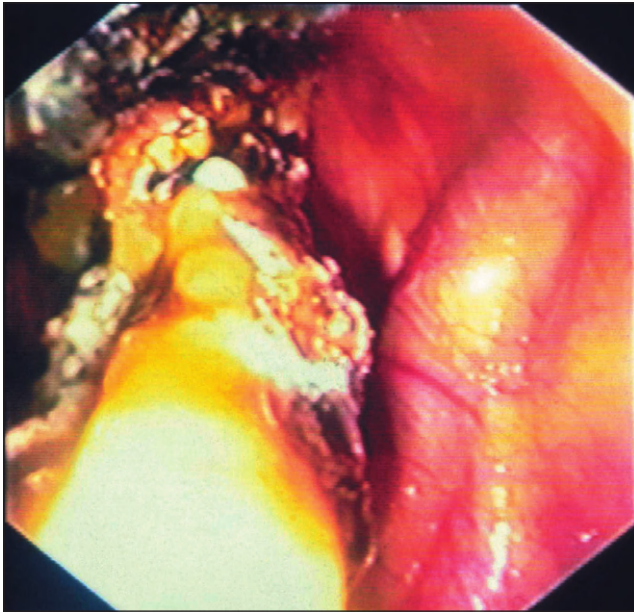
Fig. 28.5. Guttural pouch mycosis. Endoscopic view showing an extensive lesion located predominantly on the roof of the medial compartment but extending over the stylohyoid bone into the lateral compartment.

bone, stimulating formation of extensive exostoses. Lesions on the medial wall of the medial compartment can erode through the septum to affect the other pouch. Rarely, necrosis of the floor of the pouch can result in abscess formation that can spread to the supraorbital fossa or create a fistula through the nasopharyngeal recess into the nasopharynx. Dorsocaudal extension of GPM can cause atlanto-occipital infection (Dixon & Rowlands 1981, Walmsley 1988).

Clinical signs of GPM

Mycotic infection of the guttural pouch usually progresses unsuspected until invasion of neurovascular structures results in hemorrhage or neurological deficits, although in retrospect, the owner may occasionally report a preceding fetid smell from the nose, resentment to handling the base of the ear, or neck pain or stiffness.

The most common clinical sign is moderate to severe epistaxis, caused by erosion of the ICA in most cases, or less commonly, of the external carotid and maxillary arteries. The first episode of severe epistaxis is often preceded by minor hemorrhages resulting in a small quantity of blood at one or both nostrils. If undiagnosed and untreated, exsanguination is the probable final outcome. It is unusual for the first episode of severe epistaxis to be fatal but the course of the disease from first to final hemorrhage rarely spans more than 3 weeks and may be a matter of days.

Dysphagia as a result of damage to the motor or sensory (or both) pharyngeal branches of IX, X and XI is the most frequent neuropathological sequel to GPM. The severity of the dysphagia in GPM cases probably requires damage to all three cranial nerves. The study by Klebe et al (2005) showed that although the glossopharyngeal nerve (IX) has important sensory and motor contributions to pharyngeal function during swallowing, it is not essential in otherwise normal horses. Damage to X with laryngeal hemiplegia is the next most frequent cranial nerve deficit but is rarely the only sign observed by the owner. GPM may produce a wide range of other signs referable to the head and neck, including dorsal displacement of the soft palate, facial paralysis, lingual hemiplegia, Horner syndrome, stiffness of the upper neck, reluctance to lower the head to the ground, parotid pain, otorrhea, epiphora, and photophobia.
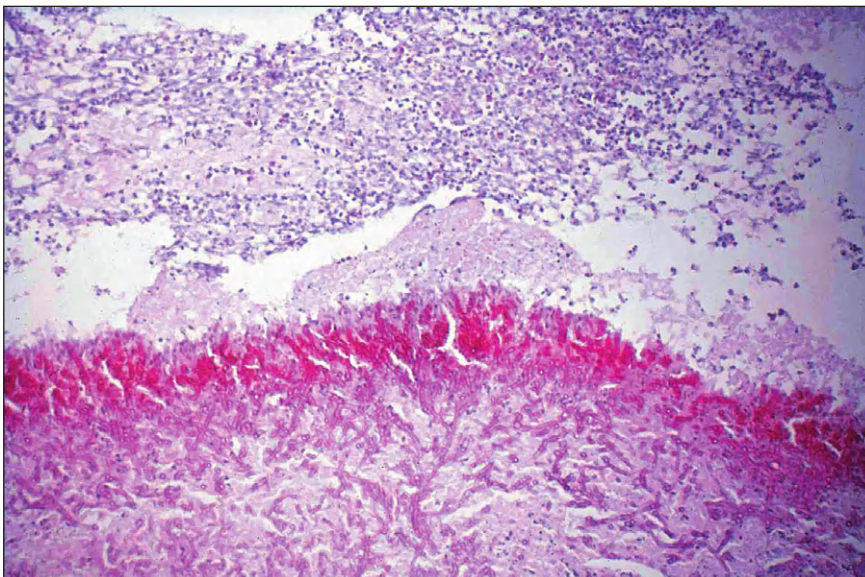
Fig. 28.6. Guttural pouch mycosis. Histological section of the internal carotid artery showing invasion of its wall by fungal hyphae; periodic acid–Schiff stain.

Fig. 28.7. Guttural pouch mycosis. (A) Bilateral epistaxis – the hemorrhage was more copious from the left nostril. (B) The lateral view shows ptosis and sweating below the ear, indicating sympathetic trunk involvement, thus incriminating the internal carotid artery as the source of the hemorrhage.

Diagnosis of epistaxis caused by GPM

Whenever a horse presents with spontaneous epistaxis, the possibility of GPM should always be explored, because delayed treatment may result in a fatal outcome. When severe, hemorrhage will be bilateral but it will be more copious from the nostril on the affected side (Fig. 28.7). In many cases, the bleeding will have stopped by the time the horse receives veterinary attention or does so soon afterwards. However, if it continues unabated, steps must be taken to prevent exsanguination. Acepromazine may be administered to lower the blood pressure, but while this is often successful, it is not without risk, and once it is administered, the horse should be left quietly in its stable. Any attempt to move it may result in fatal collapse. An alternative but unevaluated treatment that has been used by some veterinarians is the intravenous administration of 50 ml of 10% formalin in 2 liters of physiological saline solution.

A definitive diagnosis can only be made by endoscopic examination of the interior of the pouch but this procedure should not be performed until the bleeding has stopped, and the horse's general condition has stabilized. After the hemorrhage has stopped, endoscopy of the nasopharynx will usually identify which pouch is involved. A finger-like blood clot can usually be seen emerging from the ostium on the affected side, and distension of the overlying pouch may also be evident. Blood may also be seen at the other ostium (Fig. 28.8). While this could indicate a bilateral problem, in most cases it is the result of aspiration of blood during swallowing, when severe hemorrhage was occurring, or is the result of the presence of a septal fistula.

Some clinicians (GBE) prefer to delay occluding the ICA until endoscopy of the pouch is possible to confirm where the fungal plaque is and thus identify which artery is involved. Therefore endoscopy may be delayed for 1–3 days, because visibility is poor after a recent hemorrhage and because introduction of the endoscope may dislodge the blood clot and precipitate another episode of severe hemorrhage. Feeding the horse off the ground or leading it out to graze facilitates drainage of blood during this period. However, there is always the risk that the horse may hemorrhage again (even fatally) within 48–72 h, before the pouch has cleared of blood.

In view of the risk of delaying surgery, other clinicians will immediately proceed with arterial occlusion without endoscopic confirmation of which artery is affected. Whilst the administration of intravenous hypertonic saline or a blood transfusion may be essential with severe hypovolemia, these measures risk precipitating further hemorrhage from the damaged artery and some clinicians (GBE) prefer not
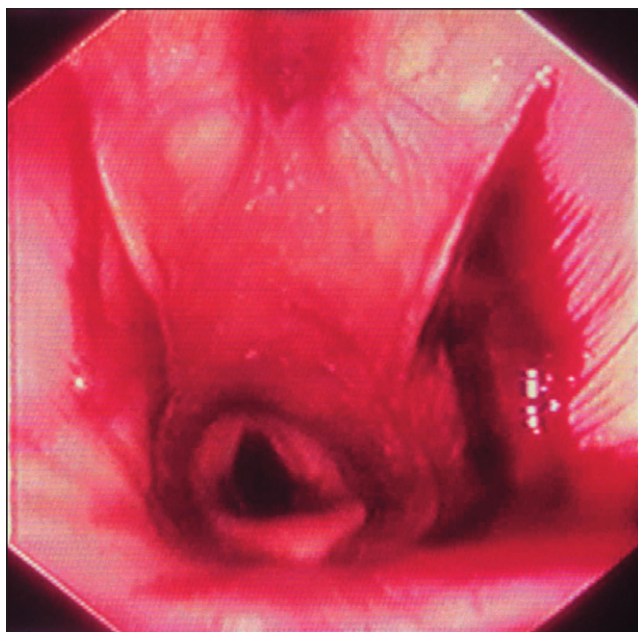
Fig. 28.8. Guttural pouch mycosis. Endoscopic view of nasopharynx showing a large blood clot at the ostium on the affected left side and a small amount of previously aspirated blood at the right ostium.

to do so, unless absolutely essential. In the case of horses showing evidence of involvement of the sympathetic trunk (i.e. ptosis and unilateral head sweating) it can be confidently assumed that the ICA is involved (Fig. 28.7). When evidence of Horner syndrome is absent, the decision to ligate the ICA may be taken on the basis that it is involved far more frequently than the external carotid or maxillary arteries. Of the 125 cases reported in the literature between 1968 and 1987, 120 involved lesions of the ICA.

Treatment options

The natural progress of primary arterial disorders is toward thrombosis, but this is a slow process with or without antimycotic treatment. As a result of the risk of further hemorrhage before thrombosis occurs, surgical occlusion of the affected artery is advised. None of the vessels commonly involved is an end artery so the potential exists for retrograde flow (e.g. from the circle of Willis) following ligation only, on the cardiac side of the lesion.

Ligation of the internal carotid artery

The ICA can be exposed at its origin from the common carotid artery via the previously described hyovertebrotomy approach. This extradiverticular ligation has the advantage that the affected pouch is not invaded but the ligature is some distance from the lesion in the arterial wall. Such ligation of the ICA close to its origin may be sufficient to prevent a fatal hemorrhage. However, if adequate thrombosis of the vessel does not occur beyond this site during

the week or so following surgery, severe, sometimes fatal, hemorrhage may occur because of retrograde flow. Although prevention of back-flow by ligation of the vessel either side of the mycotic lesion via a modified Whitehouse approach to the pouch has been described, poor surgical access to, and also great difficulty in identifying, the artery, which is obscured by the mycotic plaque or hematoma, may result in failure to occlude the vessel, or the ligature may incorporate the sympathetic trunk, causing Horner syndrome (Owen 1974).

Occlusion of the distal segment with a balloon-tipped catheter, combined with proximal ligation of the ICA, is superior to ligation alone (Freeman & Donawick 1980a,b). In the majority of horses, the ICA and the occipital artery are separate, but closely related, branches of the common carotid artery. The ICA branches off the common carotid artery more caudally and passes beneath the occipital artery in the direction of the base of the ear (Fig. 28.9). In some cases, periarterial edema resulting from the more proximal inflammatory process helps to identify the ICA. The occipital artery passes dorsally in a more caudal direction. In a small proportion of horses, the two vessels have a common trunk. Usually, the bifurcation of the common trunk of the caudally directed occipital artery and the cranially directed ICA is within the field of view, but the bifurcation can be more cranial than the surgical site. The ICA is isolated near its origin from the common carotid artery, dissected free from the surrounding tissue, and ligated. A small incision is made into its lumen distal to the ligature and a balloon-tipped catheter is inserted for a distance of 13 cm. In a 450-kg horse, this places the balloon tip at the second flexure of the sigmoid and, therefore, distal to the mycotic lesion (Fig. 28.9). The balloon is inflated with physiological saline solution, and the catheter is secured in position by a ligature placed distal to the site of insertion. The catheter is ligated and transected a few centimeters caudal to its point of entry into the artery, and the redundant part is buried in the deeper tissues when the incision is closed.

Occasionally, the tip of the catheter may pass through the defect in the ICA causing severe hemorrhage. In such cases, withdrawing the catheter for a short distance before advancing it again may enable the catheter to be passed along the artery beyond the defect. If the catheter cannot be advanced beyond the defect, it should be inserted through the arterial defect into the guttural pouch; then the balloon should be inflated and the catheter pulled back firmly against the external wall of the artery and fixed in this position with a ligature just distal to its site of insertion into the artery.

Embolization microcoils can also be placed in the affected artery to bring about therapeutic occlusion (Leveille et al 2000). These microcoils have been used to occlude the ICA as well as branches of the ECA. They are commonly placed with the aid of dynamic fluoroscopy,
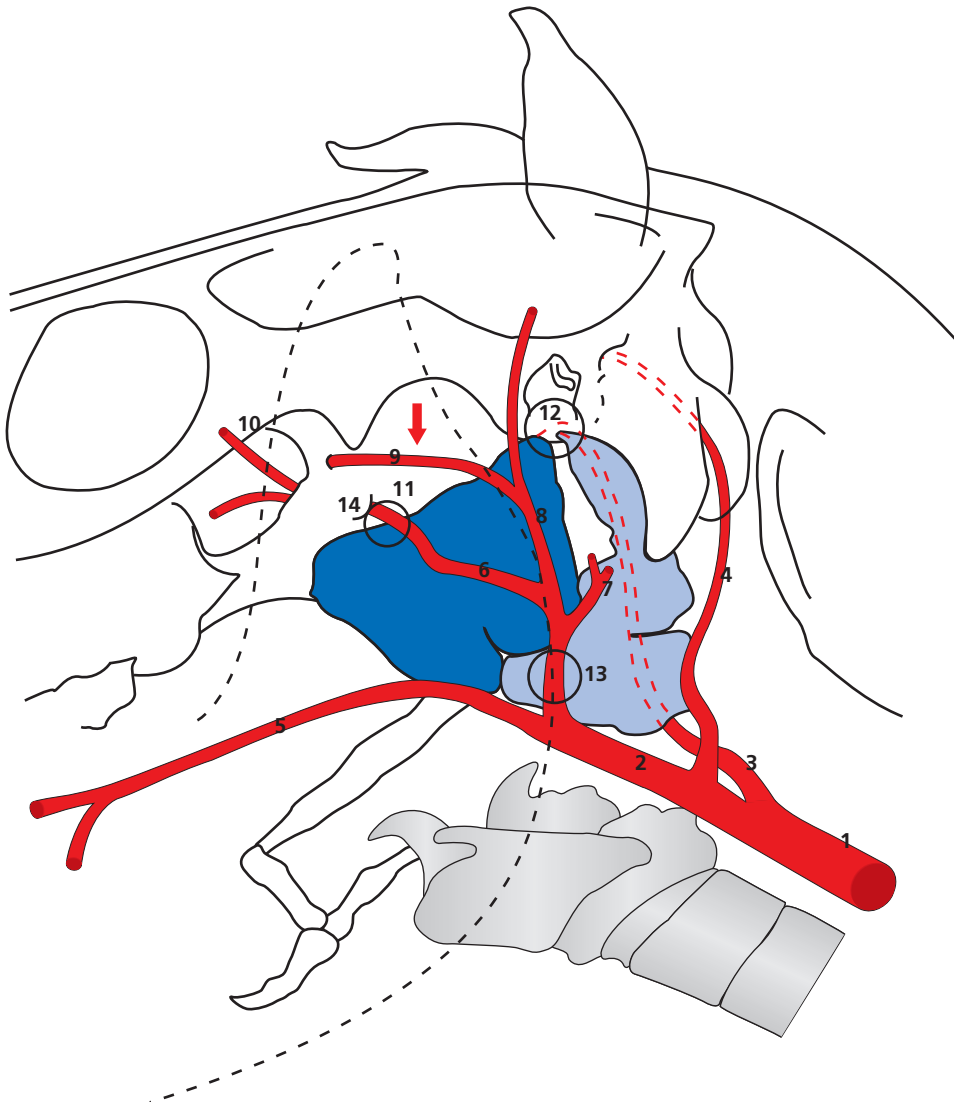
Fig. 28.9. Diagram of the major arteries related to the left guttural pouch and sites of balloon-catheter occlusion. 1 = common carotid artery; 2 = external carotid artery; 3 = internal carotid artery (ICA); 4 = occipital artery; 5 = linguofacial trunk; 6 = maxillary artery; 7 = caudoauricular artery; 8 = superficial temporal artery; 9 = transverse facial artery; 10 = external ophthalmic artery; 11 = caudal alar foramen; 12 = balloon located at sigmoid flexure of ICA; 13 = balloon located in the major palatine artery immediately caudal to caudal alar foramen; 14 = balloon inserted into the transverse facial artery at arrow and directed into the ECA, where it is inflated close to the floor of the guttural pouch. Redrawn from Smith and Barber, 1984, Caron et al, 1987 and Freeman, 1992, with permission.

although they can be positioned using a technique similar to that used to place a balloon-tipped catheter. Contrast arteriography will confirm correct placement of the microcoils and occlusion of the intended artery, but will add expense and time to the procedure.

Although complication rates as high as 46% have been reported with use of long indwelling catheters, most complications, such as incisional infection or catheter breakage, are not life-threatening. Because an infected artery with a transmural lesion is in contact with the non-sterile environment of the guttural pouch, the passage of a catheter, contrast material or lavage fluid past the lesion has the potential to cause thrombi, bacteria or fungi to flow toward the brain. Ragle (2003) reported that one horse out of more than 30 treated using microcoil techniques developed neurological signs 9 days after surgery because of a brain abscess caused by *Streptococcus equi*. At other clinics, horses have died from brain infarction following arterial occlusion, but this sequel has also been recorded in untreated horses with GPM.
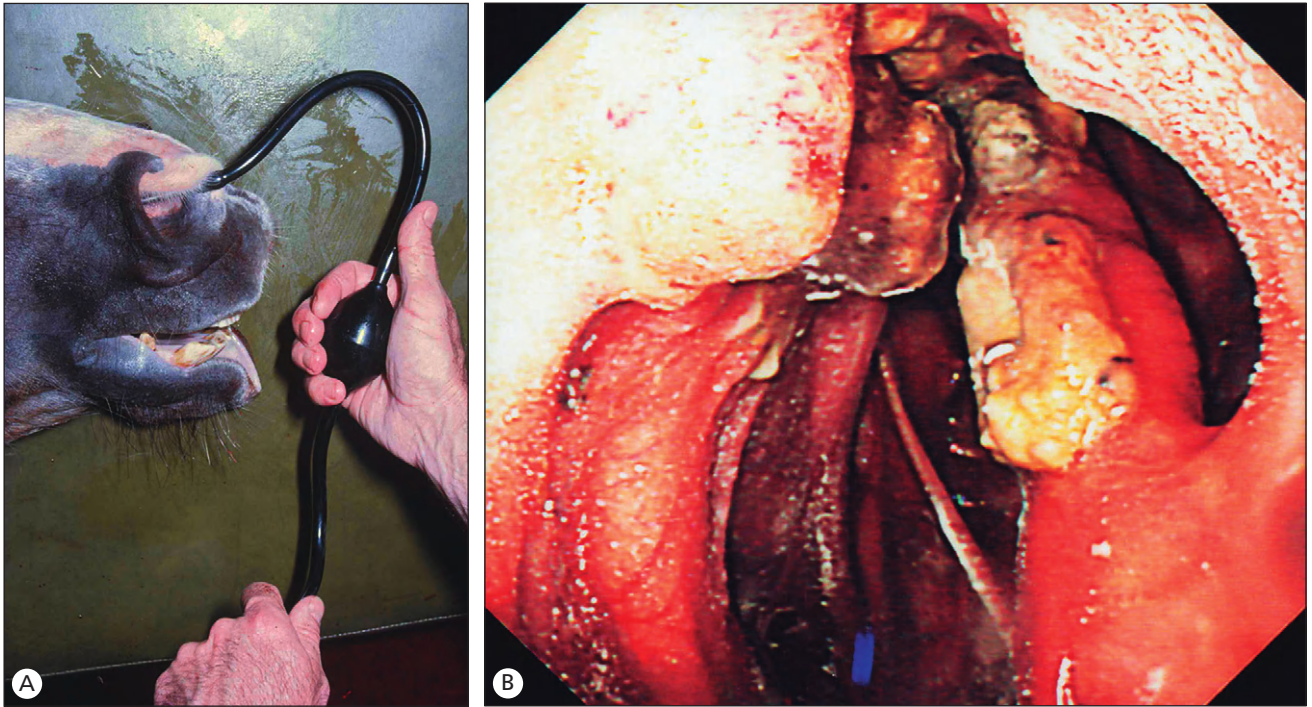
Fig. 28.10. Guttural pouch mycosis. (A) Method of insufflating nystatin powder into the guttural pouch using a Neilson's catheter and Higginson's syringe. (B) Endoscopic view immediately after insufflation showing the powder dispersed throughout the pouch.

Aspergillus fumigatus and *A. nidulans* are opportunistic, angiotrophic pathogens and require damage to the mucosal barrier to permit binding to fibrinogen on the mucosal surfaces. Resolution of mycotic plaques can occur after arterial thrombosis with no other treatment, regardless of whether the thrombosis occurs as the result of surgery or naturally. Nevertheless, one author (GBE) considers that topical application of antimycotic medication, in conjunction with arterial occlusion, is of considerable benefit in accelerating the resolution of infection, and in preventing the development of additional neurological disorders, particularly when the lesion is extensive, whilst the other author (TWG) does not.

Difficulty in applying antimycotic agents (e.g. nystatin, miconazole, or ketoconazole) to the lesion on the roof of the medial compartment of the pouch has been a major reason for topical therapy not being used more frequently. However, insufflation of one of these drugs in powder form via a Neilson's catheter, using a Higginson's enema syringe, coats the whole of the lining of the pouch with the drug. The catheter is placed along the side of the horse's face with the tip level with the lateral canthus of the eye and is then held between finger and thumb at the nostril. It is now passed along the ventral nasal meatus, with the curved tip directed ventrally until the finger and thumb holding it are at the nostril. The catheter is then rotated through 90°, so that the curved tip is in contact with the lateral wall, and it is advanced through the ostium into the guttural pouch. Successful entry of the catheter into the pouch leaves the end of the catheter at the nostril, whereas if the catheter has not entered the pouch, the tip will engage the back of the pharynx leaving several centimeters of the catheter protruding from the nostril. Once the end of the catheter is in place in the guttural pouch, the antimycotic agent is placed in the end of the enema syringe from which the plastic tip has been removed. The enema syringe is attached to the catheter, and the balloon of the syringe is compressed sharply twice. This results in a cloud of antifungal powder leaving the two openings at the end of the Neilson's catheter to effectively coat the whole of the interior of the guttural pouch (Fig. 28.10). The insufflation process may be repeated by detaching the Higginson's syringe and refilling it with the antifungal agent. The procedure, which is well tolerated by the horse, is repeated daily for 7–10 days.

If a mycotic lesion in the lateral wall of the lateral pouch has led to epistaxis, the ECA and its branches must be occluded. Although the ECA can be ligated distal to the origin of the linguofacial trunk through a hyovertebrotomy approach, this procedure is generally unsuccessful because the external carotid and maxillary arteries have numerous collateral channels that allow retrograde flow to the perforated segment.

The palatine artery, which is a large continuation of the maxillary artery, is the most likely source of retrograde flow to the ECA and its branches. This artery can be

occluded by inserting a 6-F Fogarty venous thrombectomy catheter into its lumen 3 cm caudomedial to the upper corner incisor tooth (Triadan 103, 203) (Freeman et al 1989). The catheter is passed retrograde for approximately 40 cm in a 450-kg horse. The balloon is partially inflated with saline, and the catheter is gently retracted until some resistance is felt, at which point it is assumed that the balloon is wedged at the caudal alar foramen. It is subsequently fully inflated with saline. At this site, the balloon should obstruct all retrograde flow to the maxillary artery.

Normograde flow can be prevented either by ligation of the ECA distal to the origin of the linguofacial trunk, which requires deep dissection, or by inserting a balloon-tip catheter through the transverse facial artery (Freeman et al 1989). To perform the latter procedure, a catheter is inserted into the transverse facial artery 3 cm rostral to the articular tubercle of the temporal bone and advanced in retrograde fashion until its tip enters the ECA, which is approximately 12 cm from the arteriotomy site in a 450-kg horse. The balloon is inflated with saline and the redundant ends of the catheters in the transverse facial and palatine arteries are taped and incorporated in a stockinget hood. The catheters are removed after 7–10 days (Fig. 28.9).

Fig. 28.11. Guttural pouch mycosis. Endoscopic view of nasopharynx showing presence of food material as a result of pharyngeal hemiplegia, and left recurrent laryngeal neuropathy.

Management of horses with neurological damage

The second most common clinical sign of GPM is dysphagia manifested in its severe form as discharge of masticated food, saliva, and water from the nostrils and is caused by mycotic damage of the pharyngeal branches of the glossopharyngeal and vagus nerves. The glossopharyngeal nerve is vulnerable because of its prominent position in the fold of mucous membrane that contains the other cranial nerves and the ICA.

On endoscopy, food material can be seen in the nasal cavities or nasopharynx, and there may also be laryngeal hemiplegia (confirming involvement of the vagus) (Fig. 28.11). Persistent dorsal displacement of the soft palate may also be noted. Collapse of the roof of the nasopharynx may be evident in a proportion of horses with GPM. Failure of the guttural pouch ostium on the affected side to open when swallowing is stimulated by touching the epiglottis is more convincing evidence of neuromuscular dysfunction of the nasopharynx. Radiological assessment of deglutition by fluorography or even by simple contrast radiography of the pharynx can also confirm the diagnosis of pharyngeal dysphagia.

Examination of the interior of the pouch will reveal mycotic plaques that are often extensive, extending from the roof of the medial compartment and obscuring the nerves. In the authors' experience, it is rare for a horse to exhibit both dysphagia and epistaxis. This suggests that the ICA may become occluded as the result of chronic inflam-

mation at the site of the mycotic plaque. Occasionally, a black focus in a mycotic plaque overlying the ICA may be seen, indicating that slight hemorrhage has occurred. In these cases arterial ligation should be performed because of the risk of a severe hemorrhage before the mycotic infection has resolved.

Horses with neurological deficits caused by GPM are treated by topical treatment of the lesion with nystatin as described. Although Church et al (1986) suggested that the prognosis for horses with this complication is hopeless, Cook (1968) reported that seven of 17 affected horses recovered from dysphagia, and Lane (1989) reported recovery of five of 17 dysphagic horses following successful treatment of the mycotic lesion. The period from onset of dysphagia to resolution of coughing and nasal reflux of ingesta ranged from 3 to 14 months. Dysphagia is the most difficult aspect of GPM to manage and requires considerable patience and vigorous supportive therapy to meet the nutritional requirements of affected horses.

It is not clear from the literature how often horses with laryngeal hemiplegia regain normal function. Cook (1987) considered that this type of laryngeal hemiplegia was invariably permanent, but Greet (1987) described a single case that recovered laryngeal motility spontaneously. Horses that fail to recover laryngeal motility can be surgically treated as if they had the idiopathic form of laryngeal paralysis. Some horses with Horner syndrome and facial paralysis may slowly regain normal function of the affected nerves.
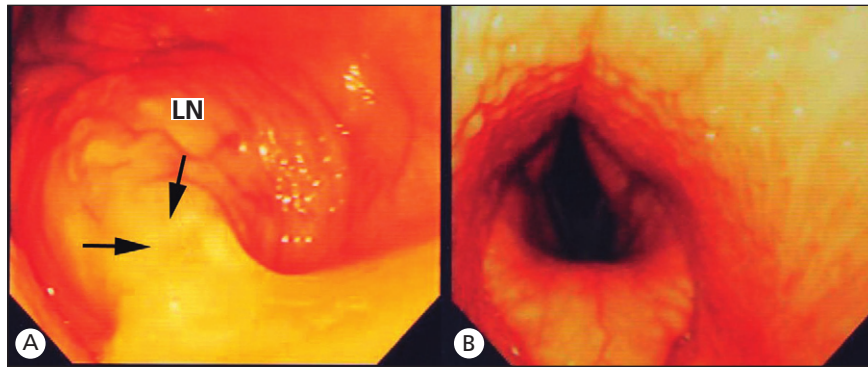
Fig. 28.12. Endoscopic images of a horse with strangles that is suffering from upper airway obstruction because of bilateral collapse of the nasopharynx. (A) An abscessed lymph node (LN) on the floor of a guttural pouch that is discharging pus, (B) the depression on the nasopharyngeal roof caused by these abscessed lymph nodes. (Figure courtesy of P.M. Dixon.)

Guttural pouch empyema

Because they share a common respiratory mucosa with the rest of the airway, inflammation of the guttural pouch mucosa accompanies most upper respiratory tract bacterial and viral infections. However, the normal mucosal defense mechanism and mucociliary clearance mechanism that serve to drain the guttural pouches usually allow rapid resolution of this inflammation. Failure of mucus and/or pus within the pouches to drain satisfactorily leads to guttural pouch empyema (GPE). Empyema of the guttural pouches can affect horses of any age but usually occurs in young animals. Upper respiratory infections, especially those caused by *Streptococcus equi*, and rupture of abscessed retropharyngeal lymph nodes into the guttural pouch are the most commonly implicated causes of GPE (Fig. 28.12). Topical guttural pouch treatment with irritant drugs, fracture of the stylohyoid bone, and congenital stenosis of the pharyngeal orifice are much less frequent causes.

Clinical signs

The clinical signs of GPE include intermittent purulent to mucopurulent nasal discharge, parotid swelling and pain, extended head carriage, and difficulty in swallowing and breathing.

The most common clinical sign of GPE is a muco-purulent nasal discharge, often persisting after the other signs of the upper respiratory tract infection have resolved. Usually only one pouch is involved, which results in an asymmetric nasal discharge, with the heavier discharge emanating from the ipsilateral naris. The discharge may be intermittent and usually increases when the horse lowers its head. External pressure on the parotid area when the head is lowered frequently results in a marked increase in the volume of discharge. The pouches can expand a great deal before enlargement can be seen externally. In the acute stages, when the mucopurulent material is quite fluid

in nature, swelling in the parotid region is absent. However if dysfunction of the guttural pouch drainage occurs, the purulent material becomes thicker and in chronic cases can develop the consistency of cottage cheese. Drainage of this viscous material into the nasopharynx is poor, and accumulation of exudate may eventually result in gross distension of the affected pouch, displacing air from the normal pouch and causing compression of the roof of the nasopharynx, and even severe obstructive dyspnea and dysphagia. If dyspnea is severe, the head and neck are extended to reduce resistance to airflow (Fig. 28.13), and although a harsh inspiratory noise may be audible, very little movement of air can be felt at the nostrils. So-called chondroids, a longer term sequel to inspissation, are ovoid masses of variable size caused by compression of the pus within the guttural pouch by head movement.

Diagnosis

Endoscopy will often show thick, purulent material at the pharyngeal ostium of the affected pouch or pouches. In severe cases, the nasopharyngeal lumen is almost completely obliterated because of depression of its roof by the grossly distended guttural pouch (Fig. 28.14). Any attempt at passing the endoscope into the pouch is usually thwarted by the inspissated material.

Lateral radiographic images of the head will confirm the loss of air contrast with the guttural pouch. If the purulent material is still fluid, an air–fluid interface will be evident, while in cases with inspissated pus the guttural pouch will appear as a large, radiodense area with little or no gas present at its apex. The degree of pharyngeal obstruction can best be evaluated, and is greatest, if the radiograph is taken during inspiration, when subatmospheric pressure produces further collapse of the airway. Chondroids can usually be recognized as marble-like densities within the pouch, aided by the contrast provided by the air present above them (Fig. 28.15). Distension of the parotid region

Fig. 28.13. Typical posture adopted by a horse with airway obstruction caused by severe guttural pouch distension.

and compression of the pharynx are often less marked in horses with chondroids compared to horses with GPE or abscessed retropharyngeal lymph nodes.

Treatment of horses affected with GPE
Non-surgical treatment
In patients with acute GPE, treatment with systemic antibiotics coupled with feeding off the floor to encourage natural drainage of the guttural pouches often brings about resolution within 10–14 days. There is some controversy concerning the administration of antibiotics to horses with strangles. If this treatment is unsuccessful,

for example because abscessed retropharyngeal lymph nodes are present in the floor of the pouch or the purulent material is too thick to drain freely, daily irrigation of the pouch with 500 ml of physiological saline or even tap water is indicated. This procedure is facilitated by placing an indwelling Foley catheter or a coiled catheter created from a 10-French polypropylene male dog catheter (White & Williamson 1987) into the affected pouch(es). The catheter is threaded with 18-gauge wire and the end is coiled around a 5-ml syringe case four times and all are then placed in boiling water for 10 min, then cold water for a further 10 min to obtain a permanent spiral configuration.
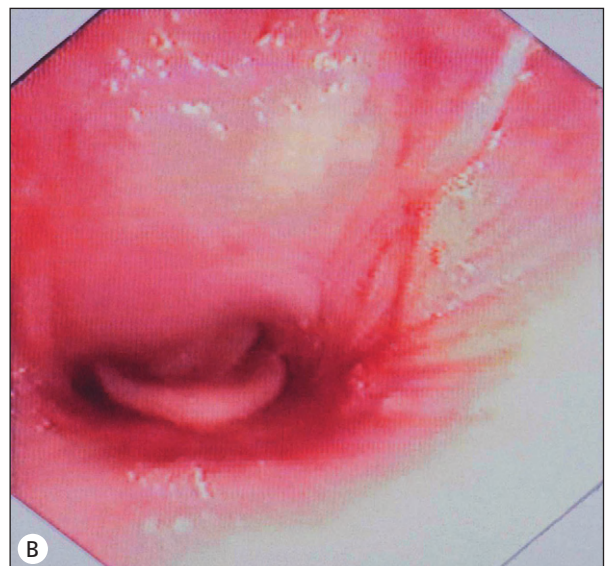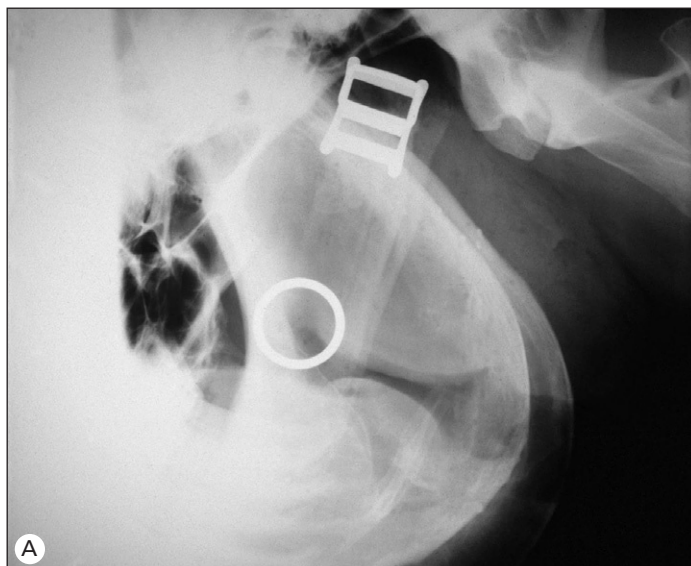
Fig. 28.14. Guttural pouch empyema. (A) Lateral radiographic projection showing opacity of the guttural pouch and almost complete obstruction of the nasopharynx. (B) Endoscopic view of the nasopharynx in the same horse.
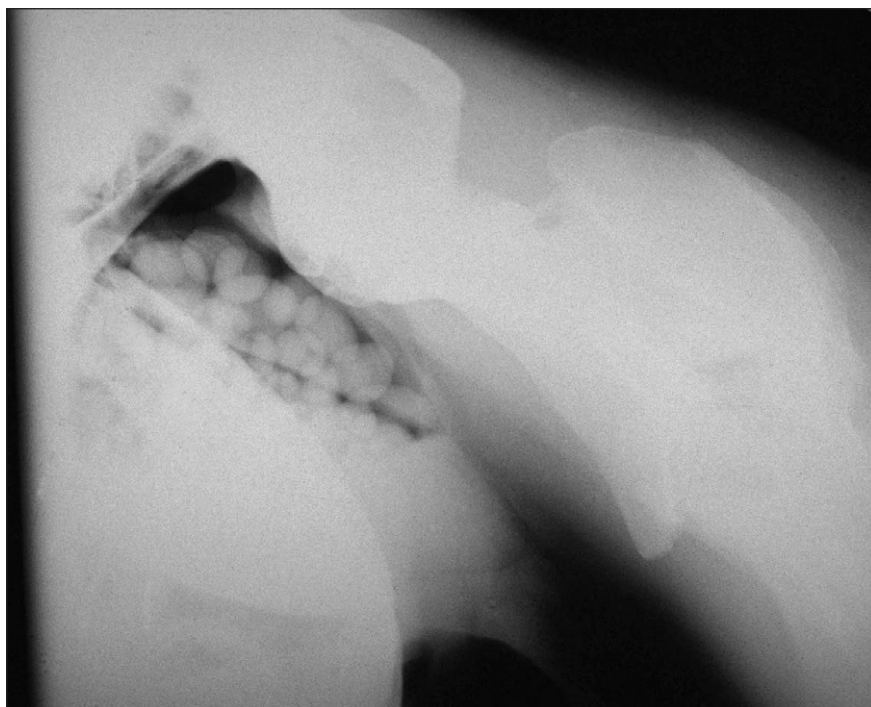
Fig. 28.15. Guttural pouch empyema. Lateral radiograph of the guttural pouch showing a large number of chondroids.

The catheter is straightened with the wire still in place and placed into the guttural pouch. Removal of the wire causes the end of the catheter to resume its spiral shape and it is then retained within the pouch.

This lavage technique has been used to remove chondroids, when the chondroids are small and few in number (Adkins et al 1997). Daily lavage is necessary to bring about dissolution of the aggregated masses of purulent material. Care should be taken to avoid using hydrogen peroxide or antiseptic solutions because they are irritant and can possibly damage the cranial nerves but the addition of acetyl cysteine can be beneficial. Transendoscopic removal of chondroids using a snare has been reported, but is difficult and very time-consuming unless small numbers of chondroids are present.

Surgical treatment of horses affected with GPE

Horses with a large number of chondroids or a large amount of inspissated pus require surgical evacuation of the pouch, and those showing severe dyspnea as a result of pharyngeal compression should be treated promptly. No attempt should be made to administer a general anesthetic until a temporary tracheotomy tube has been inserted. The authors insert a 14-mm, cuffed endotracheal tube in the mid-cervical trachea, just before induction of anesthesia (Fig. 28.16), and use this tube to administer the gaseous anesthetic. If inserted higher in the trachea, the tube tends to encroach on the surgical site.

The objective of surgical treatment is the complete removal of all the exudate. Retention of semi-solid material

Fig. 28.16. Guttural pouch empyema. Endotracheal tube placed in the trachea prior to induction of anesthesia.

or a single chondroid may predispose the horse to a recurrence of GPE, and will probably result in the horse being a carrier of *Streptococcus equi* subsp. *equi* in those cases where this is the causal organism. Several surgical approaches have been employed, but the authors consider the modified Whitehouse approach, described earlier, to be the most effective. When the pouch is very distended, there is little difficulty in identifying it in the depth of the incision. If the pouch is less distended, introducing air into it via an endoscope or an enema syringe attached to a Neilson's catheter can be helpful. Once the pouch is entered, its contents are carefully removed. A spoon is often useful for this purpose, particularly for removal of chondroids. Gentle handling of the pouch wall is imperative to avoid iatrogenic neurovascular injury. Inspissated material can be removed by copious lavage and suction. The pouch should be examined very carefully to ensure that no material, particularly chondroids, is left behind.

An indwelling Foley catheter can be inserted via the surgical incision to allow daily lavage of the pouch. After it has been removed, the surgical wound is left open to drain and heal by second intention.

Endoscopic examination of the pouch after treatment of empyema reveals thickening of the mucous membrane lining that tends to have a dull, yellowish appearance; this may obscure the underlying vessels and nerves, which are normally clearly visible. After the infection is resolved, the mucous membrane will regain its normal appearance and any dysphagia (that was probably mechanical rather than neurological) will soon resolve. Very rarely, some degree of dysphagia will remain that may be neurological in origin.

Chronic diverticulitis

As noted earlier in this chapter, chronic inflammation of the guttural pouch mucosa is common in horses with chronic GPE, but similarly thickened mucosa may also be encountered even though the horse has no history to suggest prior streptococcal respiratory infection. Cases of chronic diverticulitis without the presence of empyema may present as a series of neuropathies where a combination of deficits of the glossopharyngeal, vagus, facial, spinal accessory, and sympathetic nerves may be present. Endoscopy reveals a similar roughened thickening of the guttural pouch lining, suggesting that the nerve pathways are damaged by extension of the inflammatory process within the wall of the pouch.

Other disorders of the guttural pouch

Guttural pouch hemorrhage can also be associated with rupture of the rectus capitis ventralis and longus capitus muscles (i.e. the ventral straight muscles of the head) or avulsion fracture of the basisphenoid bone where these muscles attach to it. Rearing over backwards and trauma to the base of the skull are often associated with rupture of these two muscles. On endoscopic examination blood may be seen emerging from the ostia of one or both guttural pouches and the guttural pouch may be blood-filled. It is important to inspect both the rectus capitis muscles endoscopically (if visible) and the occipital and basisphenoid bones radiographically to distinguish this condition from GPM.

Temporohyoid osteoarthropathy

Temporohyoid osteoarthropathy is a disorder that can result in acute onset of vestibular disease, which is often accompanied by facial paralysis. Other clinical signs include head shaking and pain associated with palpation of the base of the ear. Endoscopic examination of the guttural pouch is useful because osseous proliferation or healing fractures of the proximal third of the stylohyoid bone often accompanies the disease. Temporohyoid osteoarthropathy was thought to originate from a low-grade otitis media, which then progressed to an osteitis of the tympanic bulla and temporohyoid joint (Power et al 1983, Blythe & Watrous 1997), but otitis is now not considered to be present in most such cases.

Vestibular disease and facial nerve (all branches) paralysis occur when osseous proliferation impinges on the VII (facial) and VIII (vestibulocochlear) nerves, or if the petrous temporal bone fractures, as described below. The spontaneous development of cranial nerve VII and/or VIII dysfunction in horses should prompt an examination of the guttural pouch. Progression of the disease leads to ankylosis of the temporohyoid joint. With the loss of mobility of the joint, the normal forces generated from movement of the tongue and larynx and transmitted through the bones of the hyoid apparatus can cause an acute stress fracture of the petrous temporal bone, or, less frequently, a mid-shaft fracture of the stylohyoid bone that will also be endoscopically evident in the guttural pouch. Blythe et al (1994) and Blythe and Watrous (1997) described a technique for partial resection of the stylohyoid bone that created a pseudoarthrosis to prevent the sequelae of petrous temporal bone fracture. Following regrowth of the stylohyoid and recurrence of symptoms, Pease et al (2004) developed an alternative technique of ceratrohyoidectomy, which they considered to be a safer, easier, and more permanent procedure.

Otitis media/interna caused by bacterial infection is not commonly encountered in horses, and fungal infection is even rarer (Firth 1977). Although treatment of horses with otitis media/interna caused by bacterial infection with antimicrobial therapy is reported to be successful (Mayhew 1989), there are no reports of successful treatment of fungal-induced otitis media (Newton & Knottenbelt 1999).
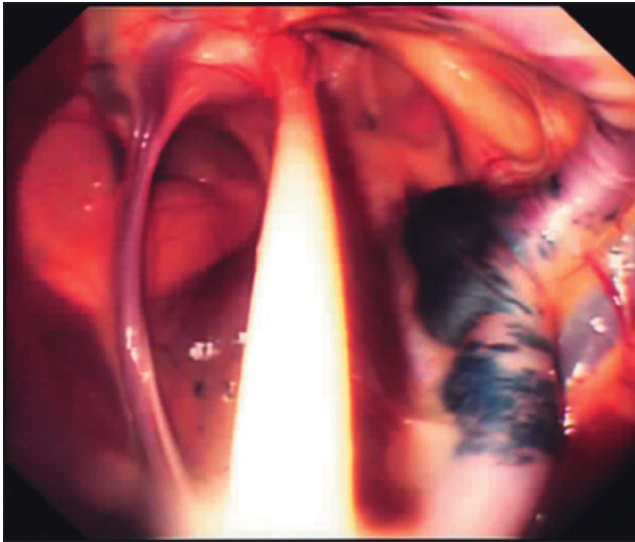
Fig. 28.17. Endoscopic view of a guttural pouch with melanomas.

Neoplasia

Neoplasia of the guttural pouch is uncommon. Reported tumors include parotid melanoma, squamous cell carcinoma (Trigo & Nickels 1981, Moulton 1990), round cell sarcoma (Cook 1973), fibroma (Merriam 1972), hemangioma (Greene & O'Connor 1986), and hemangiosarcoma (Raker 1982).

Clinical signs of neoplasia include non-specific signs of guttural pouch disease. Endoscopy may reveal dorsal pharyngeal compression, mucopurulent discharge, drainage from the pharyngeal ostium, or single or multiple masses within the pouch.

Melanotic lesions are often present in the guttural pouches of gray horses (Fintl & Dixon 2001), more particularly in the lateral compartment (Fig. 28.17). At this site two distinct types of lesion can be identified. The first are benign-looking, small, flat, irregular patches of black pigment, often in large numbers that are located on the walls of the major vessels. These are probably not tumors but represent abnormal accumulations of normal melanocytes (i.e. a hamartoma). Larger, well-defined tumor-like masses of black tissue represent a more serious and an obviously neoplastic disorder (Knottenbelt 1994). These masses are often located high in the lateral compartment. Their significance is equivocal, but extension from the pouch to the parotid lymph nodes and lymphoid tissue of the salivary gland is possibly responsible for the externally obvious form of the disease. Alternatively, spread may occur in the opposite direction (Fintl & Dixon 2001).

REFERENCES

Adkins AR, Yovich JV, Colborne CM 1997 Non-surgical treatment of chondroids of the guttural pouch of the horse. Australian Veterinary Journal 75: 332–333

Baptiste KE, Naylor JM, Bailey J et al 2000 A function for guttural pouches in the horse. Nature 403: 382–383

Blazyczek I, Hamann H, Deegan E et al 2004a Retrospective analysis of 50 cases of guttural pouch tympany. Veterinary Record 154: 261–264

Blazyczek I, Hamann H, Ohnesorge B et al 2004b Inheritance of guttural pouch tympany in the Arabian horses. Journal of Heredity 95: 195–199

Blythe LL, Watrous BJ 1997 Temporohyoid osteoarthropathy (middle ear disease) In: Robinson NE (editor) Current Therapy in Equine Medicine, 4th edn. Saunders, Philadelphia, pp.323–325

Blythe LL, Watrous BJ, Shires GM et al 1994 Prophylactic partial styloidostectomy for horses with osteoarthropathy of the temporohyoid joint. Journal of Equine Veterinary Science 14: 32–37

Caron JP, Fretz PD, Bailey JV et al 1987 Balloon tipped catheter occlusion for prevention of haemorrhage caused by GPM: 13 cases (1982–1985). Journal of the American Veterinary Medical Association 191: 345–349

Church S, Wyn-Jones G, Parks AH et al 1986 Treatment of guttural pouch mycosis. Equine Veterinary Journal 18: 362–365

Colles CM, Cook WR 1983 Carotid and cerebral angiography in the horse. Veterinary Record 113: 483–489

Cook WR 1968 The clinical features of guttural pouch mycosis in the horse. Veterinary Record 83: 336–345

Cook WR 1973 The auditory tube diverticulum (guttural pouch) in the horse: its radiographic examination. Journal of American Veterinary Radiology Society 14: 51–71

Cook WR 1987 Diseases of the auditory tube diverticulum (guttural pouch). In: Robinson NE (editor) Current Therapy in Equine Medicine, 2nd edn. Saunders, Philadelphia, pp.612–618

Dixon PM, Rowlands AC 1981 Atlanto-occipital joint infection associated with guttural pouch mycosis in a horse. Equine Veterinary Journal 13: 260–262

Fintl C, Dixon PM 2001 A review of five cases of parotid melanoma in the horse. Equine Veterinary Education 13: 17–24

Firth EC 1977 Vestibular disease and its relationship to facial paralysis in the horse; a clinical study of 7 cases. Australian Veterinary Journal 53: 560–565

Freeman DE 1992 Guttural pouches In: Auer JA (editor) Equine Surgery. Saunders, Philadelphia, pp.480–488

Freeman DE, Donawick WJ 1980a Occlusion of the internal carotid artery in the horse by means of a balloon tipped catheter: evaluation of a method designed to prevent epistaxis caused by guttural pouch mycosis. Journal of the American Veterinary Medical Association 176: 232–235

Freeman DE, Donawick WJ 1980b Occlusion of the internal carotid artery in the horse by means of a balloon tipped catheter. Clinical use of a method to prevent epistaxis caused by GPM. Journal of the American Veterinary Medical Association 176: 236–240

Freeman DE, Ross MW, Donawick WJ, Hamir AN 1989 Occlusion of the external carotid and maxillary arteries in the horse to prevent haemorrhage from guttural pouch mycosis. Veterinary Surgery 18: 39

Greene HJ, O'Connor JP 1986 Haemangioma of the guttural pouch of a 16 year old Thoroughbred mare. Clinical and pathological findings. Veterinary Record 118: 445

Greet TRC 1987 Outcome of treatment in 35 cases of guttural pouch mycosis. Equine Veterinary Journal 19: 483–487

Klebe EA, Holcombe SJ, Rosenstein D et al 2005 The effect of bilateral glasso-pharyngeal nerve anaesthesia on swallowing in horses. Equine Veterinary Journal 37: 65–70

Knottenbelt DC 1994 Conditions of the respiratory tract – neoplastic disorders. In: Knottenbelt DC, Pascoe RR (editors) A Colour Atlas of Diseases and Disorders of the Horse. Mosby Wolfe, London, p.155

Lane JG 1989 The management of guttural pouch mycosis. Equine Veterinary Journal 21: 321–324

Leveille R, Hardy J, Robertson JT et al 2000 Transarterial coil embolisation of the internal and external carotid and maxillary arteries for prevention of haemorrhage from guttural pouch mycosis in horses. Veterinary Surgery 29: 389–397

Mayhew IG 1989 Head tilt, circling, nystagmus and other signs of vestibular abnormalities. Large Animal Neurology: A Handbook for Veterinary Clinicians. Lea and Febiger, Philadelphia, pp.179–192

Merriam JG 1972 Guttural pouch fibroma in a mare. Journal of the American Veterinary Medical Association 161: 487

Moulton JE 1990 Tumours of the respiratory system In: Moulton JE (editor) Tumours in Domestic Animals, 3rd edn. University of California Press, Los Angeles, p.318

Newton SA, Knottenbelt DC 1999 Vestibular disease in two horses; a case of mycotic otitis media and a case of temporohyoid osteoarthropathy. Veterinary Record 145: 142–144

Owen R 1974 Epistaxis prevented by ligation of the internal carotid artery in the guttural pouch. Equine Veterinary Journal 6: 143–149

Pease AP, van Bierliet J, Dykes NL et al 2004 Complications of partial stylohyoidectomy for treatment of temporohyoid osteoarthropathy and an alternative surgical technique in 5 cases. Equine Veterinary Journal 36: 546–550

Pirie M, Pirie HM, Wright NG 1990 A scanning electron microscopic study of the equine upper respiratory tract. Equine Veterinary Journal 22: 333–337

Power HT, Watrous BJ, deLaHunta A 1983 Facial and vestibulocochlear nerve disease in six horses. Journal of the American Veterinary Medical Association 183: 1076–1080

Ragle CA 2003 Guttural pouch disease. In: Robinson NE (editor) Current Therapy in Equine Medicine, 5th edn. Saunders, Philadelphia, pp.386–390

Raker CW 1982 The nasopharynx. In: Mannsmann RA, McAllister ES (editors) Equine Medicine and Surgery. CA American Veterinary Publications, Santa Barbara, p.756

Sisson S 1975 Sense organs and common integument – Ear. In: Getty R, Sisson S, Grossman (editors) The Anatomy of Domestic Animals, 5th edn. Saunders, Philadelphia, pp.723–725

Smith DM, Barber SM 1984 Guttural pouch haemorrhage associated with lesions of the maxillary artery in two horses. Canadian Veterinary Journal 25: 239

Tate LP, Blikslager AT, Little ED 1995 Transendoscopic laser treatment of guttural pouch tympany in eight foals. Veterinary Surgery 24: 367–372

Trigo RJ, Nickels FA 1981 Squamous cell carcinoma of a horse's guttural pouch. Modern Veterinary Practice 62: 456

Walmsley, JP 1988 A case of atlanto-occipital arthropathy following guttural pouch mycosis in the horse. The use of radioisotope bone scanning as an aid to diagnosis. Equine Veterinary Journal 20: 219–220

White SL, Williamson L 1987 How to make a retention catheter to treat guttural pouch empyema. Veterinary Medicine 82: 76–82

29 Disorders of the Nasopharynx and Soft Palate

Susan J Holcombe and Norm G Ducharme

Introduction

The physiologically distinct functions of breathing, deglutition, and vocalization in the horse rely on the carefully orchestrated action and anatomic arrangement of the nasopharynx, the common conduit through which both air and ingesta pass. Simply conceived, it is a muscular tube suspended rostrally from the pterygoid and palatine bones, and anchored caudally on portions of the hyoid apparatus and the laryngeal cartilages. Its main body lacks the structural support of bones or cartilage. During strenuous exercise, airflow velocities and airway pressures fluctuate tremendously, and appropriate sensory and motor activity must occur to maintain airway patency. Failure of these anatomic structures or neuromuscular activities results in a constellation of clinical disorders, ranging from dysphagia to exercise intolerance.

Anatomy of the Nasopharynx

While the nasopharynx may be simply conceptualized as a muscular tube, it is an anatomically and functionally complicated structure composed of multiple muscle groups, innervated by several cranial nerves, and subject to the activity, anatomy, and the effects of pathological changes of surrounding structures. At the microscopic level, the nasopharyngeal mucosa is comprised of a pseudostratified columnar epithelium interspersed with goblet cells, lymphoid follicles and sensory receptors of the glossopharyngeal (CN IX) and trigeminal (CN V) cranial nerves. These are principally tactile receptors important for airway protection, stimulating the swallowing (gag) reflex, and for airflow detection and stimulating airway dilation during inhalation.

The muscular conduit itself comprises groups of constrictor muscles: i.e. the superior pharyngeal constrictors (palatopharyngeus and pterygopharyngeus), the middle pharyngeal constrictor (hyopharyngeus), and the caudal pharyngeal constrictors (thyropharyngeus and cricopharyngeus). The stability of this muscular conduit depends on the appropriate conformation and function being present in the surrounding tissue structures. Each of these structures will be considered in detail.

Dorsal pharyngeal muscles

The actions of the dorsal pharyngeal constricting muscles and the stylopharyngeus muscle are responsible for stiffening and dilating the nasopharynx (Kuna et al 1999, Feroah et al 2000, Kuna 2001). The middle pharyngeal constrictor (hyopharyngeus muscle) and superior pharyngeal constrictors (palatopharyngeus and pterygopharyngeus muscles) form the dorsal and caudolateral pharyngeal walls (Fig. 29.1) (Kuna et al 1999, Feroah et al 2000). The contraction and shortening of these muscles form a sphincter, which moves the food bolus caudally into the esophagus during swallowing. During breathing, these muscles have tonic and phasic expiratory activities that help to support the nasopharynx (Kuna et al 1999, Kuna 2001).

The major dilating muscle of the dorsal nasopharynx is the stylopharyngeus muscle (Feroah et al 2000). This muscle originates on the axial aspect of the distal portion of the stylohyoid bone and courses rostroventrally to ramify into the wall of the dorsal nasopharynx, by passing between the pterygopharyngeus and palatopharyngeus muscles (Fig. 29.2). Contraction of the stylopharyngeus muscles retracts the pharyngeal wall dorsally, to receive a food bolus into the pharynx during swallowing (Sisson 1975a). In a similar manner during breathing, contraction of the stylopharyngeus muscle retracts the nasopharyngeal wall dorsally, thereby supporting the dorsal wall of the nasopharynx and preventing dynamic collapse of this structure during inspiration (Fig. 29.3) (Feroah et al 2001). Experimentally creating stylopharyngeus muscle dysfunction causes collapse of the dorsal nasopharynx and inspiratory obstruction in exercising horses (Tessier et al 2004).

Soft palate

The nasopharynx is demarcated by the soft palate, which completely divides the pharynx into nasal and oral compartments in the horse. Because the horse is an obligate nasal breather, it is critically important that the soft palate remains ventral to the epiglottis (except during swallowing) to allow unimpeded nasal breathing. The soft palate extends caudally from the hard palate to the base of the
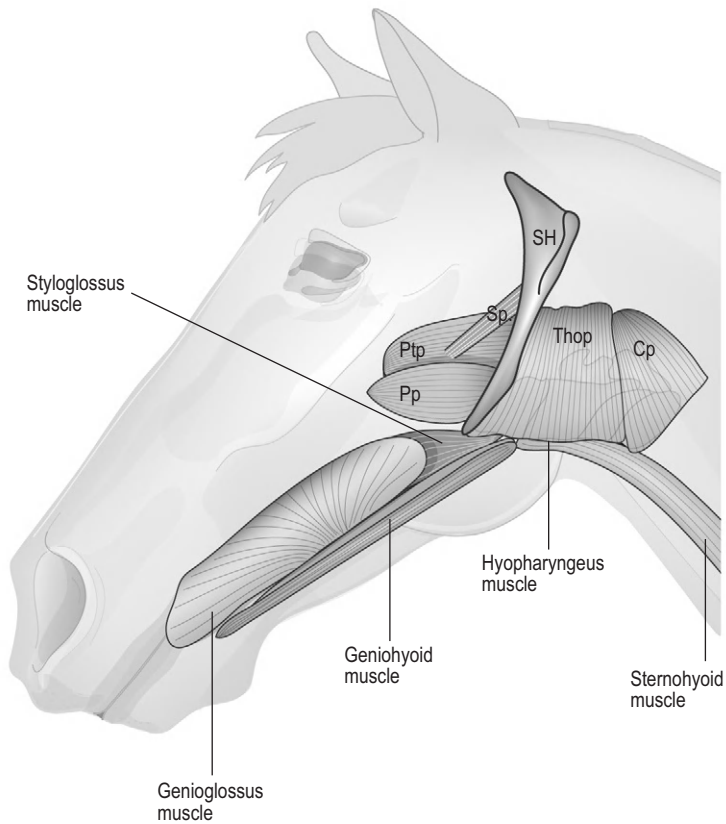
Fig. 29.1. Illustration of the lateral projection of a horse's head depicting the nasopharyngeal constricting and dilating muscles. Cp = cricopharyngeus muscle; Pp = palatopharyngeus muscle; Ptp = pterygopharyngeus muscle; SH = stylohyoid bone; Sp = stylopharyngeus muscle; Thop = thyropharyngeus muscle.
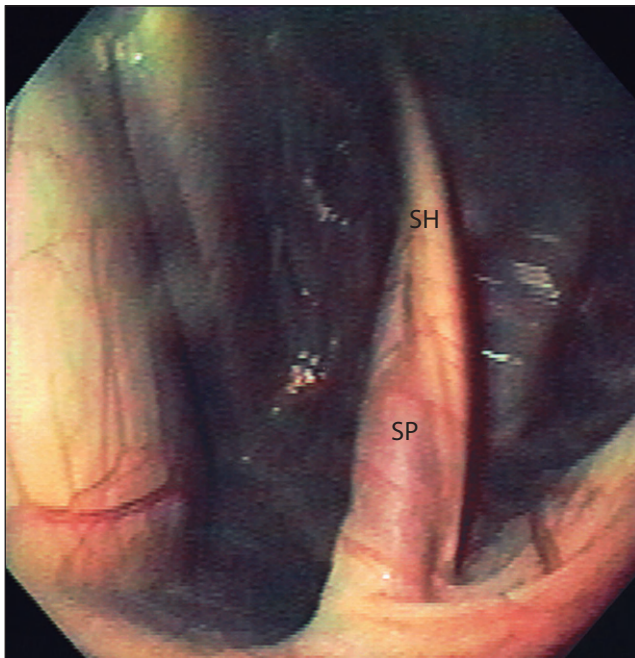
Fig. 29.2. Endoscopic image of the left guttural pouch showing the origin of the stylopharyngeus muscle (SP) on the stylohyoid bone (SH) within the medial compartment.
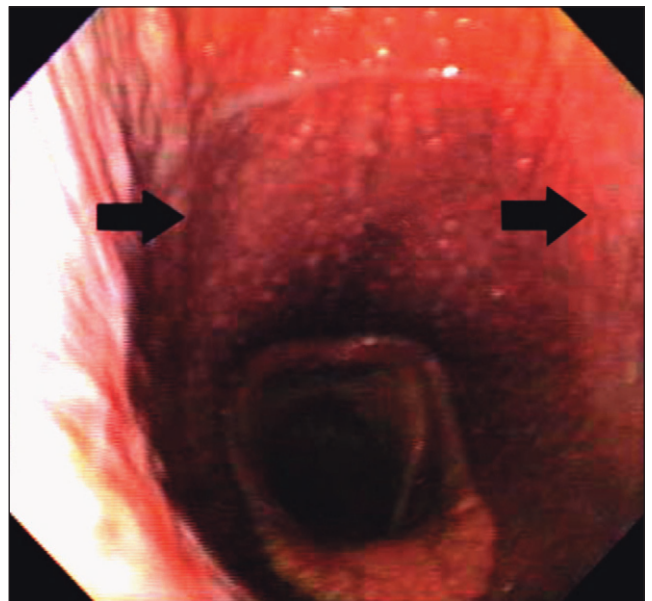
Fig. 29.3. Endoscopic image of the nasopharynx of a horse during inhalation showing the depressions (arrows) that form in the dorsal nasopharyngeal wall as both of the stylopharyngeus muscles contract, lifting the nasopharyngeal wall dorsally. This is especially evident immediately after swallowing.
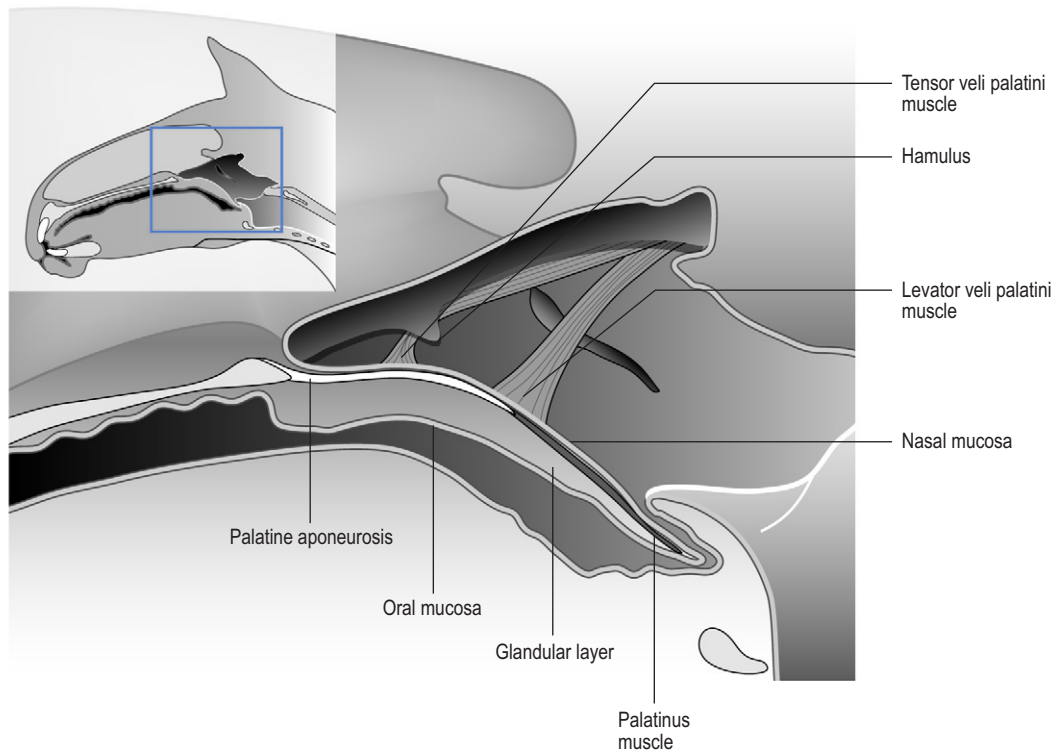
Fig. 29.4. Illustration of the cross-sectional anatomy of the soft palate.

larynx and consists of the oral mucous membrane, which contains ductile openings of the palatine glands, the palatine glands, the palatine aponeurosis, palatinus and palatopharyngeus muscles, and the nasopharyngeal mucous membrane (Fig. 29.4) (Sisson 1975a). The caudal free margin of the soft palate continues dorsally, on either side of the larynx, forming the lateral pillars of the soft palate. These pillars unite dorsal to the larynx, forming the posterior pillar of the soft palate (the palatopharyngeal arch) (Fig. 29.5).

The position of the soft palate is determined by the coordinated activity of groups of antagonistic muscles, which include the levator veli palatini, tensor veli palatini, palatinus, and palatopharyngeus muscles (Fig. 29.4) (Sisson 1975a, Kuehn et al 1982). The levator veli palatini muscle acts to elevate the soft palate during swallowing and vocalization. The action of the levator veli palatini muscle can be seen during endoscopic examination of the upper airway when the gag reflex is stimulated. A "sling" forms within the nasopharynx as the nasopharynx contracts into a sphincter (Fig. 29.6). The tensor veli palatini is a flat, fusiform muscle that travels with the levator veli palatini muscle along the lateral walls of the nasopharynx and the lateral lamina of the guttural pouch (Sisson 1975a). Its tendon is reflected around the hamulus of the pterygoid
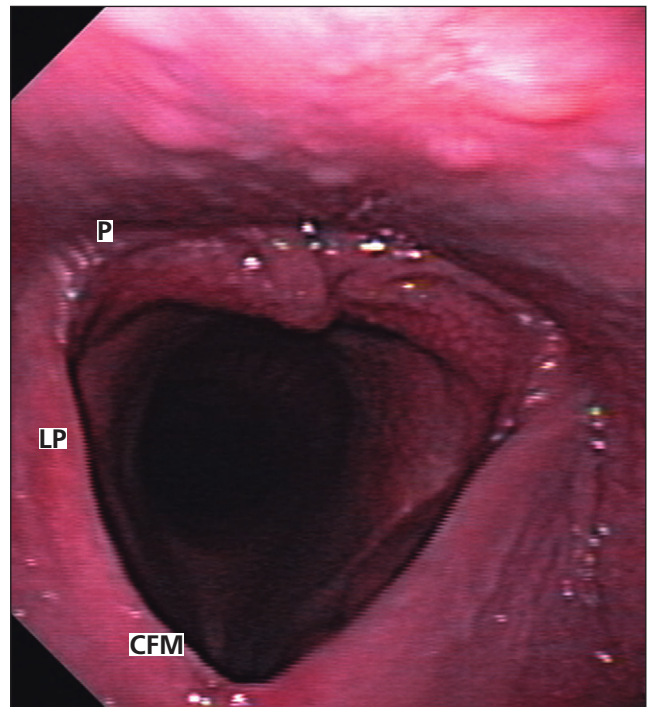
Fig. 29.5. Endoscopic image of the nasopharynx of a horse while the soft palate is displaced. Note the caudal free margin (CFM) of the soft palate, the lateral pillars (LP) and the palatopharyngeal arch (P).
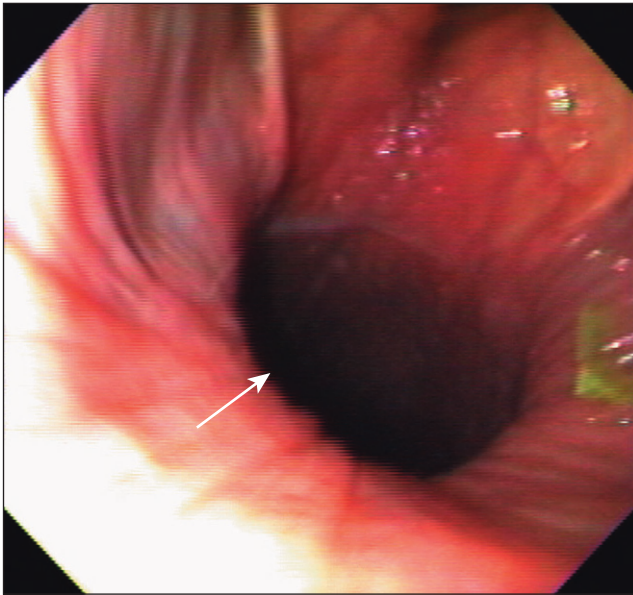
Fig. 29.6. Endoscopic image of the nasopharynx of a horse as the levator veli palatini muscles are contracting (arrow). Notice the "sling" that forms within the nasopharynx.
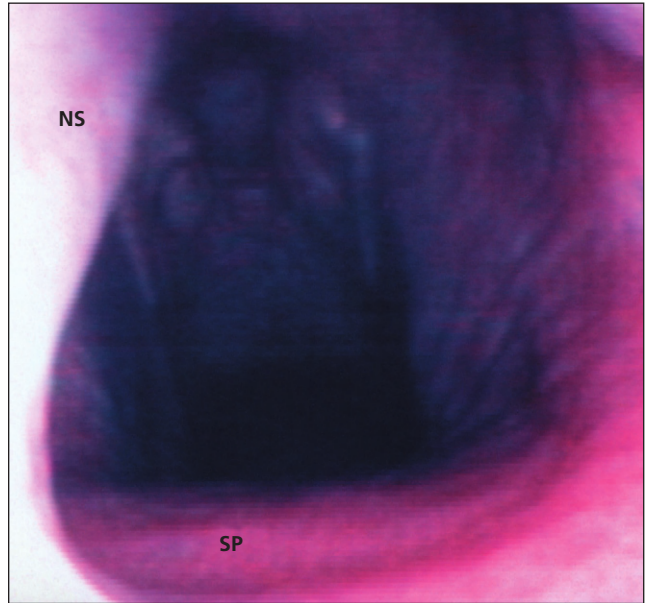
Fig. 29.7. Endoscopic image of the rostral portion of the nasopharynx showing the action of the tensor veli palatini muscle tensing and depressing the rostral portion of the soft palate (SP). NS = nasal septum.

bone, where it is lubricated by a bursa. The tendon then ramifies in the palatine aponeurosis (Sisson 1975a). Contraction of this muscle tenses the palatine aponeurosis and, therefore, the rostral portion of the soft palate, and so depresses this portion of the soft palate toward the tongue (Fig. 29.7) (Kuehn et al 1982, Sisson 1975a). Contraction of the tensor veli palatini muscle also aids in opening the pharyngeal opening of the guttural pouch (Fig. 29.8) (Baptiste 1997).

The palatinus muscle (uvula retractor muscle) consists of two fusiform muscles that lie on either side of midline, in the soft palate beneath the nasopharyngeal mucosa, extending caudally from the hard palate (Sisson 1975a). The muscles attach to the caudal aspect of the palatine aponeurosis and terminate near the caudal free margin of the soft palate. A small muscle bundle arising from the lateral aspect of each muscle continues a short distance caudodorsally into the palatopharyngeal arch. Contraction of the palatinus muscle shortens the soft palate (Sisson 1975a, Kuehn et al 1982).

The palatopharyngeus muscle originates from the palatine aponeurosis and the lateral border of the palatinus muscle (Sisson 1975a). It travels caudally along the lateral wall of the nasopharynx to the pharyngeal raphe, forming part of the superior constrictor muscle group. Contraction of this muscle shortens the soft palate and draws the larynx and esophagus toward the base of the tongue. Dysfunction of the palatinus and palatopharyngeus muscles has been implicated in the pathogenesis of intermittent dorsal displacement of the soft palate and dysphagia in horses (Holcombe et al 1998).
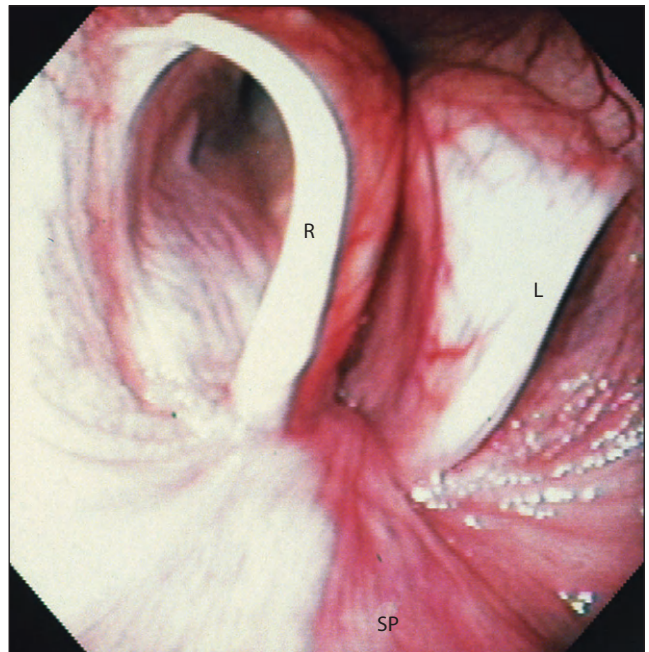
Fig. 29.8. Endoscopic image of the nasopharynx during swallowing showing opening of the right (R) and left (L) ostia of the guttural pouches, as a result of contraction of the tensor veli palatini muscles. SP = soft palate.

Tongue

The tongue is integral to positioning the hyoid apparatus, one of the key support structures of the nasopharynx. There are three intrinsic tongue muscle: the genioglossus,
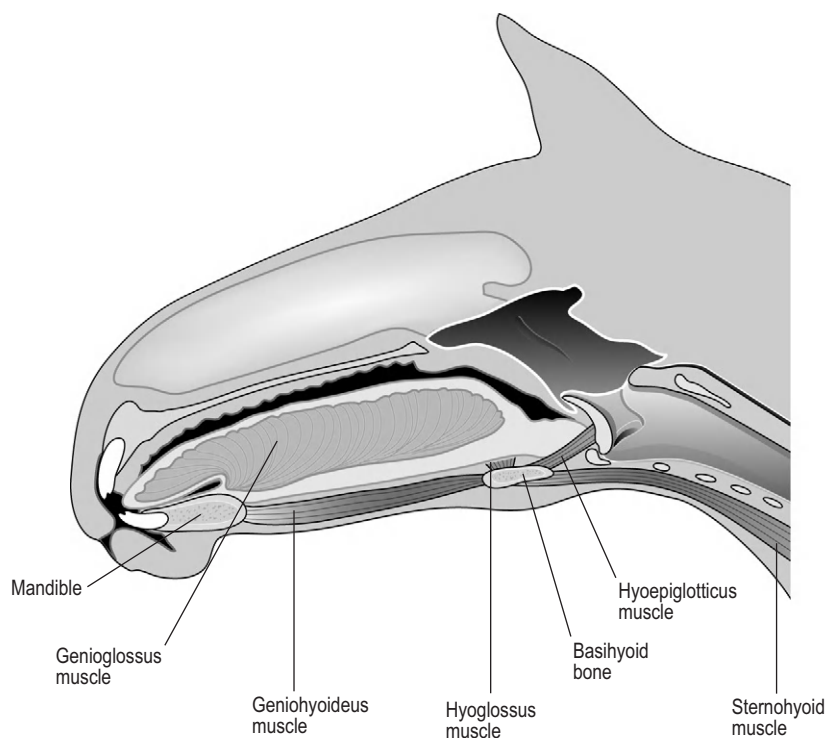
Fig. 29.9. Illustration of the cross-sectional anatomy of the hyoid region.

hyoglossus, and styloglossus. The genioglossus is the largest intrinsic tongue muscle and originates from the medial surface of the mandible, just caudal to the symphysis (Fig. 29.9) (Sisson 1975a) and its muscle fibers radiate rostrally toward the tip of the tongue, dorsally, and distally toward the root of the tongue. The hyoglossus is a flat wide muscle that lies in the lateral portion of the base of the tongue (Sisson 1975a). It originates from the lateral aspect of the basihyoid bone and from portions of the stylohyoid and thyrohyoid bones (Sisson 1975a). The styloglossus muscle originates at the distal lateral aspect of the stylohyoid bone and travels the length of the tongue, along its lateral aspect (Sisson 1975a). Near the tip of the tongue the paired muscle meets and ramifies with fibers of other tongue muscles.

Contraction of the styloglossus retracts the tongue. Contraction of the genioglossus muscle protracts the tongue and pulls the basihyoid bone rostrally. The genioglossus also acts with the hyoglossus muscle to depress and retract the tongue. Hyoglossus and genioglossus activity are synchronous with respiration, and activity of these muscles correlates well with increases in pharyngeal airway size during breathing (Brouillette & Bradley 1980, Mathew et al 1984a, Fregosi & Fuller 1997, Fuller et al 1999). Hypoxia, hypercapnia, and airway occlusion cause parallel increases in electrical activity of the protrudor and retractor muscles of the tongue. Indeed, retraction and depression of the tongue improves airflow, func-

tion, and enhanced pharyngeal stability in several species (Brouillette & Bradley 1980, Fuller et al 1999). Therefore, it seems that tongue depression may be the critical force needed to dilate and stabilize the nasopharynx in horses. This is perhaps why the use of a tongue-tie has been shown to be ineffective in expanding the dimensions of the nasopharynx or preventing dorsal displacement of the soft palate in some affected horses (Cornelisse et al 2001, Franklin et al 2002).

Hyoid muscles

Other muscles that attach to the hyoid apparatus and critically affect the nasopharyngeal architecture include the geniohyoideus, sternohyoideus, sternothyroideus, omohyoideus, and thyrohyoideus muscles. The geniohyoideus muscle is a fusiform, paired muscle that lies on the ventral surface of the tongue (Fig. 29.9) (Sisson 1975b). The geniohyoideus originates from the medial surface of the mandible (near the origin of the genioglossus muscle) caudal to the symphysis and it inserts on the basihyoid bone. The omohyoideus, sternohyoideus and sternothyroideus muscles are accessory respiratory muscles that insert on the manubrium and extend cranially. These muscles are called accessory muscles of respiration because their respiratory activity is somewhat silent during resting breathing but increases with exertion and exercise. The sternothyroideus inserts on the caudal abaxial aspect of

the thyroid cartilage, and the sternohyoideus inserts on the basihyoid bone and the lingual process of the hyoid apparatus. Contraction of these muscles results in caudal traction of the hyoid apparatus and larynx, dilating the pharyngeal region (Sisson 1975b). The omohyoideus muscles originate on the subscapular fascia near the shoulder joint and also insert on the basihyoid bone and the lingual process of the hyoid apparatus. Contraction of these muscles produces caudal traction of the hyoid apparatus (Castro et al 1999).

The thyrohyoideus is a flat rectangular muscle that originates on the lateral surface of the thyroid cartilage lamina and inserts on the caudal part of the thyrohyoid bone (Sisson 1975b). On contraction, it moves the hyoid bone caudally or the larynx rostrally and dorsally (Sisson 1975b). In studies evaluating the electromyographic activity of some "extrinsic" nasopharyngeal muscles during exercise, Ducharme et al (2003) observed decreased thyrohyoideus muscle activity prior to soft palate displacement in one horse. Investigations by Tsukroff et al (1998) reveal that transection of a combination of thyrohyoideus, omohyoideus, sternohyoideus, and hyoepiglotticus muscles results in dorsal displacement of the soft palate in horses. The soft palate displacement observed was associated with a more caudal positioning of the basihyoid bone. In subsequent studies, thyrohyoideus muscle resection caused intermittent dorsal displacement of the soft palate in 7 out of 10 exercising horses (Ducharme et al 2003). Additionally, the insertion of a thyrohyoideus muscle prosthesis (created by placing a suture through the basihyoid bone and the thyroid cartilage) returned airway function to normal such that dorsal displacement of the soft palate no longer occurred in any of these horses (Ducharme et al 2003). These data clearly suggest that more cranial positioning of the larynx relative to the hyoid bone improves soft palate stability during exercise and that thyrohyoideus muscle dysfunction may be a cause of intermittent dorsal displacement of the soft palate in horses.

In summary, it is not clear how deficits of individual muscle groups result in diminished airway patency, though this causal relationship has been well studied in laboratory animals and humans. Nasopharyngeal patency results from a synergistic association of extrinsic muscles with opposing actions that affects the position of the larynx and hyoid bone. Indeed the hyoid moves rostrally when the geniohyoid and genioglossus muscles contract and in the opposite direction when the sternohyoid and sternothyroid muscles contract (Sisson 1975a, Kuna et al 1999, Feroah et al 2000, 2001, Kuna 2001, Ducharme et al 2003, Tessier et al 2004). The complex results of the activity of these muscles yield a more cranioventral position of the basihyoid bone, and an increase in the diameter and stability of the nasopharynx in exercising horses (Rehder 1992, Castro 1999, Tessier et al 2004).

Inflammatory Disorders

Follicular pharyngeal hyperplasia (pharyngitis)

Etiology

Young horses very commonly develop follicular pharyngeal hyperplasia. The location of the nasopharynx at the entrance of the airway exposes it to multiple types of irritant particles, allergens, and viral or bacterial agents. The local lymphoid tissue responds to these stimuli by secreting mucus to entrap inhaled particles and by producing local immunoglobulins. As young horses begin their performance careers, enter training barns, and travel, they become exposed to multiple new infectious, irritant, and antigenic stimuli.

Thus, this type of pharyngitis is very common in young horses and its prevalence decreases as horses age, as documented by Hobo et al (1995) who reported a 37% prevalence of pharyngitis (grade 3–4) in 2-year-old thoroughbred racehorses that decreased to nearly 0% in horses aged 6 years or more. Auer et al (1985) reported that 68 out of 70 young thoroughbred racehorses had evidence of pharyngeal lymphoid hyperplasia, or pharyngitis. Similar to Hobo's work, they found that 2-year-old horses had the most severe pharyngeal inflammation when compared to other age groups. In the study by Auer et al (1985), none of the affected horses had a history of diminished racing performance. The results of this study suggest that pharyngeal lymphoid hyperplasia may be a normal response to new inhaled environmental irritants or antigens or infectious agents in young horses. Therefore, because pharyngitis is frequently self-limiting and has not been definitively associated with poor performance, this disease is usually not treated. However, there are anecdotal concerns that pharyngitis may be a prelude to dynamic upper airway obstruction and the sequelae of nasopharyngeal inflammation may be more performance limiting than the initial bout of pharyngitis. Some clinical evidence suggests that regional inflammation of the upper airway may predispose individuals to obstructive upper airway disease, such as nasopharyngeal collapse, dorsal displacement of the soft palate, and aryepiglottic fold collapse.

Diagnosis

It is important to evaluate the extent of pharyngeal follicular hyperplasia, which can be classified into five grades (including 0) based on the degree of severity (Raker & Boles 1978). A pharynx with a few small white follicles over the dorsal walls is classified as grade 1 (Fig. 29.10). Numerous small follicles interspersed with occasional hyperemic follicles on the dorsal pharyngeal wall and extending ventrally over the lateral nasopharyngeal walls characterize grade 2 pharyngitis. This degree of follicular
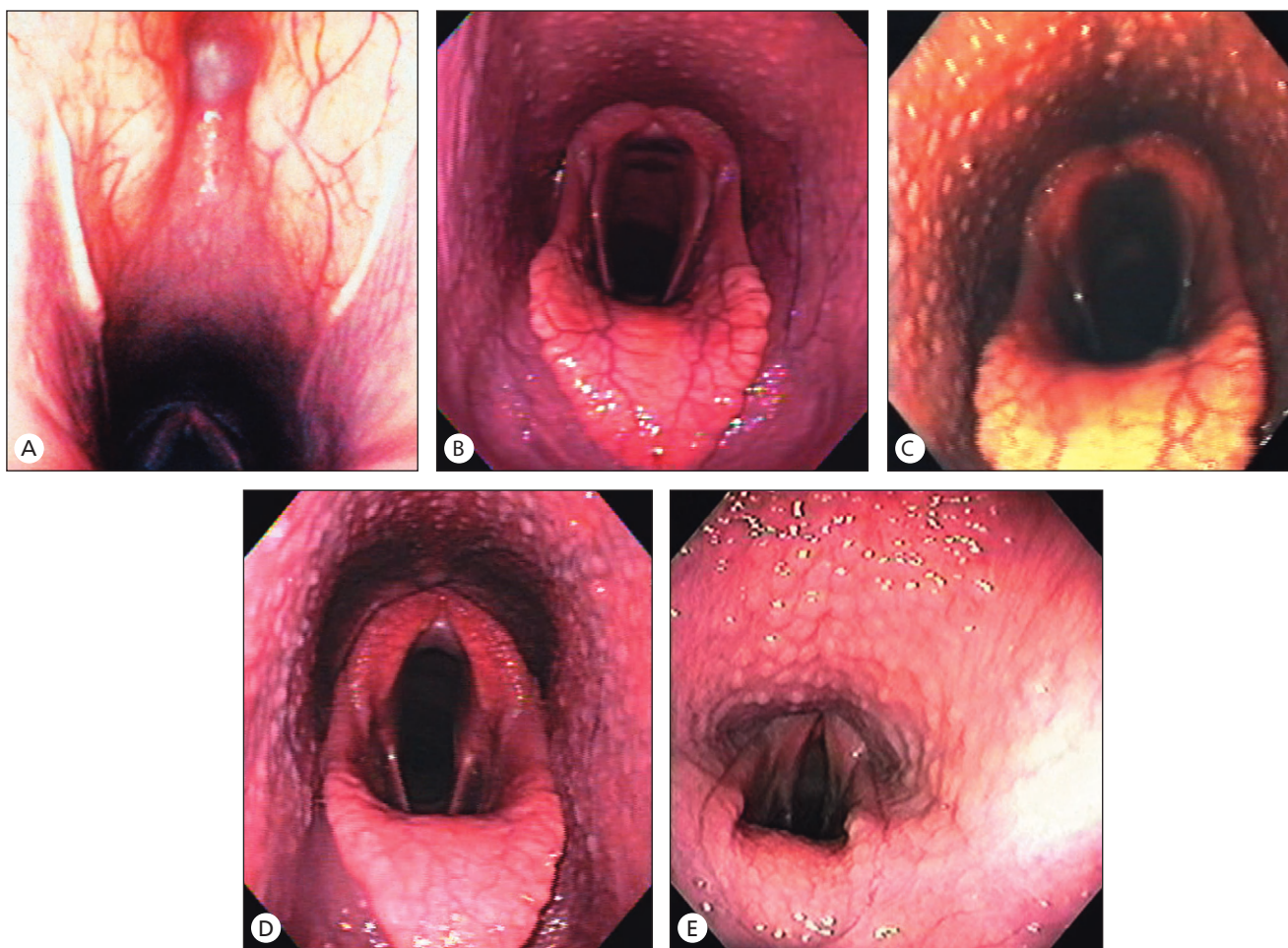
Fig. 29.10. Grading pharyngitis. A score of between 0 and 4 can be used to grade pharyngeal lymphoid hyperplasia. Here, the five endoscopic images show (A) grade 0 pharyngitis, (B) grade 1 pharyngitis, (C) grade 2 pharyngitis, (D) grade 3 pharyngitis, and (E) grade 4 pharyngitis.

hyperplasia is normal for 2-year-old horses. Grade 3 pharyngitis is diagnosed when more hyperemic follicles coalesce over the entire dorsal and lateral walls of the nasopharynx. Grade 3 pharyngitis is often seen in association with other abnormalities such as epiglottic flaccidity and dorsal displacement of the soft palate. The most severe form of pharyngitis is grade 4 and is characterized by large, edematous, hyperemic follicles that frequently coalesce into broad-based and polypoid aggregates. One reason why culture and sensitivity of the nasopharynx is rarely performed in horses with follicular pharyngeal hyperplasia is because specific bacterial nasopharyngeal infections [such as β-hemolytic group A streptococcal infections in children (Gerber & Shulman 2004)] are rarely a concern in horses with pharyngeal hyperplasia. However, if a horse with follicular hyperplasia is showing signs of clinical illness, such as depression, fever, and inappetence,

culturing the pharyngeal area may be warranted, though growth of certain *Streptococcus* spp. would be expected in nasopharyngeal swabs of normal horses.

Treatment

Generally, pharyngitis of grade 2 or less is not treated if this follicular pharyngitis is the only clinical complaint. If pharmacological therapy is to be administered, anti-inflammatory therapy may prove useful in the treatment of this pharyngitis. Systemic and inhaled corticosteroids have been used to treat pharyngitis. Following a thorough physical examination, complete blood count, and fibrinogen assay to rule out possible bacterial or viral infection, systemic corticosteroid therapy may be initiated. Therapies include prednisolone [oral dose of 0.6 mg/kg once a day (s.i.d.) for 7 days, followed by 0.3 mg/kg s.i.d. for 7 days,

and then 0.3 mg/kg every other day for five treatments] or dexamethasone [0.02–0.04 mg/kg *per os* (PO) or intravenous (IV), s.i.d for 3 days, then 0.01–0.02 mg/kg PO or IV, s.i.d. for 3 days, and then every other day for 3 days of treatment]. Topical anti-inflammatory therapies have also been used, including administration of 20 ml of a mixture comprising 250 ml glycerin, 250 ml dimethylsulfoxide 90%, nitrofurazone 500 ml, and 50 ml prednisolone (25 mg/ml) that can be sprayed on the nasopharynx twice daily. If the pharyngitis is bacterial in origin or accompanies infectious pulmonary disease, appropriate antimicrobial therapy should be used.

If a viral etiology is suspected, treatment may incorporate interferons, a family of proteins that have antiviral and immunomodulatory activity. Oral administration of a low dose (0.1 IU/kg) of human interferon-α once daily for 5–7 days reduces tracheal and nasopharyngeal exudate in racehorses with inflammatory airway disease. Oral administration of human interferon-α may modulate the activity of oropharyngeal lymphoid tissue in the oropharynx. The horse should be rested during this time, and either turned out in a pasture or worked lightly for 6–8 weeks. The airway inflammation often resolves within 7–10 days.

Fig. 29.11. Endoscopic image of the rostral portion of the nasopharynx showing collapse of the rostral portion of the soft palate into the airway. SP = soft palate; NS = nasal septum; ER = ethmoid region.

Functional Disorders

Nasopharyngeal collapse

Factors that affect the nerves and muscles that control the nasopharynx may result in dorsal, lateral, or circumferential nasopharyngeal collapse as a result of changes in the compliance of this region. Fitness and maturity of development of the musculature contribute to the stability of the nasopharynx and appropriate neural function is critical to maintain airway responses and tone. Therefore, horses with clinical signs of nasopharyngeal collapse should be evaluated for neuromuscular or primary muscle disorders, such as equine protozoal myoencephalitis, selenium and vitamin E deficiency, and hyperkalemic periodic paresis (HYPP). In quarter horses, HYPP disrupts the normal tone of the nasopharyngeal musculature even at rest (Carr et al 1996). Medications such as muscle relaxants and sedatives are known to alter nasopharyngeal muscle function. Mechanical pharyngeal disorders, including those caused by the presence of cysts within the soft palate, cleft palate tumors, or scarring from chronic inflammatory conditions, may prevent subepiglottic positioning of the caudal edge of the soft palate and result in generalized dysfunction of the nasopharynx. Inflammation of the nasopharynx that affects the pharyngeal plexus of nerves, the principal motor innervation to the nasopharyngeal muscles, has been implicated in the pathogenesis of nasopharyngeal collapse and in more severe forms of nasopharyngeal dysfunction such as dysphagia. Despite this long list of disorders known to cause nasopharyngeal collapse, the precise etiology of nasopharyngeal collapse in individual cases is usually unknown.

Models of nasopharyngeal collapse

Research models of the collapse of various regions of the nasopharynx have been created which may aid our understanding of the pathogenesis of these disorders but they have not yet led to an effective treatment. Instability of the rostral portion of the soft palate, or "billowing" of the soft palate occurs following bilateral tenectomy of the tendons of the tensor veli palatini muscles (Holcombe et al 1997). As mentioned previously, when this muscle contracts, it tenses the palatine aponeurosis, depressing the rostral portion of the soft palate slightly and maintaining its stability during inspiration. Following tenectomy, the rostral portion of the soft palate is unable to resist the collapsing subatmospheric pressures that occur in the nasopharynx during inspiration, and, as a result, the rostral aspect of the soft palate billows dorsally into the airway during inhalation and obstructs airflow (Fig. 29.11) (Holcombe et al 1997). Horses with dorsal billowing of the soft palate make a respiratory noise during exercise. This dysfunction has occasionally been described as a prelude
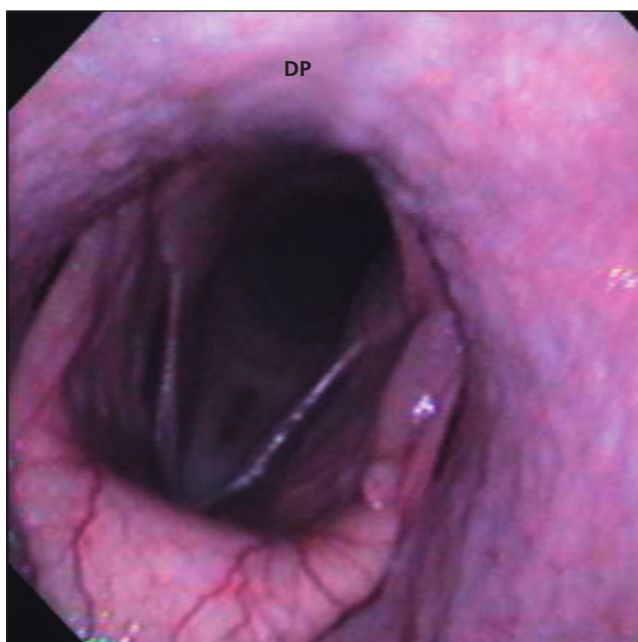
Fig. 29.12. Endoscopic image of the nasopharynx showing collapse of the dorsal pharyngeal wall. Note that the corniculate processes of the arytenoid cartilages are not visible. DP = dorsal pharynx.

to dorsal displacement of the soft palate. However, in experimental horses and clinical cases, dysfunction of the rostral soft palate can occur without dorsal displacement of the soft palate as a sequel (Holcombe et al 1997).

Collapse of the dorsal nasopharynx may be induced by anesthetizing the glossopharyngeal nerves and so creating stylopharyngeus dysfunction (Tessier et al 2004). Following this procedure, horses suffered collapse of the dorsal nasopharynx when the nares were occluded and also during treadmill exercise as inspiratory pressures increased (Fig. 29.12). The nasopharyngeal collapse did cause inspiratory obstruction based on upper airway pressure measurements. However, the degree of collapse produced by this model was modest in contrast to clinical cases of nasopharyngeal collapse, which frequently exhibit circumferential or more lateral collapse of the airway (Fig. 29.13). The etiology of clinical nasopharyngeal collapse remains unknown, though abnormalities of afferent innervation may play a causal role. Numerous mucosal pressure receptors cover the laryngeal mucosa and these mechano-receptors can sense subatmospheric pressures within the airway (Sant'Ambrogio et al 1983, Mathew et al 1984b). Increased activity of these receptors reflexly enhances the tone of upper airway dilating and stabilizing muscles, thus preventing dynamic collapse of the airway (Mathew et al 1984b). Following topical anesthesia of the laryngeal mucosa, horses exhibited collapse of the nasopharynx both

during treadmill exercise and at rest while the nares were occluded (Holcombe et al 2001). The collapse was circumferential in most cases, consistent with the presentation of most clinical cases.

Diagnosis

Horses with nasopharyngeal collapse have a history of exercise intolerance and inspiratory respiratory noise, which escalates with increasing exercise intensity. Observation of nasopharyngeal collapse when both nostrils are obstructed in the resting horse supports this diagnosis, but horses can have induced nasopharyngeal collapse during nasal occlusion and still have appropriate nasopharyngeal function during exercise. This may occur because the local reflex contraction of nasopharyngeal muscles, stimulated by nasal occlusion, results primarily from stimulation of receptors within the laryngeal mucosa. During exercise, multiple factors affect the activity of upper airway dilating muscles, including locomotion, neuroendocrine stimuli, hypercarbia and hypoxemia (Bartlett 1979, Brouillette & Thach 1979). In normal horses, the roof of the nasopharynx slightly projects ventrally into the lumen of the nasopharynx at the end of expiration, as a result of positive end-expiratory pressure within the guttural pouches, and this finding should not be considered abnormal (Rehder 1992). The floor of the guttural pouch forms part of the roof of the nasopharynx and therefore positive pressure within the guttural pouches results in mild collapse of the roof of the nasopharynx. Additionally, if a horse has been sedated for endoscopic examination, evaluation of nasopharyngeal function has no diagnostic value because the effect of sedation on muscular function may yield an erroneous result. In conclusion, a definitive diagnosis of nasopharyngeal collapse can only be made during treadmill video-endoscopy although in the future, respiratory sound analysis may prove to be a useful diagnostic aid (see Chapter 17).

Treatment

There is no current treatment for nasopharyngeal collapse. Frequently, horses are exercised with their tongues tied and/or wearing figure-of-eight nosebands in an attempt to help stabilize the airway. This treatment strategy is rarely effective. Occasionally, the disease is self-limiting, for example in horses that are not fully fit or are suffering intercurrent short-term disease, and some of these horses will recover normal nasopharyngeal function without treatment. If the horse has suffered from a respiratory viral infection or pharyngitis, alleviating the airway inflammation may improve nasopharyngeal function within a few weeks to months. Horses that are HYPP-positive respond to acetozolamide therapy (Carr et al 1996).
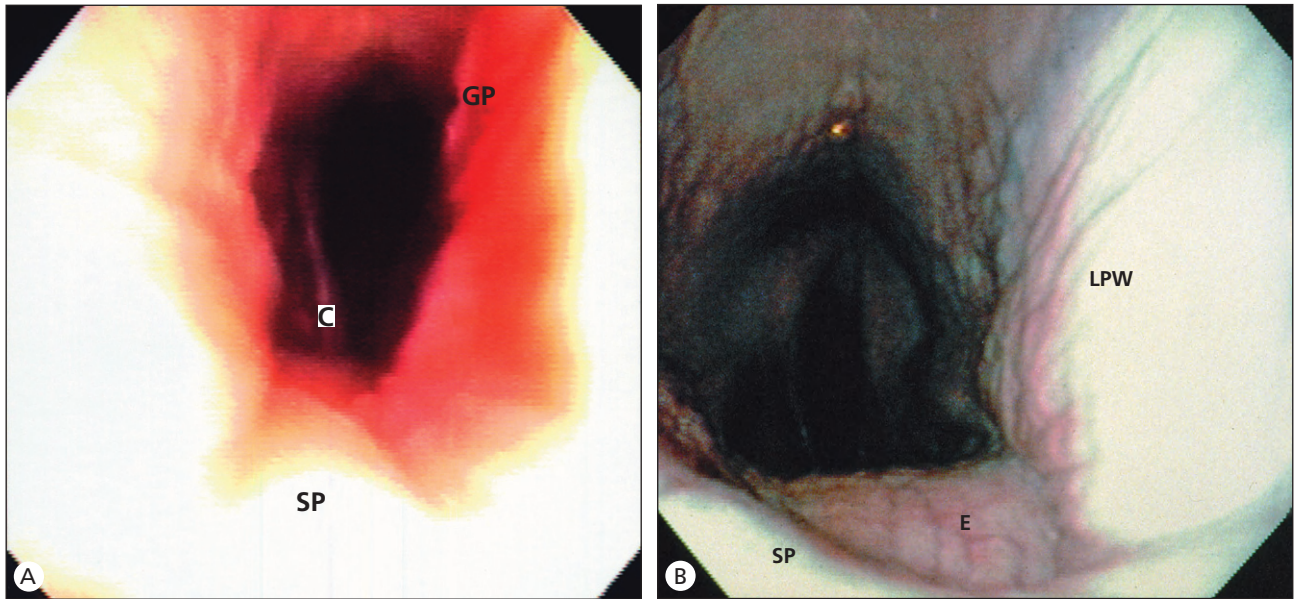
Fig. 29.13. (A) Endoscopic image of a horse during an episode of nasopharyngeal collapse. Note how the nasopharynx collapses into a sphincter. SP = soft palate. C = right vocal cord; GP = left nasopha- ryngeal opening of the guttural pouch. (B) Endoscopic image of the nasopharynx showing collapse of the lateral wall of the nasopharynx (LPW) as well as bulging of the soft palate (SP) around the epiglottis (E).

Nasopharyngeal paralysis, possibly the most severe form of nasopharyngeal collapse, can occur along with dysphagia (the inability to swallow). Differential diagnoses for nasopharyngeal paralysis in the horse include many of the same diseases speculated to cause nasopharyngeal collapse. The list includes botulism, equine protozoal myoencephali- tis, equine herpesvirus 1 infection, guttural pouch mycosis, guttural pouch empyema, and iatrogenic inflammation of the guttural pouch caused by lavage with caustic solutions (DeLahunta 1977, Mayhew 1989). Horses with naspha- ryngeal paralysis frequently have persistent or permanent dorsal displacement of the soft palate. Inflammation from fungal (guttural pouch mycosis) or streptococcal infection (guttural pouch empyema) that may affect branches of the vagal and glossopharyngeal nerves may result in ipsilateral hypesthesia of the pharyngeal mucosa and pharyngeal paresis with dysphagia (DeLahunta 1977, Kipper & Frees 1993). In one study, 12 of 35 cases of guttural pouch mycosis had dysphagia (Greet 1987). Following treatment, one horse had continuing low-grade dysphagia, five horses died or were euthanased following treatment for guttural pouch mycosis and six recovered completely. The total recovery from pharyngeal dysphagia in these horses suggested that the underlying neurological deficit is often neuropraxia rather than necrotizing inflammation of nerves (DeLahunta 1977, Greet 1987). Microscopic study of affected nerves from horses with guttural pouch mycosis revealed active neuritis. The involvement ranges from slight swelling of myelin sheaths and Schwann cells with

dilatation of intraneural capillaries to heavy leukocytic infiltration of the nerves and necrosis (DeLahunta 1977). Chromatolysis and degenerative swelling and vacuolization of neurons in the cranial cervical ganglion occur in some animals (DeLahunta 1977).

Dorsal displacement of the soft palate (DDSP)

Horses with intermittent DDSP are exercise intolerant and most (70–80%) affected horses will make an abnormal noise during exhalation at fast work (Derksen 2001). The displaced soft palate billows dorsally during exhalation as air flows beneath the soft palate (Fig. 29.14). Horses often produce a "snoring" noise when the soft palate is displaced, and this is caused by a low frequency fluttering of the caudal margin of the soft palate during expiration (Derksen 2001, Franklin et al 2004). However, in approximately 30% of horses with DDSP, noise is not reported (Franklin et al 2004). The caudal edge of the soft palate in each horse has a different stiffness, and this may be why 20–30% of horses are "silent displacers" (Franklin et al 2004). Dorsal displacement of the soft palate should still be considered as a possible diagnosis in horses with a sudden decrease in performance and no history of respiratory noise. This disease is more common in racehorses, especially 2- to 4-year-olds, but in Europe it is also common in older National Hunt racehorses (P.M. Dixon, personal communi- cation). It is an uncommon disease of show hunters and western pleasure horses but does affect horses that carry
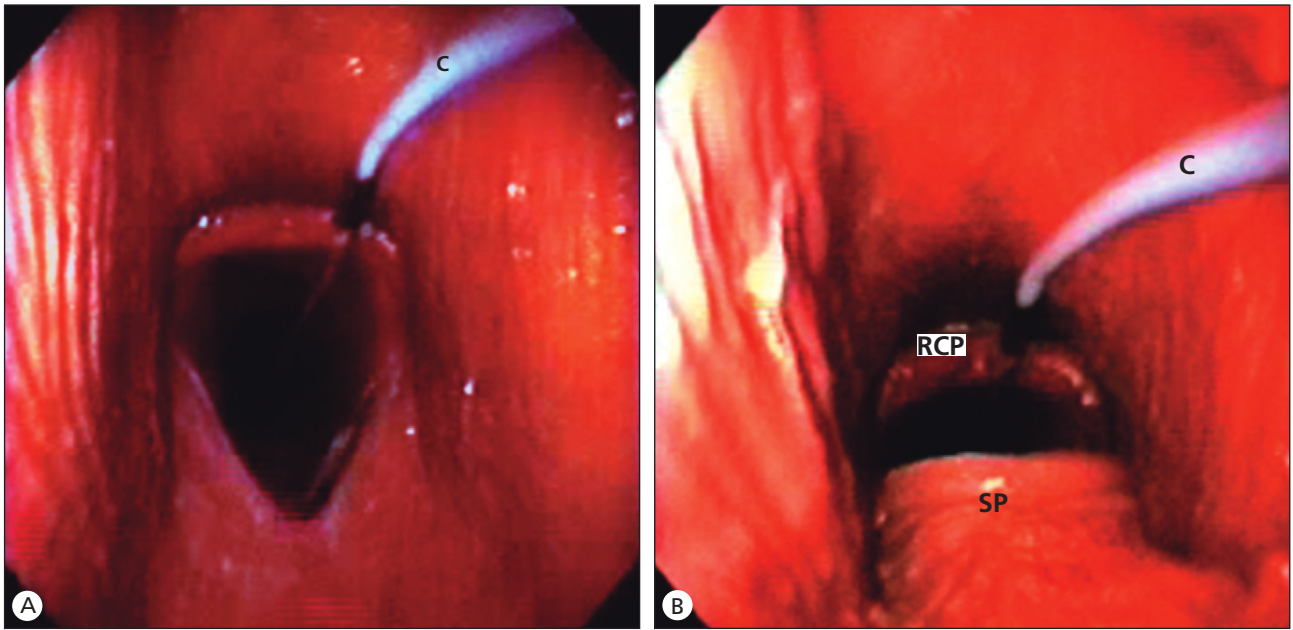
Fig. 29.14. (A) Endoscopic image of the nasopharynx of a horse on a treadmill during an episode of dorsal displacement of the soft palate. This image was taken during inspiration, documented by sub-atmospheric pressure measured with the tracheal catheter (C). (B) This image was taken during exhalation, documented by positive pressures measured with the tracheal catheter (C). Notice how the caudal free margin of the soft palate billows across the airway, obstructing the rima glottidis. SP = soft palate; RCP = right corniculate process of the arytenoid cartilage.

their head and neck in a flexed position, such as upper level dressage horses and saddlebreds.

Dorsal displacement of the soft palate is an expiratory upper airway obstructive syndrome that can cause increased expiratory impedance, decreased minute ventilation, hypoxia, and hypercarbia (Rehder et al 1995, Holcombe et al 1998). When the soft palate displaces with respect to the epiglottis during exercise, it undergoes dorsal and ventral excursion during the respiratory cycle. During inhalation, the soft palate rests dorsal to the epiglottis but does not cause obstruction because inspiratory pressures maintain it in a relatively stable position on the floor of the nasopharynx. However, during exhalation the soft palate billows dorsally into the nasopharyngeal lumen, thus diverting some flow of air through the oropharynx and mouth. This flow pattern is associated with decreased expiratory airflows and increased expiratory impedance (Rehder et al 1995, Holcombe et al 1998). Some horses suffering from DDSP exhibit the characteristic sign of mouth breathing during exhalation, recognized by fluttering of the cheeks as air is diverted underneath the soft palate through the mouth.

The cause of intermittent DDSP is unknown, but many theories exist to explain the etiology of this condition which is likely multifactorial. Some theories focus on dysfunction of the nerves and muscles that control the soft palate, and others concern the stability and proximity of the epiglottis and larynx to the soft palate.

Dysfunction of nasopharyngeal muscles has been implicated in the pathogenesis of DDSP. In a study evaluating the electromyographic activity of some "extrinsic" nasopharyngeal muscles during exercise, Ducharme et al (2003) observed decreased thyrohyoideus muscle activity prior to soft palate displacement in one horse. In the same study, bilateral resection of the thyrohyoideus muscles caused intermittent DDSP during exercise (Ducharme et al 2003). Creating a thyrohyoideus prosthesis by imbricating the thyroid cartilage to the basihyoid bone ("tie-forward procedure") corrected DDSP in these horses. The specific function of the thyrohyoideus muscle in preventing DDSP is not well understood, but because contraction of the thyrohyoideus muscles apposes the larynx and basihyoid bone, we can infer that the position of the larynx relative to the soft palate is likely to be important in the pathogenesis of DDSP.

Neuromuscular dysfunction of the structures controlling the position of the soft palate may occur secondary to inflammation of the upper airway. Some of the nerves that are important in coordinating nasopharyngeal function course through the guttural pouch to the pharyngeal plexus, which ramifies within the dorsal wall of the nasopharynx. As previously discussed with respect to nasopharyngeal collapse, infection or inflammation of this area could affect the innervation and thus the function of the intrinsic soft palate muscles. The pharyngeal branch of the vagus nerve provides motor innervation to the

palatinus and palatopharyngeus muscles, two muscles that control the position of the caudal portion of the soft palate. Experimentally desensitizing the pharyngeal branch of the vagus nerve bilaterally caused persistent DDSP and dysphagia in horses (Holcombe et al 1998). Biopsies taken of the palatinus muscle from horses with DDSP showed evidence of chronic denervation including fiber type grouping, mild atrophy, moth-eaten fibers and target fibers (Holcombe 2001). It is therefore possible that denervation atrophy of the palatinus may cause DDSP in some horses.

Some authors have implicated anatomical aberrations such as epiglottic hypoplasia (reduced size or rigidity of epiglottis) in the pathogenesis of DDSP. These authors speculate that in some horses the epiglottis is not sufficiently rigid to maintain its position dorsal to the soft palate. No conclusive evidence exists to support epiglottic hypoplasia as the cause of DDSP, and its association with DDSP and poor racing performance has not been confirmed (Stick et al 2001). However, epiglottic malformation or chondritis have been reported to result in permanent or persistent DDSP. Other anatomical abnormalities such as the presence of masses or cysts on the caudal free edge of the soft palate or beneath the epiglottis may impede subepiglottic placement of the soft palate, resulting in DDSP. Epiglottic entrapment with epiglottic deformity can also cause intermittent or persistent DDSP in horses. These horses are not dysphagic and a lateral radiograph of the laryngeal region or *per os* endoscopy or palpation of the epiglottis (under sedation or anesthesia) can confirm the diagnosis. Relieving the epiglottic entrapment will correct the DDSP in some horses. Unfortunately, re-entrapment, epiglottic deformity, and chondritis, as well as the continuation of persistent or intermittent DDSP represent alternative outcomes following treatment of the epiglottic entrapment.

Diagnosis at rest

The presence or absence of DDSP during an endoscopic examination of the nasopharynx at rest does not necessarily correlate with the development of DDSP during strenuous exercise on a high-speed treadmill (Parente et al 1994). In the practice situation however, the diagnosis must often be made based on history and endoscopy of the soft palate and epiglottis at rest. Some horses have permanent DDSP at rest, thus preventing epiglottic examination. The epiglottis must be evaluated using the previously described, alternative techniques in those horses. If the horse displaces its soft palate dorsal to the epiglottis and, despite multiple swallows, maintains the palate in a displaced position, it may be assumed that the horse is likely to exhibit DDSP during exercise. Therefore, a potentially useful strategy is to induce DDSP and

evaluate the horse's response to displacement. Soft palate displacement can be induced in some horses by obstructing the nostrils during endoscopy, thus challenging nasopharyngeal stability. Alternatively, passing the endoscope into the proximal aspect of the trachea will induce temporary displacement of the soft palate in most horses.

Evidence of trauma, inflammation, or pathology of the palate or epiglottis may support a diagnosis of DDSP. Ulcers on the axial aspect of the caudal free edge of the soft palate also indicate that DDSP occurred during exercise. An epiglottis that is abnormal (flaccid, short or hypoplastic) or deviated to one side provides some evidence to support a diagnosis of DDSP, although the association may not be causal because DDSP seems to occur independent of epiglottic conformation. In addition, a "choke ring", i.e. bruising of the roof of the nasopharynx in the area of the guttural pouch openings (in the mid portion of the nasopharynx), is consistent with DDSP.

A complete examination of the caudal portion of the soft palate to evaluate its contour, which should be somewhat concave, as well as an assessment of possible masses or cysts should also be performed. Subepiglottic cysts and masses can be located underneath the soft palate, so the patient should be observed during repeated swallows and efforts should be made to induce the cyst to move to a visible location.

Previous attempts at surgical correction of DDSP should be noted. Evidence of such surgery includes indentation in the cervical musculature, where a sternothyrohyoid myectomy was performed. It is more difficult to identify horses that have had excision of the caudal margin of the soft palate or sternothyroid tenectomy and myectomy at the muscle's origin. Local palpation and/or clipping the hair over the ventral aspect of the cricoid cartilage may permit identification of a surgical scar indicative of a prior laryngotomy procedure.

Diagnosis during exercise

The current gold standard for diagnosis of DDSP is observation of intermittent DDSP during strenuous exercise using high-speed treadmill video-endoscopy (Fig. 29.14). Following acclimation to the treadmill, horses are exercised at incrementally increasing speed until fatigue (usually 13–14 m/s at a 0° incline). One successful strategy used by some clinicians involves exercising the horse at maximum speed for 30–60 seconds, decreasing the speed to a moderate level for 30 seconds, and then increasing the speed to maximum speed. This protocol is sometimes effective because some horses exhibit DDSP during changes in exercise intensity. However, the absence of DDSP at exercise does not rule out this diagnosis because the condition is intermittent in nature and therefore not always demonstrable with treadmill evaluation. The use of

sound recordings with spectral analysis frequency peaks in the 20–80 Hz range is considered good evidence of DDSP (Derksen 2001, Franklin et al 2004).

Treatment

In 2-year-old horses, or any horse that has active or previous upper or lower airway inflammation, the initial therapy should focus on decreasing this inflammation. If bacterial upper airway infection is diagnosed, systemic antibiotics (usually penicillin G, ceftiofur or sulfamethoxazole–trimethoprim) may be administered along with non-steroidal anti-inflammatory drugs. Upper airway inflammation is treated in a plethora of different ways, including systemic administration of corticosteroids (dexamethasone); non-steroidal anti-inflammatory medication; topical anti-inflammatory throat sprays containing agents such as glycerin, dimethyl sulfoxide, and nitrofurazone; systemic administration of interferon; and gutteral pouch lavage with balanced polyionic solutions with or without dimethyl sulfoxide and corticosteroids. Oral interferon (50–200 IU/day for 10–14 days) has also been used.

An appropriate treatment regimen for moderate to severe nasopharyngeal inflammation without bacterial infection might initially include systemic corticosteroids such as prednisolone (not prednisone) or dexamethasone and topical anti-inflammatory pharyngeal spray for 2–4 weeks. A common pharyngeal spray administered transnasally at the rate of 20 ml, q 12 h consists of glycerin 250 ml, 250 ml dimethyl sulfoxide 90%, nitrofurazone 500 ml, prednisolone 50 ml (25 mg/ml). Horses should be rested (light training without fast speed work) for 10–30 days and the upper airway function should be re-evaluated periodically over this time. Normal function may not return for 3–4 months, if the cause of the DDSP was neuromuscular dysfunction related to airway inflammation. Owners and trainers of 2-year-old horses should consider waiting until the following year before pursuing any surgical treatment of DDSP because maturity may alleviate the need for treatment.

Tack modifications such as the use of a bit that maintains the position of the tongue (i.e. a "W" bit, Serena bit), tongue-ties, and the figure-of-eight noseband are traditional approaches that may be of value in reducing the occurrence of DDSP (Barakzai & Dixon 2005). There is no evidence to support the use of a tongue-tie in the prevention of DDSP or the improvement of airway mechanics in exercising horses (Cornelisse et al 2001, Franklin et al 2002). An external device, called a "Cornell Throat Support Device", is being tested in the field and focuses on preventing caudal movement of the basihyoid bone and larynx during exercise. In experimental treadmill studies, the device has been shown to be effective in six out of seven horses (Woodie et al 2005a).

Surgical treatment alternatives are numerous and include staphylectomy or trimming the caudal free margin of the soft palate, various myectomy and tenectomy procedures (sternohyoid and sternothyroid, alone or in combination), epiglottic augmentation, multiple tension palatoplasty, thermal and laser procedures to cauterize the soft palate, and the tie-forward procedure (Cook 1981, Harrison & Raker 1988, Peloso et al 1992, Ahern 1993, Anderson et al 1995, Llewellyn 1997, Hogan et al 2003, Woodie et al 2005b). These procedures are performed alone or in various combinations e.g. staphylectomy, sternothyrohyoideus myeotomy, and ventriculectomy (Barakzai et al 2003).

Staphylectomy

Staphylectomy or trimming the caudal free margin of the soft palate was developed by Dr Quinlan in 1949 (Cook 1981). Theoretically, this procedure is therapeutic for two reasons: it reduces the amount of soft palate tissue available to cause airway obstruction during episodes of DDSP and it may increase the stability of the palate, because of scarring of the caudal free margin. Staphylectomy is performed through a laryngotomy incision, with the horse anesthetized and placed in dorsal recumbency. A laryngotomy is performed by incising the cricothyroid ligament and underlying mucosa along the midline and extending the incision from the cricoid cartilage to the thyroid cartilage (Fig. 29.15). Care should be taken not to incise the thyroid or cricoid cartilage. The caudal free edge of the soft palate is identified, and a crescent section of the soft palate (0.5–1.5 cm at the midline and tapering to each side) is resected using Satinsky or Metzenbaum scissors. The laryngotomy incision can be closed or left to heal by second intention. If closure is elected, the cricothyroid membrane and epithelium are re-apposed using 3.5 metric polyglactin 910 in a simple continuous pattern. Suture placement in the cartilages should be avoided to minimize granuloma formation. Closure of the rest of the laryngotomy is optional, but may increase morbidity because of local incisional infection and potential septic mediastinitis. Perioperatively, parenteral non-steroidal anti-inflammatory drugs and broad-spectrum antibiotics are administered for 3–7 days. Training can resume within 2–3 days for standardbreds and 2–3 weeks for thoroughbreds. The prognosis for improvement in racing performance following this procedure is approximately 60% (Anderson et al 1995).

Sternothyrohyoid myectomy

This procedure was first proposed by Cook to prevent caudal retraction of the larynx (Cook 1981). When performed with the horse standing and sedated, local anesthetic is infiltrated subcutaneously at the junction of
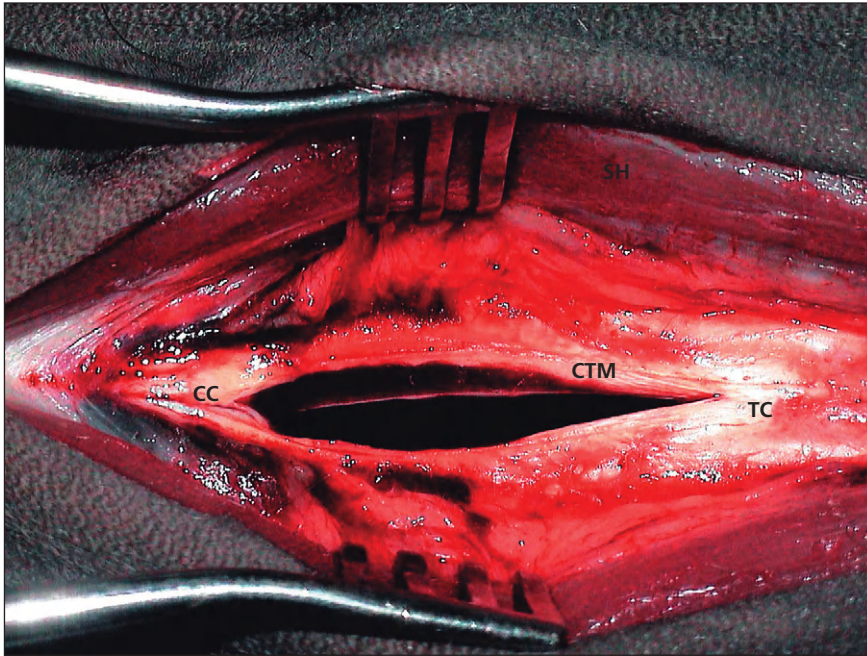
Fig. 29.15. Laryngotomy approach. SH = sternohyoideus muscles; CTM = cricothyroid membrane; TC= thyroid cartilage; CC = cricoid cartilage.

the mid and proximal cervical areas. Alternatively it can be performed under general anesthesia with the horse in dorsal recumbency. A 10-cm ventral midline skin incision is made and extended through the subcutaneous fascia to expose the sternohyoid muscle. Using curved forceps, a 7.5- to 10-cm section of the sternohyoid muscles is elevated and its proximal aspect is transected. The muscle is grasped, pulled cranially, and transected distally (Fig. 29.16). Next, the smaller sternothyroid muscles are exposed and resected. The incision is closed primarily. The surgical site may be bandaged until the skin sutures are removed to minimize swelling, and non-steroidal
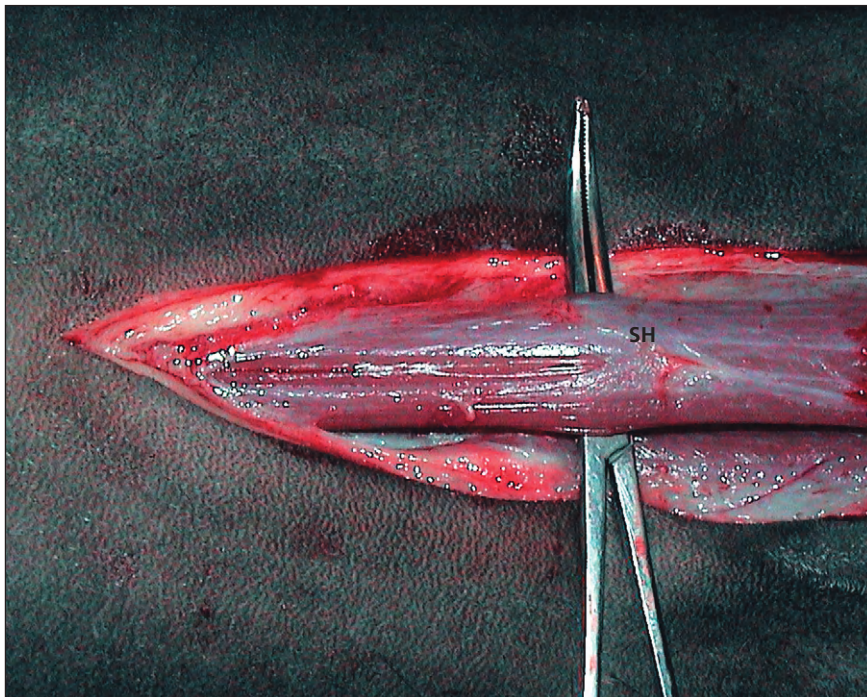
Fig. 29.16. Sternothyrohyoideus myectomy being performed in a standing horse. SH = sternohyoideus muscle.

anti-inflammatory and broad-spectrum antimicrobial therapy may be given as described above. Stall rest with daily hand walking is recommended for 2 weeks. Training can be resumed 2 weeks after surgery.

If resection of the omohyoid muscle is elected, the horse must be placed in dorsal recumbency under general anesthesia. The omohyoideus is separated from the linguofacial and jugular vein and partial resection is performed as described for the sternothyroid/hyoid muscles. Careful evaluation and ligation of all vessels is made to prevent later hematoma formation, which would be more clinically significant because of the amount of dead space resulting from the partial resection of the sternohyoid, sternothyroid, and omohyoid muscles bilaterally. The subcutaneous tissue and skin are closed after placement of Penrose drains. Currently, the omohyoideus muscle is rarely resected because of the morbidity associated with this procedure.

Complications are usually minor and are related to incisional seromas or abscesses that require appropriate drainage. This latter complication is more common if the omohyoid muscles are removed. There are no notable long-term complications except for the cosmetic defect associated with the lack of strap muscle at the operated site. The prognosis for improved performance following sternothyrohyoid myectomy is approximately 60% (Harrison & Raker 1988, Anderson et al 1995).

Combined staphylectomy and sternothyroideus tenectomy

This technique was introduced by Llewellyn and Petrowitz in 1997 (Llewellyn 1997). The combined staphylectomy and sternothyroid tenectomy procedure causes scarring of the caudal free margin of the soft palate and also prevents caudal traction of the larynx. The horse is placed under general anesthesia in dorsal recumbency, and a 10-cm ventral midline incision is made centered over the cricothyroid membrane. The incision is extended through the subcutaneous tissue and paired sternohyoid muscles on the midline. A self-retaining retractor is placed to laterally displace the sternohyoid muscles and blunt dissection is performed lateral to the thyroid cartilage exposing the tendon of insertion of the sternothyroideus muscle on the thyroid cartilage (Fig. 29.17).

Transection of the sternothyroid tendon is performed 1 cm caudal to its insertion on the thyroid cartilage to prevent inadvertent incision of the vascular pedicle of the cranial thyroid artery. A section of the muscle and its tendon is usually removed and the procedure is performed bilaterally. The cricothyroid membrane can then be incised and a staphylectomy can be performed as described above, although currently, staphylectomy is fairly unpopular and infrequently performed. Perioperative medications and rehabilitation instructions are as described for staphylec-
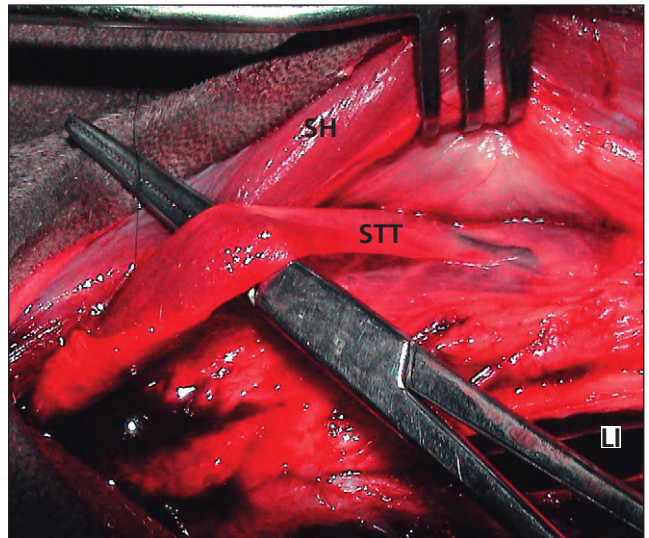
Fig. 29.17. Tendon of insertion of the sternothyroideus muscle (STT) attaching to the thyroid cartilage. SH = sternohyoideus muscle; LI = laryngotomy incision.

tomy and myectomy above. The prognosis for resolution of DDSP following combined staphylectomy and sternothyroid tenectomy is approximately 60% (Anderson et al 1995).

Epiglottic augmentation

Epiglottic augmentation was developed because of the anecdotal association of a flaccid epiglottis and DDSP in standardbreds (Peloso et al 1992, Tulleners et al 1997). The purpose of this procedure is to stiffen the epiglottic cartilage of horses to maintain the soft palate in a sub-epiglottic position. However, epiglottic augmentation is currently infrequently performed, because of lack of a proven association between epiglottic length and DDSP, an absence of objective evidence of its success, and, practically, because surgical grade Teflon is rarely available at present.

A laryngotomy is performed to provide access to the epiglottis. The epiglottic cartilage is inverted into the lumen of the larynx by grasping one of the aryepiglottic folds with Allis forceps and pulling this tissue toward the laryngotomy incision, exposing the ventral surface of the epiglottis. Between 3 and 7 ml Teflon paste (Mentor Polytef® paste for injection, Mentor O&O, Inc., Norwell, MA, USA) are injected submucosally. One of the authors (N.G.D.) prefers to inject 3 ml Teflon paste along the midline followed by digital redistribution/flattening over the ventral surface of the epiglottis. Postoperatively, parenteral anti-inflammatory agents are given for 5–7 days. Training can resume in 2–6 weeks. Prognosis for improved performance following epiglottic augmentations is approximately 60% (Tulleners et al 1997).

Partial sternothyroidectomy and laser cautery of caudal aspect of the soft palate

Partial sternothyroidectomy and laser cautery of caudal aspect of the soft palate has been described by Hogan et al (2003). First, bilateral sternothyroid tenectomy is performed as described above. Next, a laser is used *per nasum* to induce fibrosis on the caudal aspect of the soft palate. The resulting fibrosis is thought to increase the stiffness of the caudal soft palate, allowing the soft palate to maintain a subepiglottic position. The laser portion of this procedure is performed in the sedated, standing horse. Briefly the endoscope is passed through the right nasal passage and the soft palate is locally anesthetized with a topical application of 10 ml mepivacaine. A 600-μm bare laser fiber is passed through the biopsy channel and directed at the caudal free edge of the soft palate. Using 15 watts of power and contact technique, the fiber is applied for 1–2 seconds at 2- to 4-mm intervals along the entire free edge of the palate and extending approximately 1.5 cm rostrally. If an ulcer is present along the caudal free edge of the palate, the soft palate is displaced and the ulcer is cauterized directly with the laser fiber (Hogan et al 2003).

Postoperative rehabilitation includes hand walking for 3 days followed by resumption of jogging or galloping. Perioperative medications include topical pharyngeal spray for 14 days, phenylbutazone (4 mg/kg PO) for 5 days, and a decreasing regimen of oral prednisolone for 2–3 weeks. Horses return to normal work 1 week after surgery if endoscopic examination of the surgical area is normal (Hogan et al 2003). The prognosis for resolution of DDSP following this procedure is 90% (Hogan et al 2003).

Laryngeal tie-forward

We have performed the tie-forward procedure based on experimental data suggesting that the optimal position of the larynx during exercise is slightly dorsal to the basihyoid bone (Woodie et al 2005b). The horse is placed under general anesthesia in dorsal recumbency, and endotracheal intubation is performed. A 15-cm ventral midline incision is made, extending from the rostral aspect of the basihyoid bone to 1 cm caudal to the cricoid cartilage. The paired sternohyoideus muscles are separated bluntly on the midline, and dissection is extended to the ventral aspect of the larynx. The entire ventral aspect of the larynx is freed from surrounding tissue. The dissection is extended laterally to expose the tendon of insertion of the sternothyroideus muscles.

A small, self-retaining retractor is placed at the rostral aspect of the incision to facilitate exposure of the basihyoid bone, and a larger retractor (e.g. Balfour) is placed in the caudal aspect of the incision to facilitate exposure of the lateral aspects of the thyroid cartilage. A curette is used to remove the muscle insertion and expose the ventral aspect of the basihyoid bone. A 3.2-mm drill bit is used to create a hole in the basihyoid bone approximately 1 cm rostral to its caudal border. The sutures (no. 2 or no. 5 USP polybend suture, Fiberwire®) are first passed through the hole in the basihyoid bone. One suture is then placed through the left wing of the thyroid cartilage near the insertion site of the sternothyroideus muscle. A second suture is placed within 1 cm of it to increase the strength of the fixation in the thyroid cartilage by dividing the stress on the cartilage between two points. The procedure is repeated in the right thyroid cartilage placing the suture in the same direction. After suture placement, the horse's nose is lifted so that the head is at a 90° angle to the neck to facilitate tying the sutures.

Both before and after tying the sutures, a sterile, stainless steel ruler is used to measure the distance between the caudal aspect of the basihyoid bone and rostral aspect of the thyroid cartilage (the "BT distance") and the distance between the caudal aspect of the basihyoid bone and cranial aspect of the cricoid cartilage (the "BC distance") (Fig. 29.18). The first suture is tied so that the rostral aspect of the thyroid cartilage is dorsal to the basihyoid bone and extends up to 1 cm rostral to the caudal border of the basihyoid bone. The second suture is tied to match that tension. The head is replaced in its normal resting position, and the BT and BC distances are remeasured. Because the rostral aspect of the thyroid cartilage is either at the level of, or rostral to, the caudal aspect of the basihyoid bone, the postoperative BT distance is expressed as a negative value. The sternohyoideus muscles are re-apposed with a single continuous suture pattern that incorporates the loose fascia over the ventral aspect of the larynx. The subcutaneous tissues and skin are closed in a routine manner (Woodie et al 2005b).

Perioperatively, horses receive broad-spectrum antibiotics (ampicillin 10 mg/kg, IV t.i.d. and gentamicin 6.6 mg/kg, IV s.i.d. for 24 h followed by trimethoprim–sulfamethoxazole 30 mg/kg, PO, q 12 h) and phenylbutazone (2.2 mg/kg, PO, q 12 h) for 5–7 days. With the horse standing, a lateral radiograph of the larynx is obtained to verify the position of the larynx. Feeding at shoulder height is recommended to prevent early stress on the surgery site. Training resumes 2 weeks after surgery, pending satisfactory examination at the time of suture removal. The tie-forward procedure has a prognosis of 86% for improved racing performance (Woodie et al 2005b).

Pharyngeal cysts

Pharyngeal cysts occur most commonly in the subepiglottic region but have been reported within the soft palate and on the dorsal wall of the nasopharynx (Fig. 29.19) (Haynes et al 1990, Koch & Tate 1978). Subepiglottic cysts are likely remnants of the thyroglossal duct which forms as a ventral epithelial out-pouching from
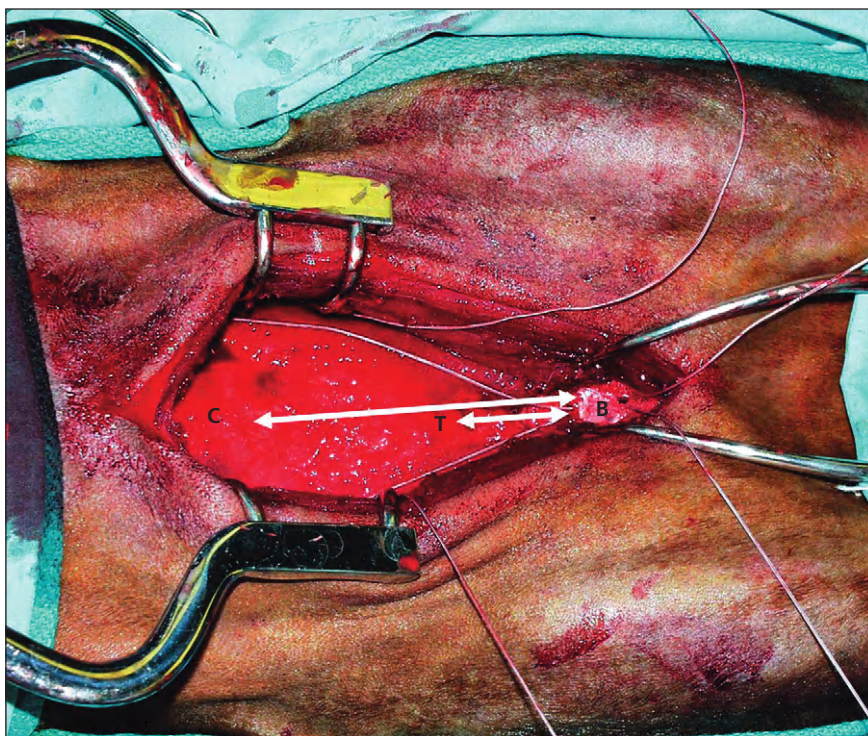
Fig. 29.18. Intraoperative view of laryngeal tie-forward after placement of sutures. Note the 3.2-mm drill hole in the base of the basihyoid bone through which both sutures are passed. The other ends of the sutures are placed in the caudal laminae of the right and left thyroid cartilages. C = cricoid cartilage; T = thyroid cartilage; B = basihyoid bone. Refer to text for explanation of arrows.

the floor of the primitive pharynx and grows caudally, forming a vesicle that divides over the trachea into two lobes of the embryonic thyroid gland (Koch & Tate 1978). Normally, the duct atrophies early in embryonic development, but rarely it persists, allowing cysts to form along its length. Cysts that form within the dorsal nasopharyngeal wall are remnants of the craniopharyngeal duct, the embryonic precursor of the adenohypophysis or anterior pituitary gland (Koch & Tate 1978). The craniopharyngeal duct originates from Rathke's pouch. Persistence of portions of the craniopharyngeal duct presumably perpetuates cyst formation within the pharyngeal walls. Cysts within the soft palate are rare and probably form as the result of the obstruction of mucus-secreting glands within the soft palate and walls of the nasopharynx, or they may have a traumatic etiology (Haynes et al 1990).

Pharyngeal cysts are more commonly diagnosed in male horses than females and are most commonly reported in young horses, between 2 and 4 years of age (Koch & Tate 1978). Clinical signs include respiratory noise, coughing, exercise intolerance, nasal discharge, dysphagia, aspiration of feed and aspiration pneumonia, and dorsal displacement of the soft palate (Koch & Tate 1978). The diagnosis is most commonly made by endoscopic examination of the nasopharynx, which reveals a raised, well-circumscribed mass covered by mucosa. Occasionally, subepiglottic cysts move from the nasopharynx to the oropharynx during

Fig. 29.19. Endoscopic image of the nasopharynx of a horse with a soft palate cyst that was causing persistent dorsal displacement of the soft palate. SP = soft palate; C = cyst.

swallowing. If a subepiglottic cyst is anticipated but not seen during endoscopic examination, the horse should be made to swallow multiple times in an attempt to displace the cyst into the nasopharynx. Additionally, radiography of the laryngeal and pharyngeal region can help to diagnose pharyngeal cysts. Contrast radiography of the region, performed by administering 60 ml of contrast material orally just prior to taking the lateral radiograph, can also help to document the size and position of the cyst within the pharynx (Haynes et al 1990).

Treatment of pharyngeal cysts includes surgical excision, which can be performed through a laryngotomy, transorally, or by use of non-contact laser ablation. The entire cyst must be extirpated because if some of the lining is left *in situ*, the cyst will likely recur. Surgical dissection of the cyst from the surrounding mucosa is facilitated by leaving the cyst intact and not aspirating the cystic contents before removal. Prognosis following removal of subepiglottic cysts is good with a reported return to athletic activity of 75% (Koch & Tate 1978). Less information is available regarding the prognosis for return to athleticism following removal of soft palate and pharyngeal wall cysts, though one report of two cases of soft palate cysts stated that both horses were unable to race but were useful as performance horses (Haynes et al 1990).

Neoplasia of the nasopharynx

Neoplasia of the nasopharynx is rare with lymphosarcoma and squamous cell carcinoma being most commonly reported. Clinical signs include dysphagia, nasal discharge, anorexia, and cachexia (Jones 1994, Sullivan & Parente 2003). Treatment is limited to surgical debulking of the mass, because excision is usually not possible, followed by intralesional chemotherapy (Jones 1994, Sullivan & Parente 2003).

Cleft Palate

Cleft palate or palatoschisis is a rare congenital anomaly affecting 0.01–0.02% of foals and represented 4% of 608 deformities diagnosed at necropsy in neonatal foals (Bowman et al 1982, Crowe & Swerczek 1985). Defects of the nasopharynx are categorized as secondary cleft palate or clefts of the soft and possibly also the hard portions of the palate. Defects of the caudal portion of the soft palate are most commonly diagnosed in horses, especially those affecting the caudal free margin of the soft palate (Bowman et al 1982, Crowe & Swerczek 1985). Cleft palates result principally from failure of the palatine processes to fuse. The development of the equine palate starts at approximately 47 days' gestation and requires development of the palatal shelves from the maxillary processes of the first arch, shelf elevation, medial edge epithelial breakdown
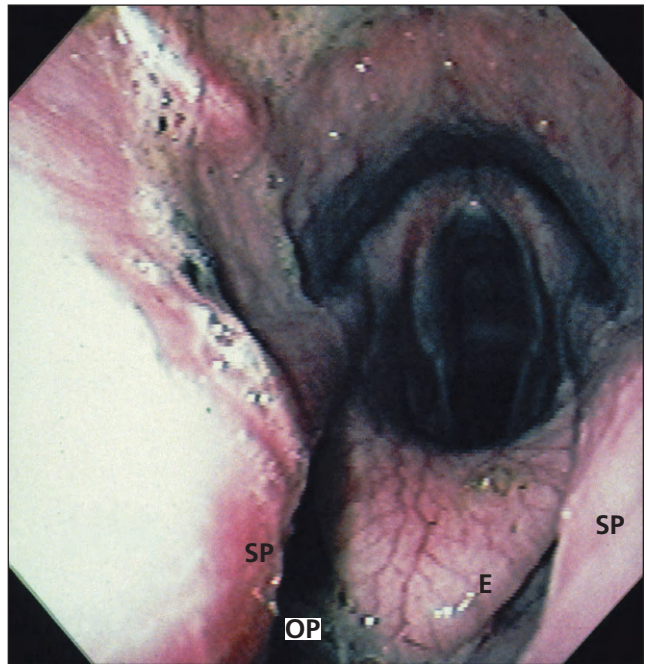
Fig. 29.20. Endoscopic image of the nasopharynx of a horse with a cleft palate. E = epiglottis; SP = soft palate; OP = oropharynx.

and mesenchyme flow with subsequent establishment of osteogenic and myogenic blastemata (Sandy 2003). This significant level of matrix turnover is partly regulated by the matrix metalloproteinases and may be affected by abnormalities in gene function (Sandy 2003). The etiology of cleft palate is still largely unknown, but mutations in candidate genes have been identified in a small proportion of cases (Wong & Hagg 2004). In other species, toxins and medications can affect the fusion of the palatine process.

Clinical evidence of cleft palate includes nasal discharge of milk during nursing and coughing as a result of aspiration of milk. Diagnosis of cleft palate is made by palpation of a deficit in the soft palate or by endoscopic observation of the cleft palate, including observing the oropharynx beneath the edges of the soft palate (Fig. 29.20). Differential diagnosis for nasal regurgitation of milk includes immature nasopharyngeal function, whereby the foal has not completely developed a coordinated swallowing reflex, which will usually develop given time. In both cases, aspiration pneumonia, failure of passive transfer, and rhinitis are potential complications.

Treatment of dysphagic foals should be restricted to surgical repair of the cleft palate or euthanasia. Before surgical correction is attempted, the presence of concurrent congenital defects, such as cardiac anomalies, gastrointestinal defects, and nasal septum deviations should be ruled out. Attempts to medically manage a foal with a cleft palate and dysphagia are unrewarding and perhaps

inhumane. The result, at best, is an unthrifty horse with chronic pneumonia. Surgical approaches to the soft palate include transoral, pharyngotomy, and mandibular symphysiotomy, the last of these being most commonly used and providing the best exposure (Bowman et al 1982, Crowe & Swerczek 1985). Defects in the soft palate are closed in three layers. Tension on the soft palate can be reduced by performing osteotomy of the hamulus of the pterygoid bone, bilaterally, to decrease the pull of the tensor veli palatini muscles, or by performing tension-relieving incisions of the palate and lateral pharyngeal walls (Bowman et al 1982). Defects in the hard palate are uncommon in foals and such defects are closed with a mucoperiosteal sliding flap combined with soft palate reconstruction (Bowman et al 1982).

Complications of cleft palate repair include dehiscence of the palate reconstruction as a result of the inherent tension on the repair and motion of the foal's tongue, mandibular osteitis and osteomyelitis, aspiration pneumonia, continued dysphagia, and stunted growth of the foal.

Additionally, even if the above complications do not occur, there is little factual evidence that surgically treated foals can establish normal epiglottis–soft palate relationships and become elite athletes.

Nasopharyngeal Scarring or Cicatrix

Nasopharyngeal cicatrization has most frequently been diagnosed in horses located in the southwestern USA, in particular horses from the Texas Panhandle area (Schumacher & Hanselka 1987, McClure et al 1994). Clinical signs of nasopharyngeal cicatrix include respiratory stridor at rest or during exercise, exercise intolerance, and, in some horses, abnormal vocalization. One review of 47 cases of nasopharyngeal cicatrix reported that the disease occurred 2.7 times more frequently in females than males with an age range from 6 to 20 years (Schumacher & Hanselka 1987). Diagnosis of nasopharyngeal cicatrix is made based on endoscopic examination of the upper airway. In addition to the cicatrix, which appears as a transverse circumferential scar within the nasopharynx, most horses also have lesions of the arytenoid or epiglottic cartilages and the nasopharyngeal openings of the guttural pouches (Fig. 29.21) (Schumacher & Hanselka 1987). The cicatrix is generally not the cause of the airway obstruction, which is usually caused by arytenoid chondritis or epiglottic deformity, and therefore the cicatrix is usually not treated (Schumacher & Hanselka 1987). However, three horses treated for nasopharyngeal cicatrix by sharp transection of the cicatrix were able to resume athletic activity (McClure et al 1994). The etiopathogenesis of this condition is not known but is assumed to be a sequela to ulcerative pharyngitis and laryngitis (Schumacher & Hanselka 1987).
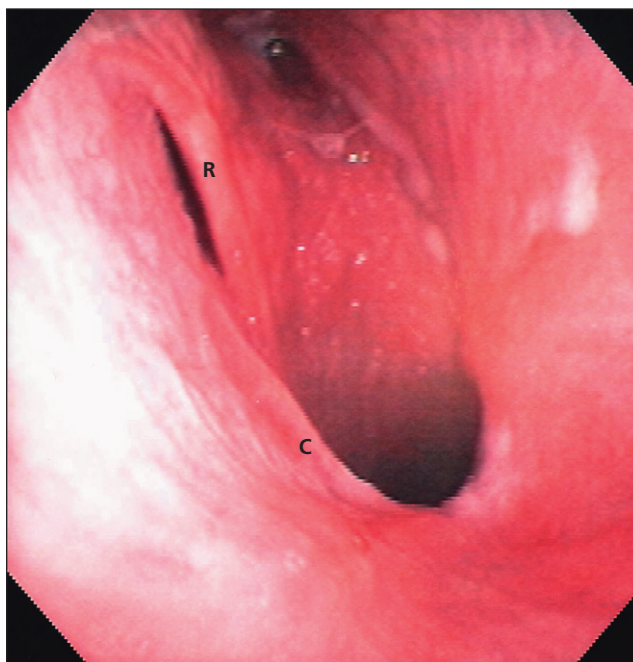
Fig. 29.21. Endoscopic image of the nasopharynx of a horse with a nasopharyngeal cicatrix (C). R = nasopharyngeal opening of right guttural pouch.

REFERENCES

Ahern TJ 1993 Oral palatopharyngoplasty; a survey of one hundred post-operative raced horses. Equine Veterinary Science 13: 670–672

Anderson JD, Tulleners EP, Johnston JK et al 1995 Sternothyrohyoideus myectomy or staphylectomy for treatment of intermittent dorsal displacement of the soft palate in race horses: 209 cases (1986–1991). Journal of the American Veterinary Medical Association 206: 1909–1912

Auer DE, Wilson RG, Groenendyk S 1985 Pharyngeal lymphoid hyperplasia in Thoroughbred racehorses in training. Australian Veterinary Journal 62: 124–126

Baptiste K 1997 Functional anatomy observations of the pharyngeal orifice of the equine guttural pouch (auditory tube diverticulum). Veterinary Journal 153: 311–319

Barakzai SZ, Dixon PM 2005 Conservative treatment for racehorses affected with intermittent dorsal displacement of the soft palate. Veterinary Record 36: 337–341

Barakzai SZ, Johnson VS, Baird DH et al 2003 The efficacy of composite surgery for treatment of dorsal displacement of the soft palate in 53 racing Thoroughbreds. Equine Veterinary Journal 36: 175–179

Bartlett D 1979 Effects of hypercapnia and hypoxia on laryngeal resistance to airflow. Respiratory Physiology 37: 293–302

Bowman KF, Tate LP Jr, Evans LH et al 1982 Complications of cleft palate repair in large animals. Journal of the American Veterinary Medical Association 180: 652–657

Brouillette RT, Thach BT 1979 A neuromuscular mechanism maintaining extrathoracic airway patency. Journal of Applied Physiology 46: 772–779

Brouillette RT, Bradley TT 1980 Control of genioglossus muscle inspiratory activity. Journal of Applied Physiology 49: 801–808

Carr EA, Spier SJ, Kortz GD et al 1996 Laryngeal and pharyngeal dysfunction in horses homozygous for hyperkalemic periodic paralysis. Journal of the American Veterinary Medical Association 209: 798–803

Castro HA, Resende LA, Berzin F et al 1999 Electromyographic analysis of the superior belly of the omohyoid muscle and anterior belly of the digastric muscle in tongue and head movements. Journal of Electromyography and Kinesiology 9: 229–232

Cook WR 1981 Some observations on form and function of the equine upper airway in health and disease: I. The pharynx. Proceedings of the American Association of Equine Practitioners 27: 355–391

Cornelisse CJ, Holcombe SJ, Derksen FJ et al 2001 Effect of a tongue-tie on upper airway mechanics in horses during exercise. American Journal of Veterinary Research 62: 775–778

Crowe MW, Swerczek TW 1985 Equine congenital defects. American Journal of Veterinary Research 46: 353–358

DeLahunta A 1977 Veterinary Neuroanatomy and Clinical Neurology, 1st edn. WB Saunders, Philadelphia, pp.104–107,370

Derksen FJ 2001 Spectrum analysis of respiratory sounds in exercising horses with experimentally induced laryngeal hemiplegia or dorsal displacement of the soft palate. American Journal of Veterinary Research 62: 659–664

Ducharme NG, Hackett RP, Woodie JB et al 2003 Investigation into the role of the thyrohyoid muscles in the pathogenesis of dorsal displacement of the soft palate. Equine Veterinary Journal 35: 258–263

Feroah TR, Forster HV, Pan LG et al 2000 Reciprocal activation of hypopharyngeal muscles and their effect on upper airway area. Journal of Applied Physiology 88: 611–626

Feroah TR, Forster HV, Pan L et al 2001 Effect of slow wave and REM sleep on thyropharyngeus and stylopharyngeus activity during induced central apneas. Respiratory Physiology 124: 129–140

Franklin SH, Naylor JR, Lane JG 2002 The effect of a tongue-tie in horses with dorsal displacement of the soft palate. Equine Veterinary Journal Supplement 34: 430–433

Franklin SH, Price C, Burn JF 2004 The displaced equine soft palate as a source of abnormal respiratory noise during expiration. Equine Veterinary Journal 36: 590–594

Fregosi RF, Fuller DD 1997 Respiratory-related control of extrinsic tongue muscle activity. Respiratory Physiology 110: 295–306

Fuller DD, Williams JS, Janssen PL et al 1999 Effect of coactivation of tongue protrudor and retractor muscles on tongue movements and pharyngeal airflow mechanics in the rat. Journal of Physiology 519: 601–613

Gerber MA, Shulman ST 2004 Rapid diagnosis of pharyngitis caused by group A streptococci. Clinical Microbiology Review 17: 571–580

Greet TR 1987 Outcome of treatment in 35 cases of guttural pouch mycosis. Equine Veterinary Journal 19: 483–487

Harrison IW, Raker CW 1988 Sternothyrohyoideus myectomy in horses: 17 cases (1984–1985). Journal of the American Veterinary Medical Association 193: 1299–1302

Haynes PF, Beadle RE, McClure JR et al 1990 Soft palate cysts as a cause of pharyngeal dysfunction in two horses. Equine Veterinary Journal 22: 369–371

Hobo S, Matsuda Y, Yoshida K 1995 Prevalence of upper respiratory tract disorders detected with a flexible video-endoscope in thoroughbred racehorses. Journal of Veterinary Medical Science 57: 409–413

Hogan PM, Palmer SE, Congelosi M 2003 Transendoscopic laser cauterization of the soft palate as an adjunctive treatment for dorsal displacement of the soft palate in the racehorse. In: Proceedings of the American Association of Equine Practitioners 48: 228–250

Holcombe SJ 2001 New thoughts on URT anatomy: relevancy. Proceeding from the 29th Annual Surgical Forum. American College of Veterinary Surgeons, Washington, DC, pp.59–62

Holcombe SJ, Derksen FJ, Stick JA et al 1997 Effect of bilateral tenectomy of the tensor veli palatini muscle on soft palate function in horses. American Journal of Veterinary Research 58: 317–321

Holcombe SJ, Derksen FJ, Stick JA et al 1998 Bilateral nerve blockade of the pharyngeal branch of the vagus nerve produces persistent soft palate dysfunction in horses. American Journal of Veterinary Research 59: 504–508

Holcombe SJ, Derksen FJ, Berney C et al 2001 Effect of topical anesthesia of the laryngeal mucosa on upper airway mechanics in exercising horses. American Journal of Veterinary Research 62: 1706–1710

Jones DL 1994 Squamous cell carcinoma of the larynx and pharynx in horses. Cornell Veterinarian 84: 15–24

Kipper A, Frees K 1993 Hypoglossal neuritis with associated lingual hemiplegia secondary to guttural pouch mycosis. Veterinary Pathology 30: 547–556

Koch DB, Tate LP 1978 Pharyngeal cysts in horses. Journal of the American Veterinary Medical Association 173: 860–862

Kuehn DP, Folkins JW, Cutting JW 1982 Relationships between muscle activity and velar position. Cleft Palate Journal 19: 25–35

Kuna ST 2001 Effects of pharyngeal muscle activation on airway size and configuration. American Journal of Respiratory and Critical Care Medicine 164: 1236–1241

Kuna ST, Smickely JS, Vanoye CR 1999 Respiratory-related pharyngeal constrictor activation on pharyngeal airway function. Journal of Applied Physiology 86: 411–417

Llewellyn HR 1997 Sternothyroideus myotomy for the treatment of dorsal displacement of the soft palate. Proceedings of the American Association of Equine Practitioners 43: 239–243

McClure SR, Schumacher J, Snyder JR 1994 Transnasal incision of restrictive nasopharyngeal cicatrix in three horses. Journal of the American Veterinary Medical Association 205: 461–463

Mathew OP, Abu-Osba YK, Thatch BT 1984a Genioglossus muscle responses to upper airway pressure changes: afferent pathways. Journal of Applied Physiology: Respiration and Environmental Exercise Physiology 52: 445–450

Mathew OP, Sant'Ambrogio G, Fisher JT et al 1984b Laryngeal pressure receptors. Respiratory Physiology 57: 113–122

Mayhew IG 1989 Large Animal Neurology: A Handbook for Veterinary Clinicians, 1st edn. Lea and Febiger, Philadelphia, pp.166–177

Parente EJ, Martin BB, Tulleners EP et al 1994 Upper respiratory dysfunctions in horses during high-speed exercise. Proceedings of the American Association of Equine Practitioners 40: 81–82

Peloso JG, Stick JA, Nickels FA et al 1992 Epiglottic augmentation by use of polytetrafluoroethylene to correct dorsal displacement of the soft palate in a Standardbred horse. Journal of the American Veterinary Medical Association 201: 13931395

Raker CW, Boles CL 1978 Pharyngeal lymphoid hyperplasia in the horse. Journal of Equine Medicine and Surgery 2: 202–207

Rehder RS 1992 Equine upper airway and guttural pouch pressures during exercise. MS thesis, Cornell University

Rehder RS, Ducharme NG, Hackett RP et al 1995 Measurement of upper airway pressures in exercising horses with dorsal displacement of the soft palate. American Journal of Veterinary Research 56: 269–274

Sandy JR 2003 Molecular, clinical and political approaches to the problem of cleft lip and palate. Surgeon 1: 9–16

Sant'Ambrogio G, Mathew OP, Fisher JT et al 1983 Laryngeal receptors responding to transmural pressure, airflow and local muscle activity. Respiratory Physiology 54: 317–330

Schumacher J, Hanselka DV 1987 Nasopharyngeal cicatrices in horses: 47 cases (1972–1985). Journal of the American Veterinary Medical Association 191: 239–242

Sisson S 1975a Equine myology. In: Getty R (editor) Sisson and Grossman's the Anatomy of Domestic Animals, 5th edn. WB Saunders, Philadelphia, pp.386–387

Sisson S 1975b Equine digestive system. In: Getty R (editor) Sisson and Grossman's the Anatomy of Domestic Animals, 5th edn. WB Saunders, Philadelphia, pp.471–475

Stick JA, Peloso JG, Morehead JP et al 2001 Endoscopic assessment of airway function as a predictor of racing performance in Thoroughbred yearlings: 427 cases (1997–2000). Journal of the American Veterinary Medical Association 219: 962–966

Sullivan EK, Parente EJ 2003 Disorders of the pharynx. Veterinary Clinics of North America; Equine Practice 19: 159–167

Tessier C, Holcombe SJ, Derksen FJ et al 2004 Effects of stylopharyngeus muscle dysfunction on the nasopharynx in exercising horses. Equine Veterinary Journal 36: 318–323

Tsukroff S, Ducharme NG, Bertram JE et al 1998 Relationship of basihyoid bone and thyroid cartilage in exercising horses. Proceedings of the 1st World Equine Airway Symposium, Guelph Ontario, Canada, CD-ROM

Tulleners E, Stick JA, Leitch M et al 1997 Epiglottic augmentation for treatment of dorsal displacement of the soft palate in racehorses: 59 cases (1985–1994). Journal of the American Veterinary Medical Association 211: 1022–1028

Woodie JB, Ducharme NG, Hackett RP et al 2005a Can an external device prevent dorsal displacement of the soft palate during strenuous exercise? An experimental model. Equine Veterinary Journal 37: 425–429

Woodie JB, NG Ducharme, P Kanter et al 2005b Surgical mobilization of the larynx (Laryngeal tie-forward) as a treatment for DDSP: a prospective study 2001–2003. Equine Veterinary Journal 37: 418–423

Wong FK, Hagg U 2004 An update on the aetiology of orofacial clefts. Hong Kong Medical Journal 10: 331–336

30

Epiglottic Fold Entrapment

Kira L Epstein and Eric J Parente

Introduction

The epiglottis is a triangular-shaped, elastic cartilage that helps to protect the airway during swallowing. The tip of the triangle points rostrally and in a normal horse, the epiglottis sits dorsal to the soft palate during breathing. The epiglottis has a characteristic scalloped border and has a vascular pattern on its dorsal surface. The aryepiglottic folds are bands of tissue coursing from the corniculate process of the arytenoids to the free edge of the epiglottis, and they join the glossoepiglottic fold under the epiglottis. Epiglottic entrapment is the result of the aryepiglottic membrane enveloping the rostral aspect of the epiglottis, and when so affected, the vascular pattern and scalloped edge can no longer be seen and a free edge of tissue is noted just in front of the glottis (Fig. 30.1). Entrapping membrane thickening has been reported to occur in 20–98% of affected horses, while ulceration has been reported to occur in 45–50% of epiglottic entrapments (Tulleners 1990, Ross et al 1993). Entrapment can occur persistently, or may be observed intermittently at rest and/or during treadmill examination (Lumsden et al 1994, Kannegieter & Dore 1995).

Epiglottic entrapment has also been associated with hypoplasia of the epiglottis and dorsal displacement of the soft palate. Two studies comparing radiographic epiglottic length have shown significantly shorter epiglottic length in horses with epiglottic entrapment when compared to normal horses (Lindford et al 1983, Tulleners 1991). In one study, 31% of thoroughbreds and 36% of standard-breds with epiglottic entrapment were judged to have a hypoplastic epiglottis (Tulleners 1991). Additionally, dorsal displacement of the soft palate can occur concurrently with an entrapment (Boles et al 1978, Tate et al 1990, Lumsden et al 1994, Kannegieter & Dore 1995).

Epiglottic entrapment is primarily a disease of racing thoroughbreds and standardbreds, although it is also reported in older thoroughbreds and in other breeds. In a study of 102 horses with epiglottic entrapment, one 12-year-old thoroughbred was affected (Tulleners 1990). Another study of 56 horses with epiglottic entrapment included one Appaloosa and one American Saddlebred (Lumsden et al 1994). The incidence of epiglottic entrapment in randomly evaluated horses has been reported to be between 0.74% (Raphel 1982) and 2.1% (thoroughbred racehorses) (Sweeney et al 1991). In horses undergoing treadmill examination for investigation of poor performance or abnormal respiratory noise, the reported incidence of epiglottic entrapment ranges from 2.1% (1/46) (Morris & Seeherman 1990) to 9.5% (8/92) (Kannegieter & Dore 1995).

Diagnosis

Clinical signs

Horses with epiglottic entrapment may make abnormal respiratory noises and/or have exercise intolerance; alternatively, they may be asymptomatic. The presence of clinical signs is greatly dependent upon the extent of the

Fig. 30.1. Simple epiglottic entrapment with a clear outline of the epiglottic shape beneath the entrapping membrane. The normal vascular pattern and scalloped edge of the epiglottis are obscured by the entrapping membrane.

abnormality of the entrapping membrane and the presence of intercurrent respiratory problems. Some clinicians feel that an entrapment may precipitate dorsal displacement of the soft palate in certain horses, thus resulting in more significant noise and performance-limiting problems. It has been speculated that the membrane may be tightly adhered to the epiglottis in horses that are asymptomatic, and therefore it does not cause respiratory obstruction (Kannegieter & Dore 1995). Billowing of the entrapping membrane during expiration may cause increased airflow turbulence. This may cause abnormal respiratory noise and a decrease in the nasopharyngeal cross-sectional area, which may lead to increased upper airway inspiratory resistance and thus to increased airflow pressures, resulting in exercise intolerance (Morris & Seeherman 1990).

Objective assessments of the impact of epiglottic entrapment on respiratory function are scarce. Measurement of inspiratory upper airway pressure at maximal exercise in horses with uncomplicated epiglottic entrapment showed only slight increases (3–5 cmH$_2$O), compared with normal horses. However, in one horse with an ulcerated and thickened entrapping membrane, the increase in inspiratory upper airway pressure was moderate (15 cmH$_2$O) (Williams et al 1990). In a study of cardiorespiratory and metabolic responses to exercise, values from one horse with epiglottic entrapment were similar to those of a normal horse (King et al 1994). Despite the minimal impact on respiratory function noted during treadmill testing in the above limited studies, it is uncommon to compete a horse with epiglottic entrapment.

Endoscopy

Horses with a history of exercise intolerance and/or abnormal respiratory noise should be evaluated by resting endoscopy, and also by high-speed treadmill examination if necessary. A diagnosis of epiglottic entrapment is based on the characteristic endoscopic appearance of an entrapped epiglottis when the scalloped border and dorsal vascular pattern are not visible because of the presence of the entrapping membrane. Instead, the outline of the triangular shape of the epiglottis is apparent under the smoother, rounded margins of the entrapping membrane. As mentioned previously, variable degrees of thickening and ulceration may be present on the entrapping membrane. If the rostral tip of the epiglottis appears blunted within the entrapment, it is likely that the tip of the epiglottis is folded back on itself under the membrane (Fig. 30.2). This may result in a persistent epiglottic deformity even after the entrapment has been resolved. If a concurrent dorsal displacement of the soft palate is present, two free edges of membrane may be seen rostral to the glottis (caudal aspects of the soft palate and entrapping membrane), and a focal bulging of the soft palate may be caused by the apex of the underlying epiglottis
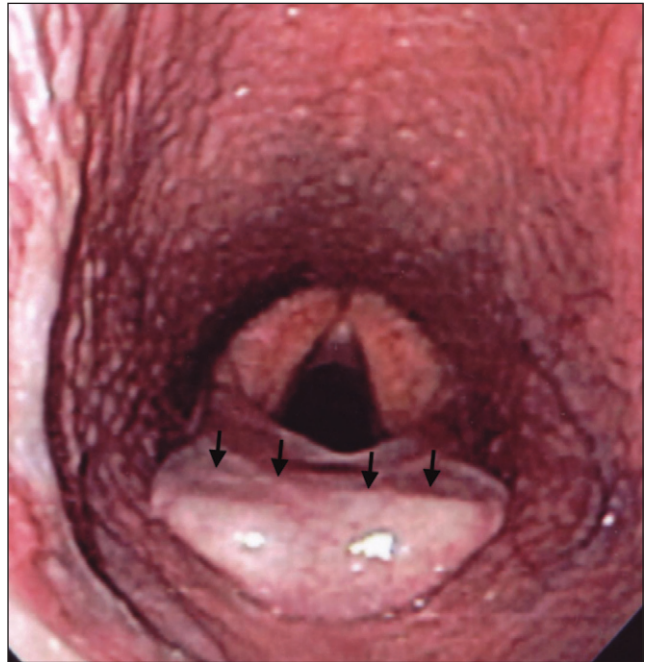
Fig. 30.2. An epiglottic entrapment with a blunted rostral tip (arrows) probably indicates that the tip of the epiglottis is rolled up into the entrapment. Even with surgical correction, epiglottic deformity may persist.

(Fig. 30.3). If entrapment is suspected, but the epiglottis is not entrapped at the time of the endoscopic examination, the horse should be induced to swallow, to try and reproduce the entrapment, or a high-speed treadmill examination should be performed.

It is important to differentiate the endoscopic appearance of epiglottic entrapment from that of (primary) dorsal displacement of the soft palate and epiglottitis. In a study of 20 horses with epiglottitis, 11 had been referred with a diagnosis of epiglottic entrapment but only one actually had concurrent epiglottic entrapment (Hawkins & Tulleners 1994). With epiglottitis, endoscopic findings may include thickening, edema, and/or discoloration of the epiglottis, ulceration and/or reddening of the epiglottic mucosa, exposed cartilage at the epiglottic tip, the presence of granulation tissue on the lingual surface of the epiglottis, and/or dorsal angulation of the epiglottis. However, it is important to recognize that with epiglottitis, the dorsal vascular pattern and scalloped border of the epiglottis, while distorted, are visible. With primary dorsal displacement of the soft palate the caudal edge of the soft palate is visible and the epiglottis is hidden.

Assessment of epiglottic hypoplasia

Both radiography and endoscopy can be useful in assessing epiglottic hypoplasia (reduced size), which should not be confused with epiglottic flaccidity. A technique for
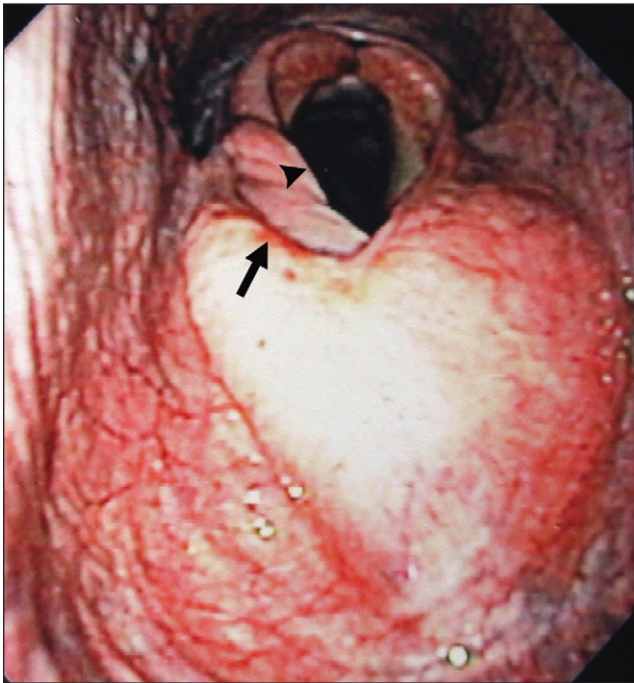
Fig. 30.3. A concurrent epiglottic entrapment and dorsal displacement of the soft palate. There are two separate edges of tissue visible, the entrapment (arrowhead), and the free edge of the palate (arrow) from the displacement. There is also a bulging of the soft palate caused by the entrapped epiglottis below it.

radiographic assessment of epiglottic length has been described by Lindford et al (1983) with good correlation to post-mortem measurements. A lateral radiograph of the pharyngeal region taken with the horse's head in a resting position can be used to measure the thyroepiglottic length. Adjustments must be made for magnification and for the additional distance from the base of the epiglottis to the thyroid cartilage to accurately assess the epiglottic length. In a study by Tulleners (1991), radiographic thyroepiglottic length in horses with epiglottic entrapment and endoscopically apparent epiglottic hypoplasia was significantly shorter than in horses with epiglottic entrapment and an endoscopically normal epiglottis. Thus, most clinicians now rely solely on the endoscopic appearance of the epiglottis.

Treatment

Local anti-inflammatory medication and rest may be effective treatments for simple entrapments when diagnosed early. However, more often, entrapments diagnosed during resting endoscopy are treated surgically. In one study, four of 38 horses with epiglottic entrapment were treated with an anti-inflammatory pharyngeal spray alone, with resolution of the entrapment without any further treatment in three of them (Greet 1995). A variety of

techniques have been described for the surgical treatment of epiglottic entrapment. Axial division of the entrapping membrane can be performed in a sedated standing horse with endoscopic guidance and local anesthesia using a curved bistoury or hook knife transnasally (Honnas & Wheat 1988, Greet 1995). Transendoscopic electrosurgery (Jann & Cook 1985, Sullins 1991), or transendoscopic laser surgery [neodymium : yttrium–aluminum–garnet (Nd:YAG) or diode lasers] (Tate et al 1990, Tulleners 1990, 1991, Greet 1995, Parente 2002) may also be used. Alternatively, axial division can be performed in an anesthetized horse under endoscopic guidance using a curved bistoury transorally (Ross et al 1993, Lumsden et al 1994).

Resection of the entrapping membrane can be performed via laryngotomy or pharyngotomy under general anesthesia (Ordidge 1977, Honnas & Wheat 1988, Lumsden et al 1994). This resection can also be performed with electrosurgery or transendoscopic laser using local anesthesia in sedated standing horses with endoscopic guidance (Sullins 1991, Parente 2002).

Performing standing surgeries alleviates the risks associated with anesthesia and recovery and can often be performed on an outpatient basis. Electrosurgery and laser surgery require specialized and expensive equipment as well as surgical technique and safety training. Additional aftercare and convalescence are required for healing of laryngotomy or pharyngotomy wounds following open surgical methods of resection.

Currently, the most commonly used surgical techniques in the USA are axial division with a laser in the sedated standing horse or axial division with a curved bistoury in an anesthetized horse.

Axial division with a laser

The most commonly used lasers for this procedure are Nd:YAG and diode lasers. They produce light of similar wavelength and the laser fibers are compatible with transendoscopic use, and both provide good hemostasis (Chapter 39). They can be used in a contact or non-contact manner, although contact is more generally used. The diode laser has the advantage of being easily portable and can be used with a standard electrical outlet. Proper eye protection should be worn when operating any laser.

Before beginning surgery, the horse should be properly sedated, placed in standing stocks, and local anesthetic should be applied transendoscopically to the epiglottis. Sedation can be achieved with an α_2-agonist such as xylazine (suggested dose 0.44 mg/kg) or detomidine (suggested dose 0.006–0.01 mg/kg) intravenously. Depending on the length of the procedure, additional half doses of the sedation may be required. Acepromazine (suggested dose 0.01 mg/kg) can also be administered, depending on the disposition of the horse and clinician preference. Opioids such as butorphanol are avoided by some clinicians because

horses may sometimes have excessive head movement following administration. Once sedated, local anesthetic solution is applied, using a transendoscopic catheter.

The entrapping membrane is divided along its midline using repeated passes of the laser fiber with mild pressure contact from a caudal to rostral direction (Fig. 30.4). The most rostroventral portion of the membrane can be difficult to divide because the membrane starts to retract beneath the epiglottis toward the end of the procedure. Therefore, several passes in the rostral region should be made in the initial stages. The laser should be activated only while the fiber is being moved to avoid excessive charring of tissue.
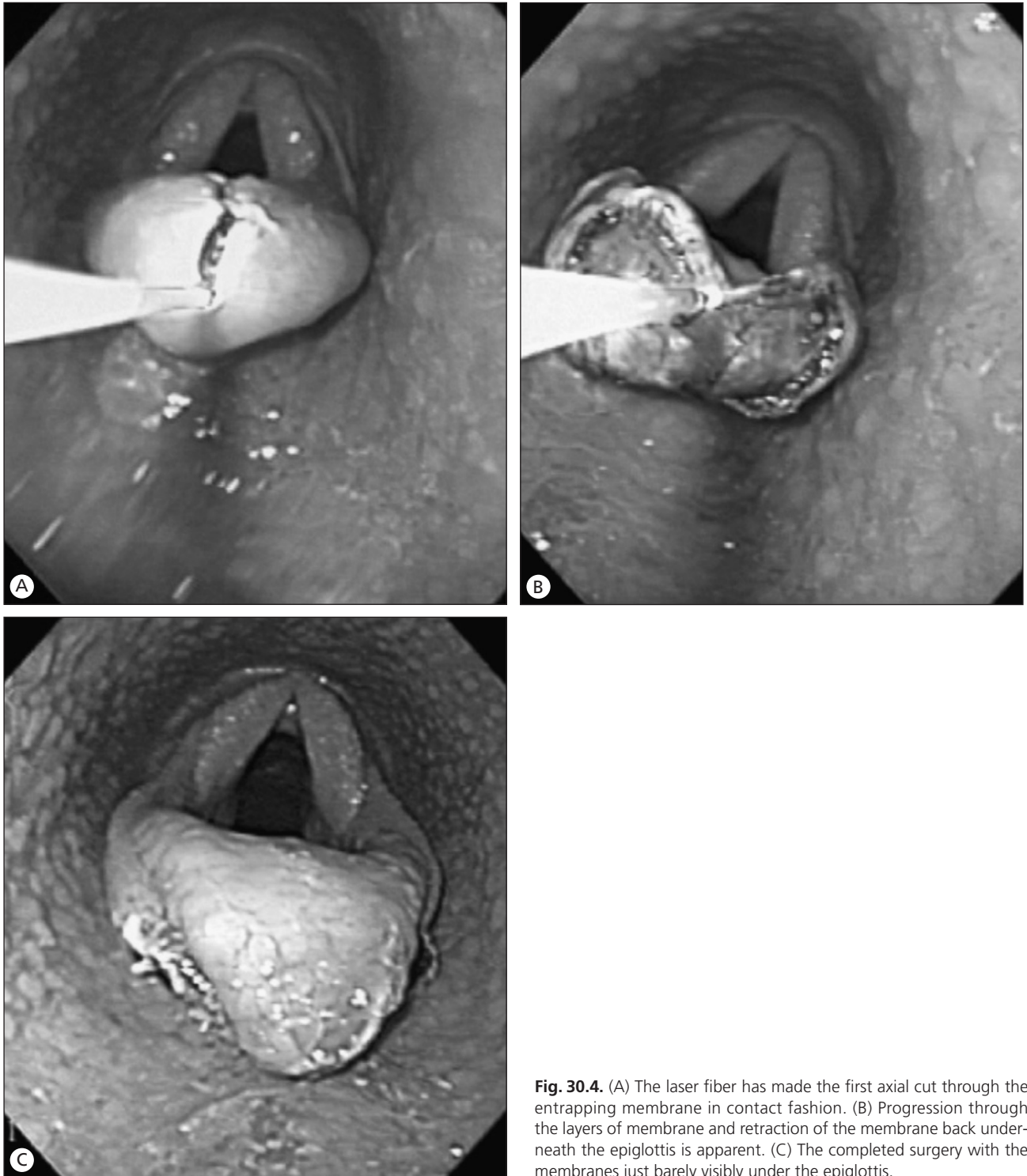
Fig. 30.4. (A) The laser fiber has made the first axial cut through the entrapping membrane in contact fashion. (B) Progression through the layers of membrane and retraction of the membrane back underneath the epiglottis is apparent. (C) The completed surgery with the membranes just barely visibly under the epiglottis.

Care should be taken to avoid inadvertent contact with and thermal damage to the dorsal surface and tip of the epiglottis. Changing the horse's head position may be helpful to allow appropriate placement of the laser fiber. Stimulation of swallowing and application of ventral pressure on the epiglottis with the endoscope tip may help retract the membrane ventrally when the cut is completed. If the membrane does not recede under the epiglottis after complete axial division, some of the entrapping membrane can be resected with the laser or alternatively, the horse can be treated successfully with local and systemic anti-inflammatory medication.

Axial division with a curved bistoury

This procedure can be performed in the standing sedated horse or under general anesthesia with a short-acting injectable anesthetic such as ketamine. With the horse in lateral recumbency, a mouth speculum is placed and the tongue is retracted and the medial aspect of the upper cheek teeth should be rasped (floated). The surgeon manually displaces the soft palate through the mouth and an endoscope is passed through the mouth to allow visualization of the epiglottis. Under endoscopic guidance, the curved bistoury (shorter than the transnasal bistoury) can be positioned between the dorsal surface of the epiglottis and the entrapping membrane on the midline. The sharp point of the bistoury should penetrate the aryepiglottic fold immediately rostral to the apex of the epiglottis and tension must be maintained at all times to avoid repositioning of the bistoury. At this time, the endoscope can be removed or operated by an assistant, because the surgeon will need both hands. One hand is inserted into the mouth to apply ventral pressure and guard the point of the bistoury, preventing damage to the base of the tongue, as the bistoury is pulled rostrally by the other hand. Complete division should be confirmed endoscopically.

As mentioned, the curved bistoury has been used *per nasum* in sedated standing horses, but there is significant additional risk of inadvertent damage to the soft palate, esophagus or other structures if the horse swallows during the procedure and therefore this technique is no longer recommended.

Resection through a laryngotomy

For resection of an epiglottic entrapment through a laryngotomy, horses are placed under general anesthesia in dorsal recumbency. While a pharyngotomy approach can be used to access the ventral aspect of the epiglottis, it is much preferred to use a routine laryngotomy approach. One side of the aryepiglottic fold is grasped with Allis tissue forceps to retrovert the epiglottis into view. Care should be taken to avoid grasping the epiglottic cartilage with the

forceps. The aryepiglottic fold is grasped bilaterally with a second and third Allis tissue forceps to allow more even tension and better visualization of the epiglottis. Metzenbaum scissors, electrocautery, or a laser can be used for resection of the entrapping membrane. The epiglottic cartilage should be identified by palpation and visualization before resection, to prevent it being cut. Ideally, the membrane should be resected within 2–3 mm of the epiglottic tip and 1–1.5 cm to either side of the midline (Stick et al 1999). The membrane can be distorted very easily with tension and great care should be exercised not to resect an excessive amount of tissue, which may increase the incidence of dorsal displacement of the soft palate postoperatively.

Aftercare

Following axial division, horses should be starved for several hours to allow the sedation or anesthesia to wear off, and so prevent dysphagia and subsequent aspiration. Antimicrobials may be indicated in cases of thickened or ulcerated entrapping membranes, or following a laryngotomy procedure. A combination of non-steroidal anti-inflammatories (phenylbutazone or flunixin meglumine typically) and corticosteroids can be administered, based on clinician preference. Additionally, topical anti-inflammatory therapy such as pharyngeal sprays containing various combinations of dimethyl sulfoxide and/or corticosteroids can be applied twice daily by transnasal catheter. In horses treated without laryngotomy, stall rest is recommended for approximately 7–14 days. Repeat endoscopy should ideally be performed before returning the horse to race training to check that healing is progressing appropriately.

Prognosis

Rates of postoperative recurrence of entrapment vary depending on the surgical technique performed (Fig. 30.5). The re-entrapment rates are as follows: 5–15% after trans-nasal axial division using a curved bistoury or hook knife (Honnas & Wheat 1988, Greet 1995); approximately 10% after transoral axial division using a curved bistoury (Ross et al 1993, Lumsden et al 1994); approximately 5% after transendoscopic laser axial division (Tulleners 1990); as high as 40% (two of five horses) after transendoscopic electrosurgical axial division (Jann & Cook 1985); and as high as 36% after resection via laryngotomy or pharyngotomy (Lumsden et al 1994). Dorsal displacement of the soft palate has been reported in 5–10% of cases following transoral axial division using a curved bistoury (Ross et al 1993, Lumsden et al 1994); in 15% of cases following transendoscopic laser division (Tulleners 1990); and in 9% of cases following resection via laryngotomy or pharyngotomy (Lumsden et al 1994). Damage to the tip of the epiglottis is a potential complication with use of the bistoury,

Fig. 30.5. Recurrent epiglottic entrapment after axial division with a curved bistoury. Note incomplete axial cut that resulted in the re-entrapment.

and with laser or electrosurgical procedures. As noted, transnasal axial division with the bistoury has caused the potentially severe complication of inadvertent injury to the soft palate, which may necessitate euthanasia.

Concurrent upper respiratory disorders, including epiglottic hypoplasia and mucosal ulceration, may complicate the correction of epiglottic entrapment and worsen the prognosis. In a study of 51 horses with epiglottic entrapment, horses with concurrent upper airway abnormalities (including, dorsal displacement of the soft palate, subepiglottic cyst, arytenoid chondropathy, and left recurrent laryngeal neuropathy) were significantly less likely to return to racing than horses with uncomplicated epiglottic entrapment (Lumsden et al 1994). In a study on transendoscopic Nd:YAG laser axial division, three out of four horses with re-entrapment and nine out of nine horses with postoperative dorsal displacement of the soft palate were judged to have a hypoplastic epiglottis (Tulleners 1990). However, a later study showed no significant association between performance before and after surgery and the presence of epiglottic hypoplasia (Tulleners 1991).

It has been suggested that severe ulceration, thickening, or scarring of the entrapping membrane may make axial division more difficult, and resection via laryngotomy or pharyngotomy may be indicated in those cases (Ross et al 1993, Lumsden et al 1994). However, with experience, resection of the tissue via transendoscopic laser surgery may yield a better prognosis than resection through a

laryngotomy. A significant advantage of transendoscopic laser resection is the normal anatomical positioning of the epiglottis while resecting the tissue using this technique, versus resection through a laryngotomy when the epiglottis is in a distorted, retroverted position. Regardless of which technique is employed, epiglottic deformity as a result of chronic entrapment will often negatively affect the postoperative prognosis.

REFERENCES

Boles CL, Raker CW, Wheat JD 1978 Epiglottic entrapment by arytenoepiglottic folds in the horse. Journal of the American Veterinary Medical Association 172: 338–342

Greet TRC 1995 Experiences in treatment of epiglottal entrapment using a hook knife per nasum. Equine Veterinary Journal 27: 122–126

Hawkins JF, Tulleners EP 1994 Epiglottitis in horses: 20 cases (1988–1993). Journal of the American Veterinary Medical Association 205: 1577–1580

Honnas CM, Wheat JD 1988 Epiglottic entrapment. A transnasal surgical approach to divide the aryepiglottic fold axially in the standing horse. Veterinary Surgery 17: 246–251

Jann HW, Cook WR 1985 Transendoscopic electrosurgery for epiglottal entrapment in the horse. Journal of the American Veterinary Medical Association 187: 484–492

Kannegieter NJ, Dore ML 1995 Endoscopy of the upper respiratory tract during treadmill exercise: a clinical study of 100 horses. Australian Veterinary Journal 72: 101–107

King CM, Evans DL, Rose RJ 1994 Cardiorespiratory and metabolic responses to exercise in horses with various abnormalities of the upper respiratory tract. Equine Veterinary Journal 26: 220–225

Lindford RL, O'Brien TR, Wheat JD et al 1983 Radiographic assessment of epiglottic length and pharyngeal and laryngeal diameters in the Thoroughbred. American Journal of Veterinary Research 44: 1660–1666

Lumsden JM, Stick JA, Caron JP et al 1994 Surgical treatment for epiglottic entrapment in horses: 51 cases (1981–1992). Journal of the American Veterinary Medical Association 205: 729–735

Morris EA, Seeherman HJ 1990 Evaluation of upper respiratory tract function during strenuous exercise in racehorses. Journal of the American Veterinary Medical Association 196: 431–438

Ordidge RM 1977 Epiglottic entrapment in the horse. Veterinary Record 100: 365–366

Parente EJ 2002 Transendoscopic axial division of epiglottic entrapment. Clinical Techniques in Equine Practice 1: 9–12

Raphel CF 1982 Endoscopic findings in the upper respiratory tract of 479 horses. Journal of the American Veterinary Medical Association 181: 470–473

Ross MW, Gentile DG, Evans LH 1993 Transoral axial division, under endoscopic guidance, for correction of epiglottic entrapment in horses. Journal of the American Veterinary Medical Association 203: 416–420

Stick JA, Tulleners EP, Robertson JT et al 1999 Larynx. In: Auer JA, Stick JA, (editors) Equine Surgery, 2nd edn. Saunders, Philadelphia, pp.349–368

Sullins KE 1991 Standing endoscopic electrosurgery. Veterinary Clinics of North America; Equine Practice 7: 571–581

Sweeney CR, Maxson AD, Soma LR 1991 Endoscopic findings in the upper respiratory tract of 678 Thoroughbred racehorses. Journal of the American Veterinary Medical Association 198: 1037–1038

Tate LP, Sweeney CL, Bowman KF et al 1990 Transendoscopic Nd:YAG laser surgery for treatment of epiglottal entrapment and dorsal displacement of the soft palate in the horse. Veterinary Surgery 19: 356–363

Tulleners EP 1990 Transendoscopic contact neodymium: yttrium aluminum garnet laser correction of epiglottic entrapment in standing horses. Journal of the American Veterinary Medical Association 196: 1971–1980

Tulleners EP 1991 Correlation of performance with endoscopic and radiographic assessment of epiglottic hypoplasia in racehorses with epiglottic entrapment corrected by use of contact neodymium:yttrim aluminum garnet laser. Journal of the American Veterinary Medical Association 98: 621–626

Williams JW, Meagher DM., Pascoe JR et al 1990 Upper airway function during maximal exercise in horses with obstructive upper airway lesions. Effect of surgical treatment. Veterinary Surgery 19: 142–147

31 Fourth Branchial Arch Defects

J Geoffrey Lane

Introduction and Definition

Congenital and developmental deformities of the equine larynx are not common and those which have been described have generally comprised defects of the cartilage skeleton which supports the moving components – the arytenoid cartilages and vocal folds – and of the extrinsic musculature which links the larynx to the upper esophagus. In the human embryo, the muscles and cartilages of the larynx form from the fourth and sixth branchial arches (Hast 1972, Zaw-Tun & Burdi 1985). The extrinsic structures, which include the wing of the thyroid cartilage and the associated cricothyroid articulation, the cricothyroid muscle and the upper esophageal sphincter muscles (i.e. the thyropharyngeus and cricopharyngeus muscles) all stem from the fourth arch. The intrinsic laryngeal muscles, and the cricoid and arytenoid cartilages form from the sixth branchial arch. There is no reason to suspect that the equine larynx develops in a different fashion from the human larynx, and the syndrome which is described here consists of aplasia, or varying degrees of hypoplasia, of one or more of the cartilaginous or muscular structures derived from the fourth arch, unilaterally or bilaterally, and is termed the fourth branchial arch defect syndrome or, in its abbreviated form, 4-BAD (Lane 1993). This congenital laryngeal deformity is not rare, but it has possibly been underdiagnosed in the past through misinterpretations of the endoscopic findings or through a failure to use other diagnostic techniques, specifically laryngeal palpation.

The 4-BAD syndrome has most often been described in isolated case reports in the equine veterinary literature under other titles and may be subject to a number of misconceptions. Under the guise of rostral displacement of the palatopharyngeal arch (RDPA) there have been descriptions of 13 previous cases; these have involved eight thoroughbreds (Cook 1974, Goulden et al 1976, Blikslager et al 1999), three warmbloods (Wilson et al 1986), one Haflinger (Deegen & Klein 1987), and one Hanovarian (Klein et al 1989). The ages at presentation ranged from 3 months to 6 years and the clinical signs predominantly related to abnormal respiratory noises (seven cases), dysphagia (one case) and recurring colic (one case). A further case, described as "cricopharyngeal laryngeal dysplasia"

(Dixon et al 1993) involved a 7-year-old thoroughbred which showed sudden-onset dyspnea and was later euthanased because of severe aerophagia. From this limited list of reports the impressions might be gained that the disorder is so rare, and that the diagnosis is so straightforward, that it is scarcely worthy of specific attention. However, more recently a series of 60 cases of 4-BAD in thoroughbreds and eight cases in other breeds has been reviewed (Lane 2003) and that study forms the basis for this contribution.

The term 4-BAD was suggested as a more appropriate description because it implies that there is a syndrome of deformities that may afflict the derivatives of the fourth branchial arch (Lane 1993). It had previously been suggested (Goulden et al 1976) that failure of development of these structures might offer an explanation for RDPA. However, in isolation, the term RDPA is misleading because it does no more than describe the endoscopic tip of an iceberg and gives little intimation of the major underlying congenital structural deformities.

The presenting signs of 4-BAD reflect the structures which are defective and fall into two broad groups – dynamic obstructions to airflow producing abnormal respiratory noises during exercise, and incompetence of the upper esophageal sphincter causing involuntary aerophagia, occasionally leading to tympanitic colic.

Normal abduction of the arytenoid cartilages and vocal folds is compromised when the mechanical stability afforded by the cartilage box structure of the larynx is defective. Thus, when there is no stable union between the thyroid and cricoid cartilages the forces applied through contraction of the cricoarytenoideus dorsalis muscle(s) may be transmitted via the vocal fold(s) to draw the body of the thyroid caudally rather than to abduct the vocal process(es) laterally. Such futile cricoarytenoideus dorsalis activity might give the false impression on endoscopy of laryngeal hemiparesis in animals with unilateral defects, or of laryngeal paresis in bilaterally diseased horses. RDPA is more easily explained: the caudal pillars of the soft palate are normally anchored at the esophageal aditus and are not visible by endoscopy *per nasum* because they are hidden behind the apices of the corniculate processes of the arytenoid cartilages. When the muscular support of the proximal esophagus is absent, the pillars lose this

anchorage and become passively displaced rostral to the apices of the corniculate processes. Involuntary aerophagia is inevitable when the proximal esophagus cannot be maintained in a closed state and there is continuity between the airspaces of the nasopharynx and the upper esophagus. It should not be surprising that horses with 4-BAD are occasionally afflicted with tympanitic colic. Normal deglutition depends upon contraction of the crico- and thyro-pharyngeal musculature to initiate primary waves of esophageal peristalsis, but in 4-BAD-afflicted horses the transfer of ingesta through the esophagus is dependent upon secondary peristaltic waves alone. Nevertheless, dysphagia is an unusual presenting sign, possibly because there is no obstruction to the passage of ingesta.

Clinical Manifestations

Although the overwhelming majority of horses identified with 4-BAD have been thoroughbreds, it is likely that any breed can be afflicted. Apart from the isolated case reports mentioned above and the previously noted 60 thoroughbreds, the only major review of the disorder included one Dartmoor pony (aged 5 months at the time of presentation), two Welsh Section A ponies (5 months and 11 months), one Warmblood (5 months), two Irish Draught horses (2 and 4 years), one Cob (5) and one part-bred hunter (6 years) (Lane 2003). There appears to be no sex predisposition to this congenital laryngeal defect.

The circumstances in which the diagnosis of 4-BAD was made in the 60 thoroughbreds were as follows:

- referred for clinical investigation of abnormal respiratory noise (37)
- referred for investigation of dysphagia (1)
- identified at prepurchase examination, including sales arbitration panel (8)
- identified during routine stud pretraining or presales screening (13)
- incidental finding during investigation of unrelated disease (1)

Thus, the most frequent presenting sign is abnormal respiratory noise at exercise, which was present in 50 out of these 60 horses. The solitary case with dysphagia was a foal that was concurrently afflicted with a unilateral hypoplasia of the soft palate. Six of the non-thoroughbreds were referred for investigation of stridor at exercise, one had shown recurrent tympanitic colic and the last non-thoroughbred (an asymptomatic entire male) was identified during a prebreeding survey. One reason why thoroughbreds may be overrepresented in the diagnosis of 4-BAD could be that afflicted horses in less athletic breeds are not exerted to the point where untoward respiratory sounds are evident and veterinary attention may not be sought unless other less frequent signs, such as colic, arise.

4-BAD most commonly exists as a right unilateral disorder and, although some cases are bilaterally afflicted, right-sided defects arise at least six times more frequently than on the left side alone (Lane 2003). In 60 afflicted thoroughbreds bilateral defects of the fourth branchial arch were identified in 15 horses, the anomalies were restricted to the right side in 39 cases, and to the left side in six of the patients.

A pretraining survey of almost 3,500 thoroughbred yearlings yielded seven animals afflicted with 4-BAD, intimating that the prevalence of the condition is in the order of two per 1,000 foaled in this breed (Lane 2003). This survey was performed over 16 years on behalf of an owner–breeder and, apart from selection for genetic excellence, the group as a whole had not been subjected to any prior screening process when the above examinations were performed. To put this incidence of 4-BAD into context, the same survey led to the identification of five yearlings with subepiglottal cysts, five with idiopathic right-sided laryngeal malfunction, and two with epiglottal entrapment.

Diagnosis and Differential Diagnosis

Palpation

Palpation is a straightforward but effective technique in the diagnosis of 4-BAD. Its purpose is to identify defects of the laryngeal cartilage skeleton. The technique depends upon assessing the space between the cricoid and thyroid cartilages. Normally this space is restricted to a small gap ventrally, corresponding to the cricothyroid ligament. As the fingers pass laterally in normal horses, the wings of the thyroid cartilage can be felt overlapping the cricoid ring. A large gap is present on the defective side(s) of those horses with absence of one or both wings of the thyroid (Fig. 31.1). The overwhelming majority of 4-BAD-afflicted horses can be identified by palpation alone – a positive finding was achieved in 55 out of 58 horses where the findings were documented, with 11 bilateral gaps, 39 right-sided defects, and only five with left unilateral defects identified (Lane 2003). However, the total number of cases with positive findings included three horses where palpation was used retrospectively after surgery had confirmed a defect of the thyroid cartilage (see below).

Endoscopy

Endoscopy of the upper respiratory tract will reveal reduced arytenoid motility on the afflicted side(s) and/or RDPA. Sometimes, in bilateral cases where a comparison between right- and left-sided movements is sought, evidence of reduced laryngeal function is more difficult to establish. Similarly, because recurrent neuropathy is an all too familiar disorder afflicting the left side of the equine larynx, the correct diagnosis of 4-BAD may be overlooked in left-sided unilaterally afflicted horses. Reduced motility
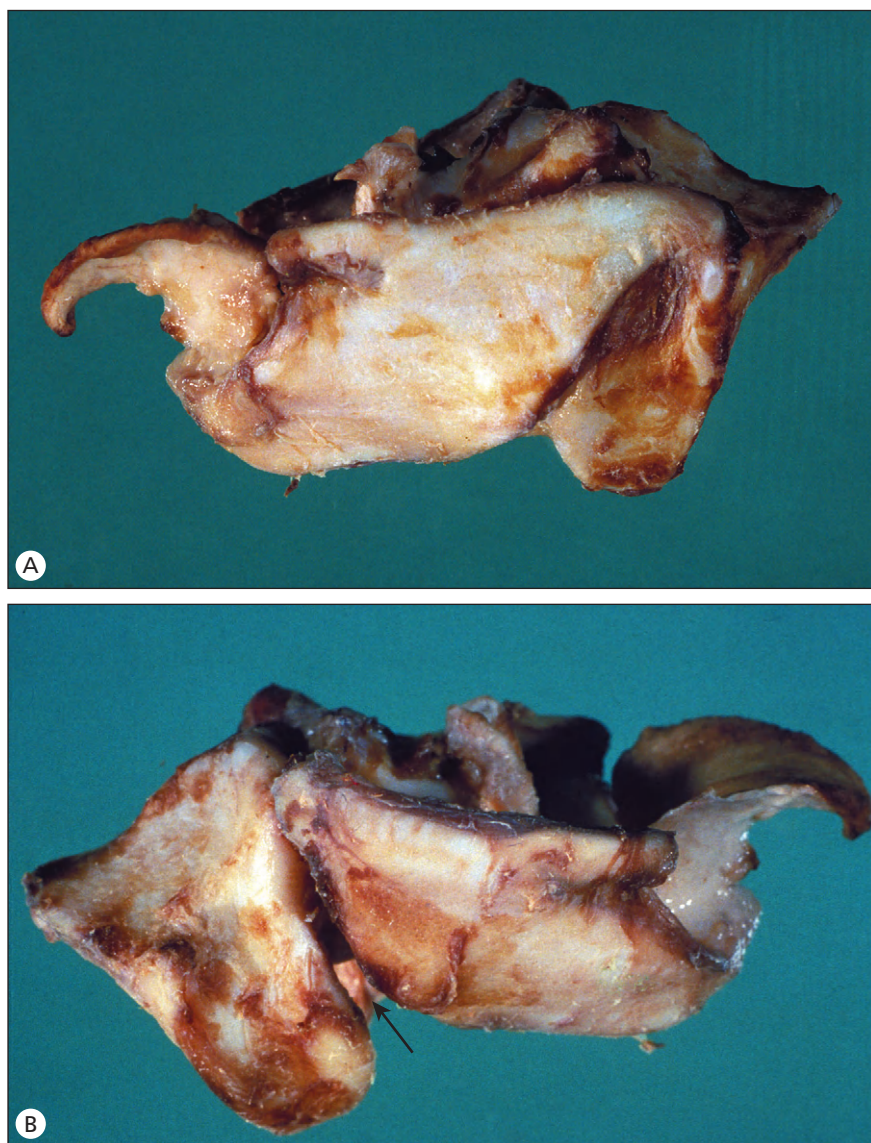
Fig. 31.1. The appearance of the laryngeal cartilages of a horse unilaterally afflicted with 4-BAD on the right side: (A) view of the normal left side, (B) view of the defective right side. Note the absent wing of the thyroid cartilage and the resultant wide cricothyroid space (arrow).

on the right side of the larynx is always more likely to sound alarm bells (Fig. 31.2) and 4-BAD is the most common cause of such a finding (Tulleners et al 1996). RDPA may be obvious to the extent that it is possible to see an open esophageal aditus (Fig. 31.3) but it is, on occasions, more subtle with no more than a "lipping" of the displaced palatal arch over the apices of the corniculate processes. RDPA will be evident only in those horses which have aplasia or hypoplasia of the thyropharyngeus and/or cricopharyngeus muscles, which accounts for slightly more than half of cases – present in 30 of the 59 horses where the technique was applied (Lane 2003). However, overall endoscopy provided positive findings in 58 out of the 59 cases of 4-BAD where it was applied, the exception being a horse with very minor defects that were only confirmed by a combination of endoscopy during treadmill exercise and surgical exploration.

Endoscopy during high-speed treadmill endoscopy provides a definitive means to identify the structures that are intruding into the airway during forceful respiration. As is frequently the case with dynamic collapse of the upper respiratory tract, the obstructions are often complex with partial collapse of the arytenoid cartilage and axial deviation of the aryepiglottal fold being the most common combination. Such information may be helpful if surgical correction of the respiratory obstruction is to be considered.

Radiography

Radiography of the pharynx and larynx takes advantage of the excellent contrast that exists in this area and seeks to show a continuous column of air between the nasopharynx and the upper esophagus. This can only arise when the upper esophageal sphincter muscles are defective,
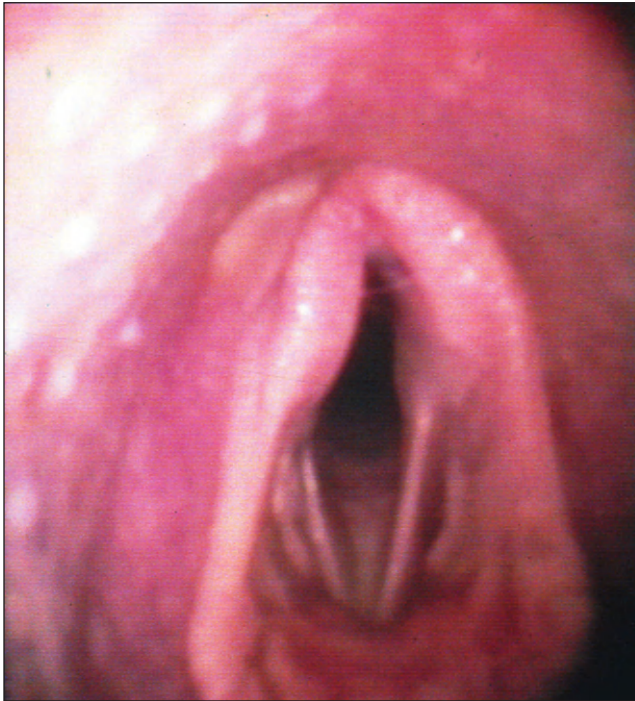
Fig. 31.2. Endoscopic view of the larynx of a 7-year-old gelding pre-senting with abnormal inspiratory noise at exercise. Note the reduced abduction by the right arytenoid cartilage and vocal fold. Congenital defects of the cartilages and musculature were only discovered when prosthetic laryngoplasty was mistakenly attempted.

Fig. 31.3. Endoscopic view of the larynx of a 2-year-old filly presenting with abnormal inspiratory noise at exercise. Note the rostral displacement of the caudal pillars of the soft palate and the open esophageal aditus.

otherwise the esophageal aditus remains closed and no air enters. In lateral projections of cases of 4-BAD with RDPA the rostrally displaced palatal pillars are seen as a "dew drop" dorsal and slightly rostral to the corniculate processes of the arytenoids and large volumes of air are present in the esophagus (Fig. 31.4). Clearly, radiography will only yield positive results in those cases where RDPA is identifiable on endoscopy. Thus, in clinical practice radiography may be regarded as a luxury rather than a necessity in the diagnosis of 4-BAD. There will be no radiological abnormalities in those afflicted horses showing reduced arytenoid motility but no RDPA (Lane 2003).

Dynamic fluoroscopic studies of deglutition (e.g. using a bran mash impregnated with barium sulfate as the contrast medium) may be used to assess the influence of 4-BAD on deglutition. In normal horses, primary esophageal peristalsis is triggered by the closure of the upper esophageal sphincter after the passage of food and fluid boluses from the oropharynx to the proximal esophagus has occurred. However, in horses with defective thyropharyngeus and cricopharyngeus musculature, this mechanism cannot apply, with the result that ingesta is moved caudally by secondary waves of peristalsis initiated by stretch receptors in the esophageal wall after a mass of ingesta has been propelled caudally by the pharyngeal "stripping wave".
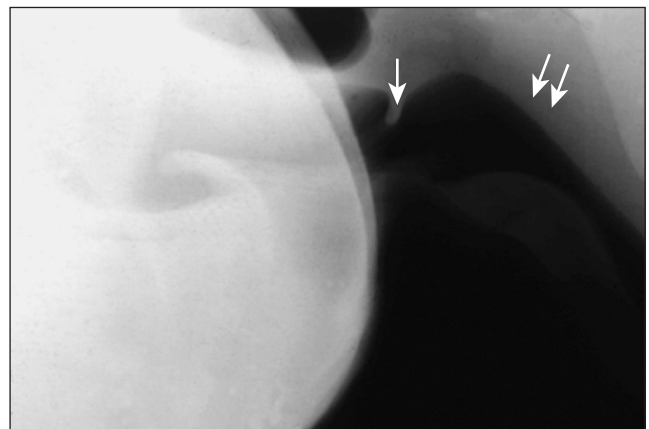
Fig. 31.4. Lateral radiograph of pharynx and larynx of the same 2-year-old filly shown in Fig. 31.3 showing a rostrally displaced palato-pharyngeal arch (single arrow) and an air column in the proximal esophagus (double arrows).

Exploratory surgery/autopsy

Clearly the full extent of the anatomical anomalies present in any individual 4-BAD-afflicted horse can only be confirmed by exploratory laryngeal surgery or autopsy. One or other of these procedures was applied to 11 of 60 horses (Lane 2003). In the two horses with endoscopic evidence of left laryngeal dysfunction, but without RDPA, an incorrect

diagnosis of recurrent laryngeal neuropathy was made and it was only when prosthetic laryngoplastic surgery was attempted that the defective laryngeal structures were discovered. One horse with a right-sided 4-BAD was similarly misdiagnosed preoperatively and the deformity was only confirmed at surgery. In all three horses, a palpable defect between the cricoid and thyroid cartilages on the affected side had been overlooked before surgery but was confirmed postoperatively.

A full autopsy was performed on six afflicted horses. The most consistent findings consisted of maldevelopment of one or both wings of the thyroid cartilage and consequently absence of the cricothyroid articulation(s). These are the features that lend themselves to diagnosis by palpation. The frequency of aplasia or hypoplasia of the cricothyroid musculature cannot be estimated other than at autopsy. In all six horses the cricothyroid muscles were symmetrical in appearance – normal in two, vestigial in two, and aplastic in two. The cricopharyngeus muscular sphincter was generally absent or hypoplastic (Lane 2003).

Differential diagnosis

The 4-BAD syndrome should be considered as an alternative diagnosis in cases presented as stereotypic "windsucking" behavior. Frequently those involved in the routine stable care of afflicted horses will report hearing "belching" sounds corresponding to the passage of air out of the esophagus. Clearly, no horse should be subjected to any form of therapy for stereotypic aerophagia until the possibility of 4-BAD has been eliminated by palpation and endoscopy.

The most likely cause for diagnostic confusion lies with those horses that show reduced laryngeal function on one or both sides but without RDPA, and that might otherwise be diagnosed as being afflicted with recurrent laryngeal neuropathy if endoscopy is used and palpation is not. Horses with right-sided 4-BAD account for the majority of cases with reduced right-sided laryngeal motility. However, it is correct clinical practice to explore other possibilities by checking the course of the recurrent nerve at the level of the ipsilateral auditory tube diverticulum, examining the ipsilateral neck for evidence of scarring, and assessing the filling of the ipsilateral jugular vein.

Horses that have recently been subjected to laryngeal surgery or have sustained trauma in this region may develop swelling in the area of the cricopharynx that can displace the caudal pillars of the soft palate forwards, resembling RDPA. Sedation also causes relaxation of the nasopharyngeal muscles that may mimic RDPA.

Treatment and Prognosis

At present, no surgical remedy has been devised to replace or reconstruct the deficient structures. However, in the past there have clearly been misunderstandings regarding the

nature of the underlying disorder because there have been reports of resection of rostrally displaced palatal pillars either by sharp instrumentation or with a cutting laser. It comes as no surprise that the results of these techniques were universally disappointing (Blikslager et al 1999).

The racing performance statistics of the afflicted horses are incomplete but of 51 affected cases that were raced, seven horses won races and three others were placed. One horse won six races between the ages of 2 and 6 years. However, none of the horses has won a group, listed or stakes race and the majority of the successes were achieved over short distances (Lane 2003). It is concluded that 4-BAD is likely to be a performance-limiting disorder and that afflicted horses cannot be recommended for purchase whenever they are identified before sale.

Discussion and Conclusions

Fourth branchial arch defects constitute an important syndrome which may arise in any breed of horse but which is more likely to be identified in racehorses simply because they are subjected to the greatest athletic exertion where any respiratory impediment will cause performance limitation. Most afflicted horses present with abnormal respiratory noise during exercise. An incidence of two cases per 1,000 thoroughbreds foaled (0.2%) means that all clinicians involved in equine practice will encounter horses with 4-BAD from time to time. Indeed, the condition is more common in thoroughbreds than other better recognized entities such as subepiglottal cyst and epiglottal entrapment, although it is conceded that the latter disorder is frequently acquired in later life.

Previous reports have often used the term RDPA as if it were a diagnosis in its own right rather than an endoscopic finding pointing to a serious underlying laryngeal deformity. As with many obstructive disorders of the upper respiratory tract, an overdependence on endoscopy of the resting horse, to the exclusion of other simpler techniques such as palpation and an exercise test, is likely to lead to a failure to achieve a complete or correct diagnosis. Laryngeal palpation should routinely be used to identify defects of the cartilage skeleton in horses with 4-BAD as well as to assess the presence or absence of atrophy of the intrinsic musculature in cases of recurrent neuropathy, and investigate the presence of scars from previous surgical interferences.

It is difficult to explain the clear overrepresentation of right-sided 4-BAD. It seems improbable that there are equal numbers of right- and left-sided cases and that the left 4-BAD-afflicted horses are simply being misdiagnosed as recurrent neuropathy. If this were the case many more structural defects would have been encountered during routine prosthetic laryngoplasty surgery. In a previous report of right laryngeal "hemiplegia" (Tulleners et al 1996) seven out of 11 thoroughbreds were found to have

congenital malformations of the laryngeal cartilages. Indeed, 4-BAD should be considered as a likely explanation whenever right-sided laryngeal dysfunction is identified in clinical practice.

Regardless of the severity of the defects, preliminary studies of race performance records suggest that afflicted horses are less likely to become effective athletes than non-affected horses. This has medicolegal implications for veterinarians, who should be vigilant for this condition at prepurchase examinations of horses, and for bloodstock auctioneers, who should take this performance-limiting disorder into account when drafting their veterinary conditions of sale. An increased awareness of the simplicity of a definitive diagnosis by palpation alone in almost all cases would be helpful.

REFERENCES

Blikslager AT, Tate LP, Tudor R 1999 Transendoscopic laser treatment of rostral displacement of the palatopharyngeal arch in four horses. Journal of Clinical Laser Medicine and Surgery 17: 49–52

Cook WR 1974 Some observations on diseases of the ear, nose and throat in the horse, and endoscopy using a flexible fibreoptic endoscope. Veterinary Record 94: 533–541

Deegen E, Klein, H-J 1987 Rostrale verlagerung des arcus palatopharyngicus beim pferd. Pferdeheilkunde 3: 303–308

Dixon PM, McGorum BC, Else RW 1993 Cricopharyngeal-laryngeal dysplasia in a horse with sudden clinical onset of idiopathic laryngeal hemiparesis. New Zealand Veterinary Journal 41: 134–138

Goulden BE, Anderson LJ, Davies AS, Barnes GRG 1976 Rostral displacement of the palatopharyngeal arch: a case report. Equine Veterinary Journal 8: 95–98

Hast MH 1972 Early development of the human laryngeal muscles. Annals of Otology, Rhinology and Laryngology 81: 524

Klein H-J, Deegen E, Stockhofe N, Wissdorf H 1989 Rostral displacement of the palatopharyngeal arch in a seven-month-old Hanoverian colt. Equine Veterinary Journal 21: 382–383

Lane JG 1993 Fourth branchial arch defects in the horse. In: Dyson SJ, Lane JG (editors) Proceedings of the 15th Bain-Fallon Memorial Lectures. Australian Equine Veterinary Association, Artamon, pp.49–50

Lane JG 2003 Non-RLN upper respiratory tract disorders found in a survey of 3497 Thoroughbred yearlings. In: Dixon PM, Robinson NE, Wade JF (editors) Havemeyer Foundation Monograph Series. R & W Publications, Newmarket, pp.49–50

Tulleners EP, Ross MW, Hawkins J 1996 Management of right laryngeal hemiplegia in horses: 28 cases. ACVS Abstract in Veterinary Surgery 25: 439

Wilson RG, Sutton RH, Groenendyk S 1986 Rostral displacement of the palatopharyngeal arch in a Thoroughbred yearling. Australian Veterinary Journal 63: 99–100

Zaw-Tun HA, Burdi AR 1985 Re-examination of the origin and early development of the human larynx. Acta Anatomica (Basel) 122: 163–184

32 Idiopathic Recurrent Laryngeal Neuropathy: Etiopathogenesis

Caroline Hahn and Joe Mayhew

Introduction

Idiopathic recurrent laryngeal neuropathy (RLN) is a prominent disease of domestic horses that is characterized by the distal degeneration of axons in the recurrent laryngeal nerve supplying the larynx, and paresis or paralysis of the left vocal fold. Respiratory impairment and inspiratory stridor ("roaring") ensue and the athletic performance of affected animals can be significantly impaired. It is widely reported throughout the world in both temperate and tropical climates (Cahill & Goulden 1987). In addition, a large proportion of clinically unaffected horses have histological changes of the intrinsic laryngeal musculature and recurrent laryngeal nerves characteristic of RLN (Cook 1989).

Despite the fact that "roaring" was described in the 18th century, a great deal remains to be learned about the pathogenesis and precise pathological changes of RLN. Fundamental questions, such as whether or not RLN is a manifestation of a polyneuropathy, whether the cause involves a mechanical component, and whether there is a prenatal onset, have not been resolved and the etiology remains a mystery.

Anatomy

The mammalian larynx acts as a modified air valve with a number of functions, including protection of the lower respiratory tract and phonation, and the curious anatomy of this organ has its origin in the evolution of air-breathing animals.

The lungfish evolved the ability to breathe air directly from the external environment about 400 million years ago, perhaps because its watery home was periodically subject to drought (Ewings 1949). A simple larynx-like slit behind the gills developed to allow air into the swim bladder when the fish was exposed to the atmosphere, and to keep water out when it was submerged. As the lungfish's descendants moved on to land, this swim bladder evolved into lungs, a multi-compartment organ with a large surface area whose sole function was gas exchange. The larynx in the meantime developed adductor and abductor muscles and lateral cartilages (such as found in

the axolotl), then separate arytenoid and cricoid cartilages (newt), primitive thyroid cartilages (alligators and their feathered relatives, the birds) and finally the complex mammalian larynx. As the survival of equids depended on their athletic capability to escape predators they evolved a larynx that when fully abducted has an aperture that is larger than the trachea itself; this is in sharp contrast to the human larynx, which allows for speech but when abducted is only half the diameter of the trachea).

An appreciation of the anatomy of laryngeal innervation is a prerequisite to understanding the pathological changes characteristic of RLN. The main source of laryngeal innervation of the equine larynx is the ipsilateral recurrent laryngeal nerve. Motor neurons of the recurrent laryngeal nerve are in the nucleus ambiguus in the caudal brainstem. This nucleus was recently localized in the horse (Hackett 2000) and was found to be a loosely organized column of cells in the ventrolateral medulla oblongata. A somatotopic distribution of adductor and abductor motor neurons was not apparent but neurons innervating the cricoarytenoideus lateralis muscle were observed throughout the nucleus, whereas neurons innervating the cricoarytenoideus dorsalis (the only laryngeal abductor) tended to be situated more rostrally.

Nucleus ambiguus axons loop around the parasympathetic nucleus of the vagus to emerge from the brainstem as axons of the internal branch of the accessory nerve, cranial nerve (CN) XI. They only join the vagus nerve (CN X) on leaving the skull through the jugular foramen and tympano-occipital fissure (Fig. 32.1).

Growth of the head and neck during embryogenesis, and differential degeneration of the sixth aortic arch, result in extremely long nerves with the left and right nerves having different pathways. The left nerve loops around the aorta while the right takes a shorter route around the right subclavian artery. Including its vagal course, the total length from neuronal cell body to larynx of the left recurrent laryngeal nerve can be up to 250 cm, making it twice as long as other motor nerves in the horse and 31 cm longer than the right recurrent laryngeal nerve.

The normal recurrent laryngeal nerve consists of medium-sized myelinated fibers with only scattered, smaller
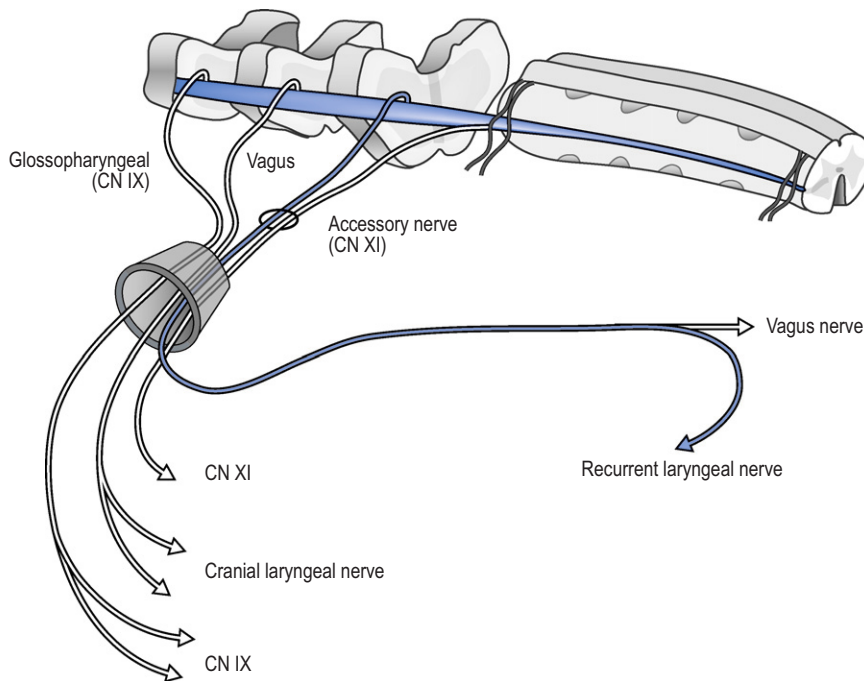
Fig. 32.1. The recurrent laryngeal nerve is supplied by axons originating in the caudal nucleus ambiguus. Redrawn from de Lahunta, 1983, with permission.

diameter fibers present. Myelinated axons in the recurrent laryngeal nerve segregate as fascicles within the vagus nerve. After these fascicles separate from the vagus as the recurrent laryngeal nerve however, the axons that are targeted to innervate a particular intrinsic laryngeal muscle are not discretely clustered within the recurrent laryngeal nerve at its origin in the thorax, but instead are mixed among the fascicles throughout its length. This implies that focal, non-selective damage ("compression") of the recurrent laryngeal nerve is unlikely to be the cause of the different severities of lesions found in specific laryngeal muscles of RLN cases.

Although the recurrent laryngeal nerve is classically thought of as a motor nerve, primary afferent ("dorsal root ganglia") recurrent laryngeal nerve neurons have been demonstrated in the proximal and distal vagal ganglia. The distal vagal ganglion is poorly described in the horse but histologically consists of scattered neurons in the vagus nerve at its bifurcation with the cranial laryngeal nerve. Involvement of sensory axons in horses with recurrent laryngeal neuropathy has not been established. The recurrent laryngeal nerve then courses cranially to provide motor innervation to the paired intrinsic laryngeal muscles, with the exception of the cricothyroideus muscle, which is innervated by the cranial laryngeal nerve (de Lahunta 1983).

The complexity and length of this pathway is likely to underlie the pathological changes in RLN.

Pathological Changes

The lesions associated with RLN have been well characterized at the light and electron microscopic levels (Cole 1946, Duncan & Griffiths 1974, Duncan et al 1978, 1991a,b, Cahill & Goulden 1986a,b,c,d,e).

The principal pathological changes are found in the recurrent laryngeal nerve and are characterized by a progressive loss of large myelinated fibers in the distal nerve (Fig. 32.2). It is worth pointing out, however, that the same trend, including the presence of whorled
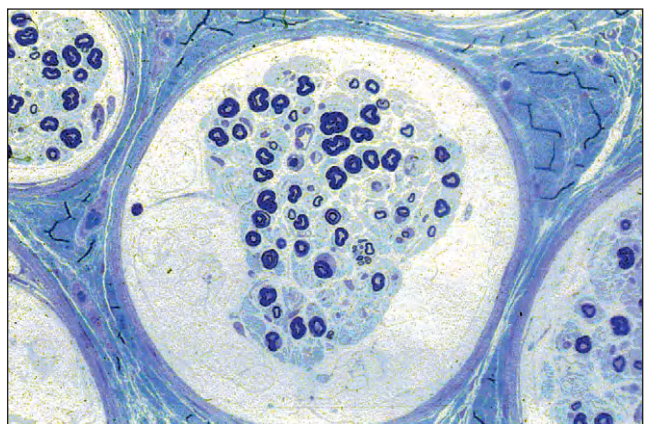
Fig. 32.2. Plastic-embedded 1-μm section of left recurrent laryngeal nerve showing loss of myelinated fibers.
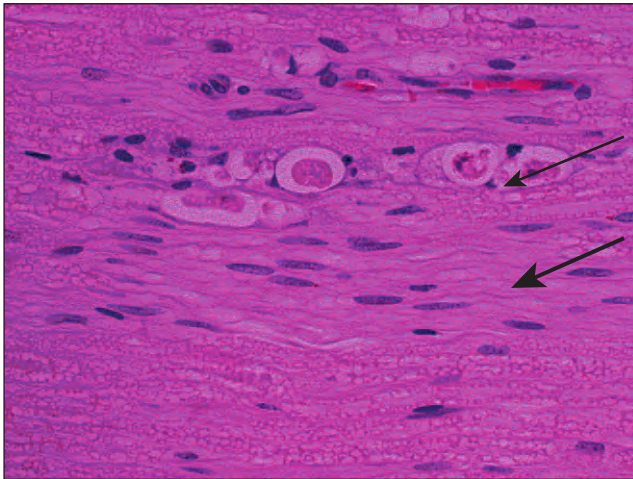
Fig. 32.3. Longitudinal section of left recurrent laryngeal nerve showing Wallerian degeneration (thin arrow) with myelin digestion chambers, axonal and myelin debris and macrophages. The thick arrow points to proliferating Schwann cells, the so-called bands of Büngner.
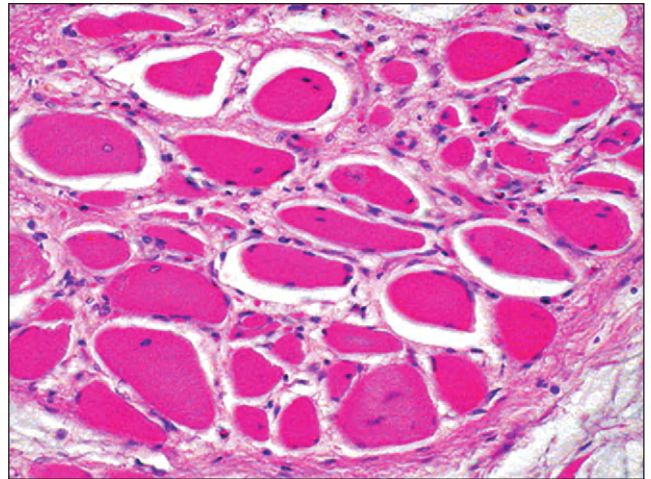
Fig. 32.4. Left cricoarytenoideus lateralis muscle. There is fibrosis, myofiber angular atrophy, and hypertrophy with multiple fibers having internal nuclei.

fibrillary structures (Renaut bodies) that has been reported in RLN cases, has been shown in "normal" horses (Lopez Plana et al 1993). Axonal loss has been found to be greatest in the distal portions of the left and right recurrent laryngeal nerves but has also been noted proximal and distal to the aorta and in the vagus nerve. Changes noted in the right recurrent laryngeal nerve are less severe than those found in the left. Lesions are presumed to be found principally in motor axons and it is unknown if sensory fibers in the recurrent laryngeal nerves are also affected. Vagal sensory ganglionic cell bodies should be examined for neuronal chromatolysis.

The primary lesion may be axonal in nature, as indicated by collapsed myelin sheaths, increased relative myelin sheath thickness (potentially as a result of axonal atrophy), regenerating Schwann cell membrane clusters and paranodal and internodal accumulations of axonal debris and organelles. The latter may be an indication that a defect in the axonal transport systems results in the eventual distal axonal degeneration. Signatures of proliferating Schwann cells (Büngner's bands and onion bulbs) are commonly found (Fig. 32.3), as are myelin digestion chambers containing central axon fragments. Teased fiber preparations show a marked variation in internodal length and diameter indicating chronic demyelination and attempted remyelination. This could be the result of either a primary myelin deficit or, perhaps more likely, underlying axonal damage.

Evidence of damage to the cell bodies of the recurrent laryngeal nerve has been sought; however, neither Cahill and Goulden (1986e) nor S. Hackett and C.W. Cummings (personal communication) were able to identify lesions in the nucleus ambiguus in the brainstem of affected horses. This is perhaps not surprising because the chromatolysis seen in lower motor neuron soma secondary to the axonal damage is influenced by the proximity of the lesion, which in the case of RLN may be over 1 m away. Chromatolysis or neuronal loss in the nucleus ambiguus would however be anticipated if the axonal changes were the result of somatic (cell body) lesions, as has been described in Bouvier des Flandres (Venker-van Haggen 1980) and Siberian Husky dogs (O'Brien & Hendriks 1986). Unfortunately, there has been no systematic work in the horse evaluating the peripheral or central pathological changes that accompany damage to long axons. Ultrastructural examination of nucleus ambiguus neurons has been attempted but is complicated greatly by the difficulty of identifying the boundaries of the nucleus in the medulla oblongata. It was believed that there was a difference in the number of neurons in horses with RLN compared to normal horses but the small number of animals examined did not allow a statistical comparison (S. Hackett, personal communication). There have been no histochemical techniques applied to identify somal changes secondary to the hypothesized transport disorder.

Lesions in the laryngeal muscles innervated by the recurrent laryngeal nerves are characteristic of neurogenic disease (Fig. 32.4). Denervation of the adductor muscles precedes abductor involvement and typical changes include scattered angular fibers and groups of atrophied fibers adjacent to hypertrophied fibers with central nuclei (Duncan & Griffiths 1974, Duncan et al 1991a). The cricoarytenoideus lateralis is among the earliest and most severely affected muscles (Lopez Plana et al 1993). The chronic or repetitive nature of the disease is further exemplified by the presence of muscle fiber type grouping, because muscle fiber type is controlled by the innervating neuron.

These pathological changes have been classified as a distal axonopathy, with the greater damage in the left recurrent laryngeal nerve being explained by its greater length. One hypothetical cause of distal axonopathy is a defect in the neuronal soma, because the axon depends on the cell body for metabolic support and trophic influences. Indeed, many of the peripheral nerve lesions that are typically found in equine motor neuron disease, a disease primarily affecting the cell body, are also observed in RLN including axonal atrophy, proliferated Schwann cell cords (Büngner's bands), loss of myelinated fibers and an increase in endoneurial collagen.

Hypothetical Etiologies

Despite many years of work we appear to be no closer to clarifying the etiology of this common equine disease. Hypotheses range from mechanical causes, such as tension and stretch to the recurrent laryngeal nerve and its blood supply during neck movement, growth, or growth of the head and neck during embryonic development, to environmental factors including toxins (reviewed by Cahill & Goulden 1987). Compression of the nerve as it loops around the aorta might be expected to result in nerve lesions at that site; however, this has not been noted, and toxins have been viewed as unlikely causes of RLN as the neuropathological changes appear to be limited to the recurrent laryngeal nerves. The effect of nerve stretch on the blood supply of the recurrent laryngeal nerve (the vasa nervorum) has not been evaluated.

A hypothetical etiology for RLN is an inherited axonopathy or myelinopathy. Comparable pathological changes have indeed been noted in foals (Duncan 1992, Harrison et al 1992) and clinical signs of left-sided hemiplegia have been demonstrated to be clinically progressive (Dixon et al 2002). There have however been no reports of left hemiplegic horses progressing to develop right-sided clinical signs (P.M. Dixon, personal communication), and horses affected with RLN clearly do not show classical clinical signs of polyneuropathy such as mega-esophagus, tetraparesis, and somatic muscle atrophy. However, involvement of other long peripheral nerves (common, deep and superficial peroneal and tibial nerves) has been reported by some workers (Cahill & Goulden 1986a, Kannegieter 1989), but was not found by Duncan et al (1978). Similarly, neurogenic muscle changes have been reported to exist in the extensor digitorum longus (Cahill & Goulden 1986d) in three out of four horses suffering from RLN. The above observations, however, are isolated, uncontrolled and have not taken into account that age-related pathological changes can be demonstrated in the distal limb nerves of horses (Wheeler 1987). A detailed study of the peripheral nerves in RLN-affected and control animals has not been undertaken.

It should be remembered that axonal degeneration, characterized by distal degeneration that spreads proxi-

mally ("dying back"), is the most common lesion seen in peripheral nerve diseases caused by a wide variety of toxic, metabolic, and infectious insults. Some of these processes affect the cell body, and it may be that the axonal dying-back process is initiated to conserve energy: how a cell can eliminate part of itself while leaving the rest intact is unknown. Localized axonal degeneration that resembles dying back can also occur in cell culture if the distal portion of the axon is deprived of nerve growth factor and a similar process may be involved in disease states. Other forms of axonal degeneration that seem distinct from typical dying back occur in various human neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases.

Pathological changes of the recurrent laryngeal nerve in RLN have been described in great detail using both light and electron microscopy, but the tools of the burgeoning science of molecular pathology have not been utilized: a detailed examination of changes in gene regulation and cytokine expression will have to be applied if further details of the pathogenesis are to be uncovered.

REFERENCES

Cahill JI, Goulden BE 1986a Equine laryngeal hemiplegia. I. A light microscopic study of peripheral nerves. New Zealand Veterinary Journal 34: 161–169

Cahill JI, Goulden BE 1986b Equine laryngeal hemiplegia. II. An electron microscopic study of peripheral nerves. New Zealand Veterinary Journal 34: 170–175

Cahill JI, Goulden BE 1986c Equine laryngeal hemiplegia. III. A teased fibre study of peripheral nerves. New Zealand Veterinary Journal 34: 181–185

Cahill JI, Goulden BE 1986d Equine laryngeal hemiplegia. IV. Muscle pathology. New Zealand Veterinary Journal 34: 186–190

Cahill JI, Goulden BE 1986e Equine laryngeal hemiplegia. V. Central nervous system pathology. New Zealand Veterinary Journal 34: 191–193

Cahill JI, Goulden BE 1987 The pathogenesis of equine laryngeal hemiplegia – a review. New Zealand Veterinary Journal 35: 82–90

Cole CR 1946 Changes in the equine larynx associated with laryngeal hemiplegia. American Journal of Veterinary Research 7: 69–77

Cook WR 1989 Recent observations on recurrent laryngeal neuropathy in the horse: applications to practice. Proceedings of the Annual Convention of the American Association of Equine Practitioners, pp.427–478

de Lahunta A 1983 Veterinary Neuroanatomy and Clinical Neurology. WB Saunders, Philadelphia

Dixon PM, McGorum BC, Railton DI et al 2002 Clinical and endoscopic evidence of progression in 152 cases of equine recurrent laryngeal neuropathy (RLN). Equine Veterinary Journal 34: 29–34

Duncan ID 1992 Determination of the early age of onset of equine recurrent laryngeal neuropathy. 2. Nerve pathology. Acta Neuropathologica (Berlin) 84: 316–321

Duncan I D, Griffiths I R 1974 Pathological changes in equine laryngeal muscles and nerves. Proceedings of the

Nineteenth Annual Convention of the American Association of Equine Practitioners, pp.97–113

Duncan ID, Griffiths IR, Madrid RE 1978 A light and electron microscopic study of the neuropathy of equine idiopathic laryngeal hemiplegia. Neuropathology and Applied Neurobiology 4: 483–501

Duncan ID, Amundson J, Cuddon PA et al 1991a Preferential denervation of the adductor muscles of the equine larynx. I: Muscle pathology. Equine Veterinary Journal 23: 94–98

Duncan ID, Reifenrath P, Jackson KF et al 1991b Preferential denervation of the adductor muscles of the equine larynx. II: Nerve pathology. Equine Veterinary Journal 23: 99–103

Ewings V 1949 The Comparative Anatomy and Physiology of the Larynx. William Heinemann, London

Hackett S 2000 The equine nucleus ambiguus: myotopic and neurotopic representations of motor and sensory components of the recurrent laryngeal nerve. PhD Thesis, Cornell University, USA

Harrison GD, Duncan ID, Clayton MK 1992 Determination of the early age of onset of equine recurrent laryngeal neuropathy. 1. Muscle pathology. Acta Neuropathologica (Berlin) 84: 307–315

Kannegieter N 1989 A study of distal hindlimb muscles and nerves in normal and laryngeal hemiplegic horse. PhD Thesis, Massey University, New Zealand

Lopez Plana C, Sautet JY, Pons J et al 1993 Morphometric study of the recurrent laryngeal nerve in young "normal" horses. Research in Veterinary Science 55: 333–337

O'Brien JA, Hendriks J 1986 Inherited laryngeal paralysis. Analysis in the husky cross. Veterinary Quarterly 8: 301–302

Venker-van Haggen AJ 1980 Investigations on the pathogenesis of hereditary laryngeal paralysis in the Bouvier. PhD thesis, University of Utrecht, The Netherlands

Wheeler SJ 1987 Structure and function of equine peripheral nerve: morphology, morphometry and clinical electrophysiology. PhD Thesis, Royal Veterinary College, UK

33

Laryngeal Paralysis with Known and Suspected Causes

Bruce C McGorum

Introduction

This chapter reviews the current state of knowledge regarding the small proportion of horses with laryngeal paralysis for which a known or suspected cause can be identified, given as 6% by Goulden & Anderson (1981) and 11% by Dixon et al (2001). The remainder of horses with laryngeal paralysis are termed idiopathic recurrent laryngeal neuropathy (RLN) cases, because their laryngeal paralysis has no known or suspected cause (see Chapter 32).

Laryngeal Paralysis Resulting from Nerve Damage

Most commonly, non-RLN laryngeal paralysis is a sequel to localized injury to the vagus or recurrent laryngeal nerves at any site along their long and circuitous pathway. Such injury may occur in disorders of the guttural pouch, pharynx, neck, and cranial mediastinum as indicated in Table 33.1.

Table 33.1. Disorders that cause laryngeal paralysis via local injury to the vagus/recurrent laryngeal nerve

Disorders of the guttural pouch
Guttural pouch mycosis
Trauma

Disorders of the pharynx
Trauma
Neoplasia

Disorders of the neck
Perivascular/perineural irritant injection reactions
Trauma
Iatrogenic nerve damage during laryngeal, esophageal, tracheal, thyroid, and vertebral surgery

Disorders of the cranial mediastinum
Neoplasia
Abscesses

Unknown site and nature of injury
Postanesthesia bilateral laryngeal paralysis (nerve stretching or compression?)

Postanesthesia Laryngeal Paralysis

Unilateral or bilateral, total laryngeal paralysis is a rare, but potentially fatal, complication of general anesthesia (Abrahamsen et al 1990, Dixon et al 1993, 2001, Southwood et al 2003). Proposed predisposing factors include dorsal recumbency with hyperextension of the neck and a dependent head position, pre-existing RLN, large body size, prolonged duration of anesthesia, hypotension, hypoventilation, hypoxemia, and postanesthetic myopathy. Contributory factors may include postoperative laryngeal edema and inflammation, and laryngeal dysfunction associated with xylazine administration (Southwood & Gaynor 2003). Postoperative laryngeal paralysis is probably caused by excessive extension of the head and neck, which could induce neural stretch injury or cause neural hypoxia via occlusion of the vasa nervorum. Alternatively, nerve damage may be a consequence of compression of the recurrent laryngeal nerve between the endotracheal tube and a rigid structure in the neck, such as the vertebrae. Affected horses develop an acute-onset, life-threatening laryngeal obstruction, with inspiratory dyspnea, stridor, and cyanosis. Extreme distress makes them difficult and dangerous to handle, and the condition can be rapidly fatal. Laryngeal obstruction may occur following extubation, when the horse is encouraged to stand during recovery, or during vocalization when the horse is walking to the stall following recovery (Southwood et al 2003). The laryngeal obstruction may also lead to potentially fatal pulmonary edema and hemorrhage (see Chapter 43). Treatments have included emergency tracheal reintubation via the oral or nasal cavities, placement of a temporary tracheostomy tube, supplemental intranasal oxygen, anti-inflammatory drugs, and in horses with secondary pulmonary edema, furosemide. Horses that survive the acute obstruction have variable recovery of laryngeal function; some horses show complete resolution within 24 h, while others have permanent laryngeal dysfunction. Southwood et al (2003) recommend avoiding hyperextension of the neck during anesthesia, and having an emergency tracheostomy kit available when recovering horses that may be predisposed to this disorder. Previously reported cases of postanesthetic death from pulmonary

Table 33.2. Generalized neuromuscular disorders that cause laryngeal paralysis
Hepatopathy Lead toxicosis Delayed organophosphate-induced neurotoxicity Australian stringhalt Plant poisoning Hyperkalemic periodic paresis

edema may in fact have been the result of bilateral laryngeal paralysis with secondary pulmonary edema (Tute et al 1996).

Generalized Neuromuscular Disorders That Cause Laryngeal Paralysis

Non-RLN laryngeal paralysis may be a manifestation of generalized neuromuscular disorders (Table 33.2). Such disorders commonly cause bilateral laryngeal paralysis.

Laryngeal paralysis associated with hepatopathy

Hepatopathy is a common cause of bilateral laryngeal paralysis (Mayhew 1989, Pearson 1991, McGorum et al 1999). Indeed bilateral laryngeal paralysis was recorded in seven of 50 horses with primary hepatic disease, all of which had encephalopathy and hyperammonemia (McGorum et al 1999). Because all horses had a loud inspiratory stridor, many were referred for investigation of suspected primary upper respiratory tract obstruction. This suggests that hepatopathy causes preferential dysfunction of the recurrent laryngeal nerve compared to other peripheral nerves. Endoscopy of affected horses reveals total bilateral paralysis, with both arytenoids being passively adducted to the midline during inspiration (Fig. 33.1). Ponies appear to be more commonly affected than horses. This may reflect the increased frequency of liver failure in ponies compared with horses, or a true predisposition of ponies to develop this type of bilateral laryngeal paralysis. The laryngeal dysfunction may be temporary, resolving with restoration of hepatic function, but worsening again when the encephalopathy recurs.

The pathogenesis of this complication is unknown. To date, no gross or histopathological lesions have been identified in the laryngeal muscles, recurrent laryngeal nerve or other peripheral nerves of affected horses. Reported cases have involved horses with hepatic failure and encephalopathy; whether it occurs in horses with compensated liver disease is unknown. While laryngeal paralysis has been reported in horses with pyrrolizidine alkaloid-induced hepatopathy (Pearson 1991, McGorum et al 1999), the causal role of pyrrolizidine alkaloids, which may be neurotoxic *per se* (Huxtable et al 1996),
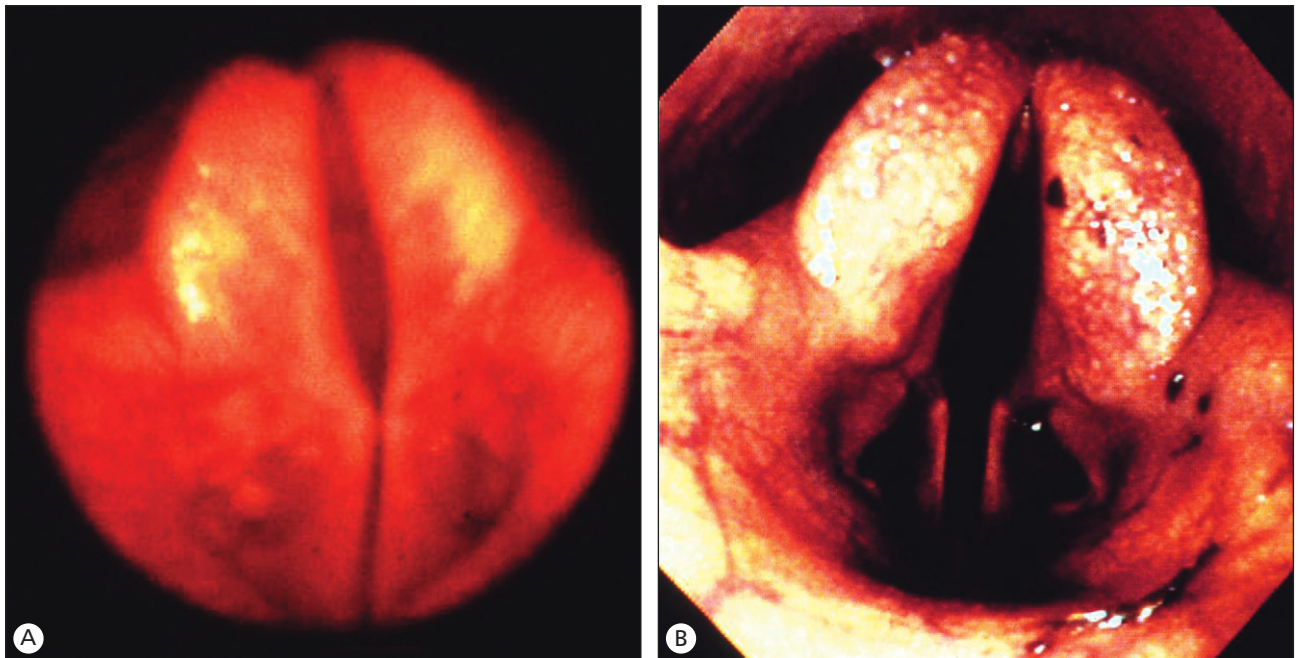
Fig. 33.1. Endoscopic view of the larynx of a pony with bilateral laryngeal paralysis which was associated with liver failure. (A) This image, captured during inspiration, reveals passive adduction of the arytenoids with resultant closure of the glottis. (B) The blood in the airway is a result of an emergency tracheostomy. Following this procedure, the arytenoids remained relatively motionless in the resting position, indicating bilateral paralysis.

in laryngeal dysfunction is unclear. Since the laryngeal paralysis may be transient, and since no neuropathology has been identified, it probably reflects neuromuscular dysfunction rather than neuromuscular pathology. Such dysfunction could potentially occur by mechanisms akin to those that cause hepatic encephalopathy. Alternatively, this form of laryngeal paralysis may represent a peripheral neuropathy, which is a common complication of hepatic disease in humans. The pathogenesis of peripheral neuropathy in humans with liver disease is incompletely understood but may involve (1) metabolic inhibition of axonal membrane function, (2) metabolic damage to Schwann cells, and/or (3) disordered insulin metabolism akin to diabetic neuropathy.

Toxic peripheral neuropathies causing laryngeal paralysis

Various toxic peripheral neuropathies may also cause laryngeal paralysis (Table 33.2). These disorders are readily differentiated from RLN because the laryngeal paralysis is commonly bilateral and it is clearly part of a generalized disorder that affects multiple peripheral nerves. Delayed organophosphate-induced toxicity causes neurodegeneration, primarily of long axons in peripheral nerves and spinal cord. The resultant laryngeal paralysis may be permanent (Rose et al 1981, Duncan & Brook 1985). Ingestion of *Lathyrus* spp. and *Cicer arietinum* (chickpea) may cause laryngeal paralysis. The toxic agents include β-*N*-oxalylamino-L-alanine, an excitatory amino acid that causes neuropathy with distal axonal degeneration. Interestingly, experimental studies indicate that, even after prolonged ingestion of *Lathyrus sativus* (Indian vetch), only a minority of horses develops laryngeal dysfunction (Hutyra et al 1938), suggesting that factors other than toxin ingestion contribute to the clinical outcome.

Sojka et al (1996) described laryngeal dysfunction in around 13% of horses with lead toxicosis.

Hyperkalemic periodic paresis

Hyperkalemic periodic paresis, a generalized myasthenic disorder, often results in episodic upper airway obstruction. While the nature of airway obstruction in this disorder appears to be variable, obstruction is attributed to laryngeal spasm or paralysis in approximately half of affected horses. Appropriate medical treatment may ameliorate the severity and incidence of upper airway dysfunction associated with this disorder (Carr et al 1996).

REFERENCES

Abrahamsen EJ, Bonahon TC, Bednarski RM et al 1990 Bilateral arytenoid cartilage paralysis after inhalation anaesthesia in a horse. Journal of the American Veterinary Medical Association 197: 1363–1365

Carr EA, Spier SJ, Kortz GD et al 1996 Laryngeal and pharyngeal dysfunction in horses homozygous for hyperkalemic periodic paralysis. Journal of the American Veterinary Medical Association 209: 798–803

Dixon PM, Railton DI, McGorum BC 1993 Temporary bilateral laryngeal paralysis in a horse associated with general anaesthesia and post anaesthetic myositis. Veterinary Record 132: 29–32

Dixon PM, McGorum BC, Railton DI et al 2001 Laryngeal paralysis: a study of 375 cases in a mixed-breed population of horses. Equine Veterinary Journal 33: 452–458

Duncan ID, Brook D 1985 Bilateral laryngeal paralysis in the horse. Equine Veterinary Journal 17: 228–233

Goulden BE, Anderson LJ 1981 Equine laryngeal hemiplegia part II: some clinical observations. New Zealand Veterinary Journal 29: 194–198

Hutyra G, Marek F, Manninger T 1938 Paralysis of the recurrent laryngeal nerve. In: Special Pathology and Therapeutics of the Diseases of Domestic Animals, 4th edn. Bailliere, Tindall & Cox, London, vol. 3, pp.401–410

Huxtable RJ, Yan CC, Wild S et al 1996 Physicochemical and metabolic basis for the differing neurotoxicity of the pyrrolizidine alkaloids, trichodesmine and monocrotaline. Neurochemical Research 21: 141–146

Mayhew IG 1989 Large Animal Neurology. Lea & Febiger, London, p.95

McGorum BC, Murphy D, Love S et al 1999 Clinicopathological features of equine primary hepatic disease: a review of 50 cases. Veterinary Record 145: 134–139

Pearson EG 1991 Liver failure attributable to pyrrolizidine alkaloid toxicity and associated with inspiratory dyspnea in ponies: three cases (1982–1988). Journal of the American Veterinary Medical Association 9: 1651–1654

Rose RJ, Hartley WJ, Baker W 1981 Laryngeal paralysis in Arabian foals associated with oral haloxon administration. Equine Veterinary Journal 13: 171–176

Sojka JE, Hope W, Pearson D 1996 Lead toxicosis in 2 horses: similarity to equine degenerative lower motor neuron disease. Journal of Veterinary Internal Medicine 10: 420–423

Southwood LL, Baxter GM, Wagner AE 2003 Severe postanesthetic upper respiratory tract obstruction in horses: 8 cases (1993–2001). Veterinary Surgery 32: 602

Southwood L L, Gaynor J S 2003 Postanesthetic upper respiratory tract obstruction. In: Robinson NE (editor) Current Therapy in Equine Medicine 5. Saunders, St Louis, pp.391–393

Tute AS, Wilkins PA, Gleed RD et al 1996 Negative pressure pulmonary edema as a post-anesthetic complication associated with upper airway obstruction in a horse. Veterinary Surgery 25: 519–523

34

Recurrent Laryngeal Neuropathy: Clinical Aspects and Endoscopic Diagnosis

Brian H Anderson

Introduction and Definition

Paralysis of the equine larynx can be bilateral or unilateral, and either partial or total. Unilateral, left-sided paralysis is most common and is termed laryngeal hemiplegia if complete paralysis is present and laryngeal hemiparesis if partial paralysis is present. Two studies showed that a definitive cause was established in only 6% (Dixon et al 2001) and 11% (Goulden & Anderson 1981a) of cases of laryngeal paralysis (or partial paralysis). Documented causes of unilateral laryngeal paralysis include injury to the left or right recurrent laryngeal nerve by inadvertent perivascular injection of irritant medication, trauma to the neck, guttural pouch mycosis, thyroid carcinoma, strangles, abscessation of the head and neck, and thymic lymphosarcoma. In addition, organophosphate poisoning, plant toxicity, lead poisoning, liver disease, and sequelae to general anesthesia have been associated with bilateral laryngeal paralysis (Cahill & Goulden 1987, Barber 1981) However, in the remaining vast majority of cases, which are predominantly left-sided hemiparesis, the cause is unknown and the disease is termed recurrent laryngeal neuropathy (RLN) (formerly idiopathic laryngeal hemiplegia)

Although the etiology of RLN remains unknown, the pathology has been well described and is discussed in detail in Chapter 32, but it is briefly reviewed here to emphasize the relationship between neuromuscular pathology of the larynx and the clinical signs demonstrated by affected individuals. Neuropathy of the left recurrent laryngeal nerve, and to a lesser extent the right, is the primary pathological feature of RLN (Duncan et al 1974, 1978, Cahill & Goulden 1986a,b,c). Progressive loss of large myelinated nerve fibers in the distal part of the left recurrent laryngeal nerve results in atrophy of the intrinsic laryngeal muscles innervated on this side. Although the adductor muscles of the arytenoid cartilage are affected earlier and more profoundly (Duncan et al 1991a), it is atrophy of the dorsal cricoarytenoid muscle (DCAM) that results in significant clinical signs. This muscle is the only abductor of the arytenoid cartilage and vocal fold and its dysfunction results in a significant reduction in airflow in exercising horses. Histopathological examination of horses with RLN shows that a repetitive, progressive denervation/reinnervation process occurs. Attempts at

repair, including collateral axonal sprouting and reinnervation of previously denervated muscle (demonstrated by fiber-type grouping) are evident (Duncan et al 1974, 1977, Anderson 1984, Cahill & Goulden 1986d). When axonal loss and muscle atrophy are extensive and repair processes are insufficient, abductory dysfunction of the left side of the larynx occurs. The term RLN is now preferred to "idiopathic laryngeal hemiplegia" because RLN describes the full range of pathology and associated clinical disease which can vary from mild to severe. Laryngeal hemiplegia is therefore the end-stage of neuropathy of the recurrent laryngeal nerve.

Inability to abduct the arytenoid cartilage sufficiently and/or maintain abduction reduces the cross-sectional area of the rima glottidis, consequently obstructing inspiratory airflow and causing abnormal inspiratory noise because of increased air turbulence. This abnormal inspiratory noise is referred to as "roaring" or "whistling" and horses so afflicted are termed "roarers" and "whistlers" respectively. Reduced volumes of oxygen reach the pulmonary capillaries and the resultant hypoxemia causes premature fatigue during strenuous exercise.

Most RLN-affected horses are presented to practicing clinicians because abnormal respiratory "noises" and in many cases impaired athletic performance are noted by owners and trainers. The diagnosis of severe RLN is not difficult but the detection of mildly affected horses is more troublesome, as is the ability to predict possible progression of this disorder or the clinical effect of this disease on affected horses.

Clinical Aspects

Prevalence

Obtaining an accurate figure of the prevalence of RLN is difficult and the results depend on the criteria and methods used to determine the presence of this disorder. Various endoscopic surveys conducted primarily on thoroughbred horses estimate significant RLN to be present in some 2.6–8.3% of adult horses (Lane et al 1987). In a more recent endoscopic survey (using a five-point grading system), which reported on the distribution of RLN in

3,494 unbroken elite thoroughbred yearlings examined over 15 years, significant RLN (including laryngeal hemiplegia) was recorded in 0.45% of cases (Lane 2003a). Horses with equivocal evidence of RLN (grade 3) comprised 17.7% of the horses examined (Lane 2003a). In selected populations, such as those in public auction sales of thoroughbred yearlings, significant RLN was recorded in 0.18% of horses (Anderson 2002) when endoscopy alone was used for diagnosis, but in 0.65% of horses (Ellis et al 2004) when the examination for the presence of a characteristic inspiratory noise during exercise was also undertaken.

In a further study (Duncan et al 1974) of mixed breeds of horses, which used histopathological criteria for the diagnosis of RLN, 30% of apparently clinically normal horses had evidence of neurogenic atrophy of the left intrinsic laryngeal muscles. Of these horses, 77% were 14.2 hands or taller. In another study (Anderson 1984), in which all the horses were thoroughbreds, 80% of clinically normal horses over 1.5 years of age without endoscopic evidence of abnormal laryngeal movements had evidence of neurogenic atrophy of the left intrinsic laryngeal muscles. These horses have been referred to as subclinical cases.

Breed, height, and body weight

In general, RLN affects heavier, taller breeds such as draft breeds, warmbloods, and thoroughbreds, and is rarely found in ponies. In one study, the odds ratios by breed for the development of significant RLN in descending order were draft breeds, warmbloods, Tennessee walkers, saddlebreds, thoroughbreds, standardbreds and quarter horses (Beard & Hayes 1993). A clinical and endoscopic survey of Clydesdale horses showed that 9% (i.e. four of 48) horses had laryngeal paralysis (Goulden et al 1985). Approximately 95% of horses affected with clinically significant RLN are over 160 cm tall at the withers (Cook 1965, Goulden & Anderson 1981b, Dixon et al 2001).

Gender

A number of clinical and endoscopic surveys have indicated that higher grades of RLN are more common in males than females but conclusive evidence is lacking (Goulden & Anderson 1981b, Dixon et al 2001). Histopathological changes typical of RLN are more frequent in male as compared to female horses (Anderson 1984).

Age of onset

Histological changes typical of RLN have been recorded in fetuses (Gunn 1973) and in male draft horse foals as young as 2 weeks of age, lending weight to the theory that the disease is hereditary and/or congenital (Duncan 1992, Harrison et al 1992). In a survey of predominantly

thoroughbred horses the histopathological changes typical of RLN increased dramatically in prevalence and severity in horses at 1–2 years of age (Anderson 1984) which supports the view that RLN is a disease of "childhood" (Cook 1970).

Despite these findings the age at which clinical signs of RLN occur can be variable. For example, RLN is often diagnosed in unbroken yearling thoroughbreds examined at the time of sale. More commonly, however, clinically significant RLN becomes evident with the start of strenuous work. In thoroughbreds, particularly those intended for flat racing, the clinical signs of RLN most commonly occur when training and racing begins in 2- to 3-year-old horses. In hunters and National Hunt racehorses, which are not broken until aged 4–5 years, the median age for diagnosis of RLN was 6 years (Dixon et al 2001). In summary, RLN can affect animals at almost any age but the most commonly noted time of clinical disease is between 1 and 6 years of age.

Diagnosis

RLN is suspected when there is a history of exercise intolerance and an abnormal respiratory noise evident at exercise. Palpation of the larynx is performed to detect evidence of atrophy of the DCAM, although this muscle may be atrophied in many large horses with normal laryngeal function. Confirmation of RLN is achieved by endoscopic examination of the larynx at rest or, if necessary, during exercise on a treadmill (Ducharme & Hackett 1991).

History and presenting clinical signs

RLN-affected horses are frequently reported to make an abnormal respiratory noise during exercise and have reduced exercise capacity.

Respiratory noise and RLN

In athletic horses, principally racehorses, an abnormal respiratory noise, typically described as a "whistle" or "roar", is reported when exercising at higher speeds, i.e. cantering or galloping in larger breeds and fast trotting or pacing in standardbred racehorses. Such noises are seldom heard at the trot except in severely affected (hemiplegia) horses. Sometimes there is a history of sudden appearance of a noise in horses, which often coincides with the onset of training. In young racehorses it is not uncommon for the noise to start immediately upon resumption of training following a break between the second and third years (Goulden & Anderson 1981a). In other cases the onset of abnormal respiratory noise is insidious.

In draft breeds used for showing, an abnormal respiratory noise is often reported but in those used for pulling

competitions an abnormal respiratory noise and exercise intolerance may be reported (Beard & Hayes 1993). Because of the low speeds at which sport horses such as showjumpers and dressage horses perform, RLN is frequently not a performance-limiting disorder. However, at elite levels it can be, and often the associated abnormal respiratory noise is, objectionable to owners and competition judges. RLN can impair three-day event horses from competing successfully over cross-country courses because of the intensity and duration of exercise required. Vocalization changes may also be noted. A softer neigh or whinny can be heard as a result of incomplete adduction of the affected arytenoid cartilage. Horses that are suddenly frightened may have a prolonged, instead of a short, "grunt" as a result of forced expiration against an incompletely closed glottis. This is the basis of the "grunt to the stick" test (Speirs 1992). Thoroughbred yearlings sold at public auction sales often whinny when being paraded in the sales ring, especially colts. An abnormal whinny may alert the potential purchaser to the presence of underlying RLN. Because RLN is not the only cause of abnormal respiratory noises, riders or drivers should be questioned carefully on the nature of any abnormal respiratory noise reported. The following questions should be asked:

- What does the noise sound like? RLN-induced noises are often musical in nature in mildly affected cases and with faster work may become harsher "wood-sawing" type noises.
- Is the abnormal noise heard during inspiration, expiration or both? It should be noted, however, that many owners and trainers will not be able to accurately differentiate between inspiratory and expiratory noises (Dixon et al 2001).
- At what level of exercise does the noise start and stop? Is it continuous or intermittent? Does it stop immediately on slowing down or does it continue even when the horse is standing quietly? With RLN, the noises tend to occur only during strenuous exercise and are continuous until the exercise slows down or stops, after which they will disappear within seconds.

Exercise intolerance and the physiological effects of RLN

It has long been recognized, especially in racehorses, that RLN causes decreased exercise performance. It has been shown that, compared to unaffected horses, those with RLN suffer from a number of ventilatory alterations including an increase in inspiratory impedance (a measure of resistance to airflow), increased inspiratory and expiratory trans-upper airway driving pressures, reduced peak inspiratory airflow and hypoventilation. In addition, exercising horses with significant RLN are more hypoxemic, hypercapnic and acidotic compared to normal horses

and the degree of dysfunction relates to the severity of RLN (Christley et al 1997). The end result is that oxygen demand exceeds oxygen supply and fatigue sets in. In many cases, horses are competitive for most of the race but in the final 400–600 m they do not accelerate and may weaken, with the rest of the field passing them. Even though obviously affected horses can be competitive over short distances, e.g. 1000–1200 m, the longer the race (1600 m or more), the more difficulty RLN-affected horses will have.

Assessment of respiratory sounds

Galloping and cantering horses normally have a 1 : 1 coupling between stride and respiration with inspiration and expiration completed within one stride cycle. When the leading limb strikes the ground to complete the stride cycle, expiration occurs. In normal animals there is little if any audible sound heard on inspiration, unless the listener is close to the horse. Thus, when listening to a horse gallop a single expiratory sound is usually noted. In horses affected with RLN, an additional audible noise is usually heard during inspiration. Therefore, two noises are noted and this biphasic sound has been compared to that heard when sawing wood. Although the quality of the inspiratory sound has been described in a variety of ways, two different sounds are commonly heard. The first is a finer, higher pitched sound which is described as a "whistle", often heard early on in exercise or throughout exercise in less severely affected cases. The other is a coarse/harsh, lower pitched sound that is usually louder and is referred to as a "roar", hence the description "whistlers" or "roarers". The precise source of the abnormal inspiratory noise within the larynx has not been identified but air turbulence caused by air moving across an incompletely abducted or collapsed arytenoid cartilage and an open laryngeal ventricle or collapsed vocal fold are involved (Attenburrow 1982).

When examining racehorses it is preferable to be positioned down-wind, and listening and watching the horse can help to determine if the abnormal sounds are made on inspiration or expiration, if they are continuous throughout the exercise period or intermittent, and if they stop abruptly with cessation of exercise or can still be heard post exercise. On cold days the expired breath is clearly visible and respiratory sounds can be matched to expiration and inspiration. With thoroughbred racehorses the horse is warmed up over 1000 m or so, starting with trotting and then cantering. Hard galloping is then performed over a further 400–600 m to stress the horse and increase respiratory rate. In the standardbred, the horse is jogged at lower speed initially until warmed up but usually is fast worked over 2000 m, aiming to complete the last 400 m in 28 seconds. The rider/driver should then pull the horse up as quickly as possible and jog or canter back to the listener. In a minority of horses with RLN the abnormal inspiratory

noise can still be heard for up to a minute or so but it usually disappears within seconds after the horse is brought to a stop. Palpation of the larynx immediately after exercise may reveal fremitus, and auscultation of the ventral aspect of the larynx with a stethoscope may reveal increased inspiratory noise. An abnormal inspiratory noise can be accentuated at this time in some horses with RLN using the arytenoid depression maneuver. The test can be performed bilaterally or unilaterally and detects reduced abductor tone (Mackay-Smith & Marks 1968, Spiers 1992). By placing fingers of each hand on the muscular processes of the arytenoid cartilages and applying pressure in the medial, rostral, and ventral directions, inspiratory noise is readily demonstrated in affected individuals. The ease with which stridor is induced can be compared between sides by depressing the muscular process of each side independently. In horses with left-sided RLN inspiratory noise can be demonstrated more easily on the left side compared to the right (Mackay-Smith & Marks 1968). These tests can also be performed in the resting horse. The results of these tests should be interpreted with caution however, because it is possible to induce a similar inspiratory noise in some normal horses (Cook 1988).

In sport horses, yearlings or horses not fit enough to gallop, lunging or riding at the canter may be enough to elicit abnormal respiratory noises (Lane et al 1987). A 15-m diameter circle is sufficient and horses should be exercised hard enough to achieve 1 : 1 stride and respiration coupling. The horse should be worked in both directions for approximately 5 min (Gerring 1985). The examiner should be positioned close to the horse, on the perimeter of the circle. The inspiratory noise made by a horse with severe RLN and complete paralysis is characteristic. However, in less severely affected horses, abnormal inspiratory noise may be less obvious, especially at submaximal exercise. Moreover, abnormal inspiratory noises can be produced by obese or unfit horses or be the result of other upper respiratory tract abnormalities such as soft palate displacement, axial deviation of the aryepiglottic folds or epiglottic entrapment.

More accurate assessment of respiratory sounds made at exercise has recently been achieved using various microphone recording systems positioned at the nostrils using a facemask, in the nasopharynx or external to the nostrils via a flexible wand attached to a cavison (see Chapter 17). Spectrogram analysis of the recorded sound can be performed and spectral patterns characteristic for specific upper airway disorders including RLN and dorsal displacement of the soft palate (DDSP) have been identified (see Chapter 17). This diagnostic tool is potentially of great benefit to practitioners who may be able to use it under field conditions. However, further work is required to verify the accuracy of sound analysis in differentiating between various upper airway disorders and within each disorder, between disease of varying severity.

External examination and palpation of the larynx

All horses suspected of suffering from RLN should have the larynx palpated. During palpation of the larynx the clinician should also check for evidence of previous upper respiratory tract surgery. If a ventral laryngotomy has been performed, fibrosis and thickening of the tissues ventral to the cricothyroid membrane may be obvious, especially if the laryngotomy closed by secondary intention. A scar may also be evident especially if the area is clipped. If a prosthetic laryngoplasty has been performed, a scar parallel and ventral or dorsal to the linguofacial vein may be evident in a minority of cases, but clipping the hair over this site is usually necessary to detect such scars.

Further evidence for the presence of RLN can be obtained by comparing the percutaneously palpated prominence of the muscular process of the arytenoid cartilage between left and right sides. The muscular processes in most horses can be palpated 2–3 cm caudal to the vertical ramus of the mandible and medial to the tendon of the sternocephalicus muscle using the flexed index finger of both hands (Fig. 34.1), except in horses with a rostrally positioned larynx that lies between the mandibles ("cock-throttled"; Hawe et al 2001). The muscular process of the normal arytenoid cartilage feels like a rounded "flesh-covered knuckle". Overlying the muscular process are the cricopharyngeus and thyropharyngeus muscles and associated with it are the insertions of the dorsal cricoarytenoid, lateral cricoarytenoid, and transverse arytenoid muscles (intrinsic laryngeal muscles). Because RLN results in atrophy of the intrinsic laryngeal muscles a more obvious, more pointed and firmer muscular process can be palpated in horses with RLN.

Some authors place great importance on this technique and even believe the diagnosis of RLN by this method is more accurate than by endoscopic examination of laryngeal movements. Cook reported that RLN could be diagnosed by palpation in up to 100% of horses selected at random (Cook 1988). Laryngeal palpation is useful in detecting totally hemiplegic horses and when an endoscope is unavailable; it also supports a diagnosis of RLN where laryngeal endoscopy is equivocal but an inspiratory noise is heard at exercise. In one study (Anderson et al 1997) the percentage of horses that had a more prominent muscular process on the left side and had abnormal arytenoid movements (39% of 18) was significantly higher ($P = 0.006$) than that for horses in which both sides were of similar prominence and in which abnormal arytenoid movements were recorded (10% of 78). In addition, it has been noted that many larger horses have some degree of left-sided intrinsic laryngeal muscle wasting but that laryngeal dysfunction is absent (Hawe et al 2001).

Maldevelopment of one or both wings of the thyroid laminae and the absence of the cricothyroid articulation(s)
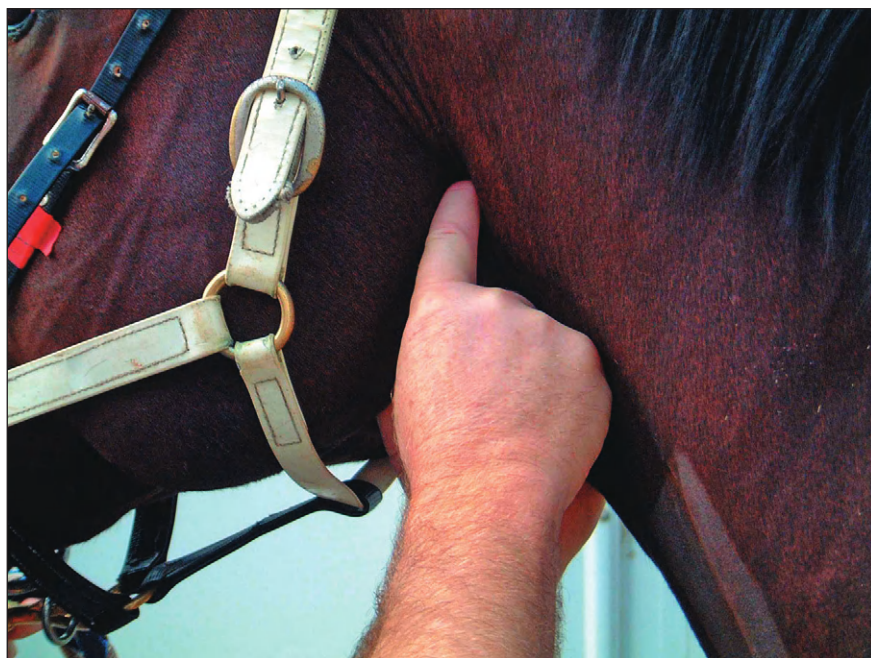
Fig. 34.1. Technique used to palpate the left and right muscular processes of the arytenoid cartilages.

may be determined digitally and indicate the presence of a congenital laryngeal disorder such as cricopharyngeal laryngeal dysplasia (fourth branchial arch defect syndrome) (Lane 1993a; see Chapter 31).

Endoscopic diagnosis

Endoscopy of the larynx is essential to confirm the presence, and determine the severity, of RLN. An endoscopic examination of the pharynx and larynx is indicated in all or some of the following situations:

- owner/trainer/jockey/driver/rider reports an unusual or excessive respiratory noise during exercise
- as part of the prepurchase examination
- as part of the examination of horses which are performing poorly.

Examination of laryngeal movements

The technique and requirements for endoscopy of the upper respiratory tract during exercise on a high-speed treadmill have been detailed in Chapter 16 – Treadmill Endoscopy. A description of the evaluation of laryngeal function for evidence of RLN is presented below but the appearance of normal laryngeal function is first briefly reviewed.

Normal laryngeal movements

It is important that the endoscopist observes the full range of arytenoid cartilage movements (Table 34.1) before deciding if there is evidence for adductor and/or abductor muscle dysfunction.

In the normal horse during quiet breathing, the arytenoid cartilages are positioned at approximately 15° to the midline of the rima glottidis (Fig. 34.2). Full symmetrical and synchronous abduction and adduction of the arytenoid cartilages indicates normal laryngeal function. When both arytenoid cartilages are bilaterally, fully abducted in normal horses, as seen after swallowing or

Table 34.1. Definitions of terminology used to describe endoscopically observed laryngeal movements (Robinson 2004)	
Abduction	Movement of the corniculate process of the arytenoid cartilage away from the midline of the rima glottidis
Adduction	Movement of the corniculate process of the arytenoid cartilage toward the midline of the rima glottidis
Full abduction	Most of the corniculate process lies horizontally (90° to the midline of the rima glottidis) (Fig. 34.3)
Asymmetry	A difference in position of the right and left corniculate processes of the arytenoid cartilages relative to the midline of the rima glottidis
Asynchrony	Movement of the corniculate processes of the arytenoid cartilages at different times. This can include twitching, shivering and delayed or biphasic movement of one arytenoid cartilage as compared to the other
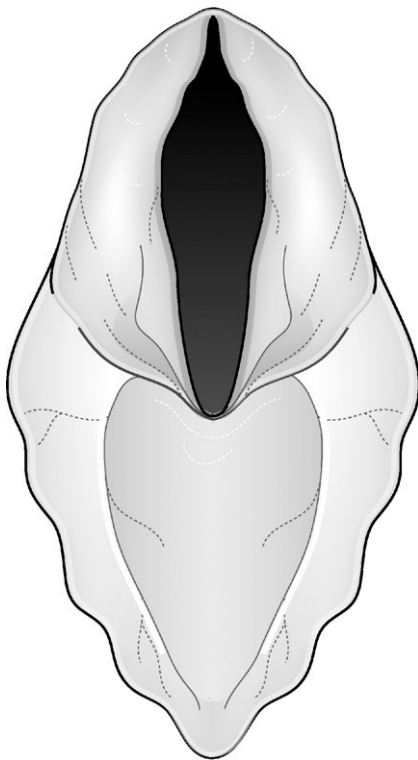
Fig. 34.2. Diagram of the endoscopic appearance of the arytenoid cartilages in the resting position during quiet breathing.

Fig. 34.3. Diagram of the endoscopic appearance of full and symmetrical bilateral arytenoid abduction as observed following nasal occlusion or swallowing in the resting horse. Much of the corniculate cartilage is in contact with the nasopharyngeal roof.

following bilateral nasal occlusion, the abaxial borders of *most* of the corniculate processes of the arytenoid cartilages are in contact with the dorsal nasopharyngeal wall and are positioned at approximately 90° to the midline of the rima glottidis (Fig. 34.3). In strenuously exercising normal horses, video-endoscopy shows that the arytenoid cartilages are further abducted and almost all are in contact with the dorsal nasopharyngeal wall and are essentially at 90° to the midline of the rima glottidis (Fig. 34.4). Bilaterally symmetrical adduction can be observed during phonation, swallowing, coughing or during bilateral nasal occlusion in normal horses (Fig. 34.5).

Techniques to stimulate laryngeal movement

To accurately assess abnormalities in laryngeal function it is necessary to view the entire range of laryngeal movements. In some horses the arytenoid cartilages may be held in complete symmetrical abduction for the duration of the examination. These horses are considered to be functionally normal. In others, the arytenoid cartilages are held in the resting position with very little movement present. To stimulate abduction and adduction all horses should be made to swallow and a nasal occlusion maneuver should be performed. It is recommended that three full and symmetrically maintained abductions of the arytenoid cartilages be observed before determining that laryngeal function is normal.

Swallowing can be induced by transendoscopic flushing of the endoscope lens or more effectively by flushing water down the biopsy channel. The palate is displaced dorsally as the horse swallows and on replacement, full and symmetrical abduction of the arytenoid cartilages occurs in the normal horse. In horses with more severe degrees of RLN, the left arytenoid cartilage is positioned on or near the midline of the rima glottidis at rest (Fig. 34.6) and no abduction is observed following swallowing. The degree of abduction achieved, however, can vary from slightly more than the normal resting position (Fig. 34.7) to where the abaxial margin of the corniculate process is at approximately 75° to the midline (Fig. 34.8). In other horses with lower grades of RLN, full abduction may be achieved after swallowing, but is very transient.

To perform the nasal occlusion maneuver, both nostrils are occluded by placing the hand over the nasal bone and while the thumb compresses one nasal cavity the fingers compress the other. This temporary asphyxiation (if held for 30 seconds or more) results in deep respiratory efforts that causes near-maximal arytenoid cartilage abduction frequently followed by complete arytenoid cartilage adduction. This technique induces subatmospheric tracheal and pharyngeal pressures that are equal to or exceed those occurring during maximal exercise but
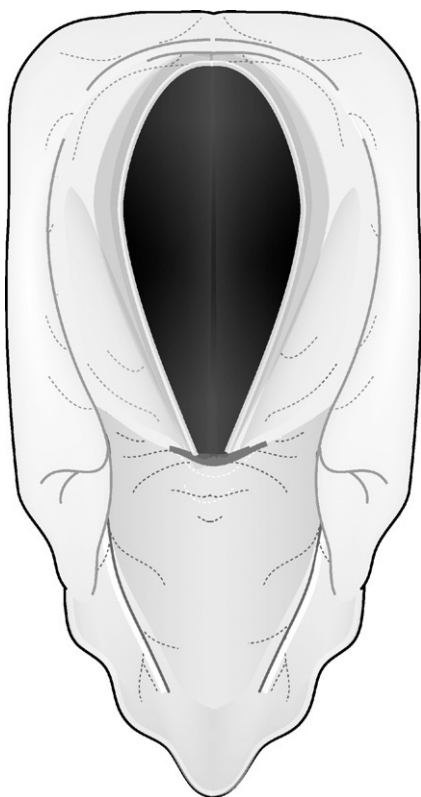
Fig. 34.4. Diagram of the endoscopic appearance of full and symmetrical bilateral arytenoid abduction as observed during strenuous exercise on a treadmill with a video-endoscope in the pharynx. Most of corniculate cartilage is in contact with the nasopharyngeal roof.
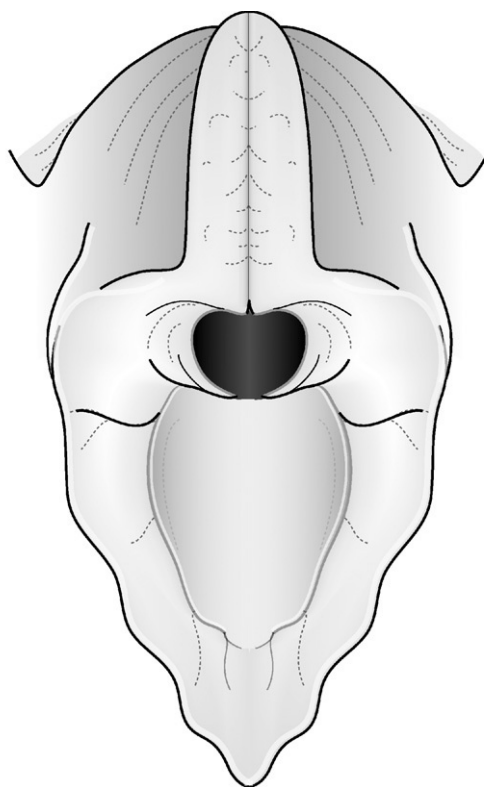
Fig. 34.5. Diagram of the endoscopic appearance of bilaterally symmetrical adduction of the arytenoid cartilages that can be observed during phonation, swallowing, coughing or during bilateral nasal occlusion in normal horses.

without the associated airflow; nevertheless it is useful for assessing upper airway neuromuscular function (Holcombe et al 1996). Horses affected with RLN are frequently unable to fully and symmetrically abduct the left arytenoid cartilage during this procedure. Evidence of adductor muscle dysfunction can also be observed in some RLN cases when incomplete adduction of the left arytenoid cartilage is noted.

One drawback to the procedure is that some horses, particularly thoroughbred yearlings, do not tolerate this procedure and can become violent, striking with the front feet or attempting to jump out of stocks. In such horses an inability to abduct both arytenoid cartilages should be interpreted with caution because of the shortened duration of nasal occlusion. Another difficulty is interpretation of laryngeal function when full abduction is not achieved following nasal occlusion but is achieved following swallowing. To examine this, Parente and colleagues performed endoscopy of the upper respiratory tract at rest and then during intensive treadmill exercise in 150 horses (Parente & Martin 1995). The ability to fully and symmetrically abduct both arytenoid cartilages after swallowing was a more accurate predictor of maintaining full arytenoid abduction during strenuous exercise than

was the use of nasal occlusion. All nine racehorses that could achieve full symmetrical arytenoid cartilage abduction following swallowing, but could not do so after nasal occlusion, were able to maintain full abduction when exercising on a treadmill at 12–14 m/s. Conversely, when 14 racehorses which could not achieve full symmetrical arytenoid cartilage abduction after swallowing were evaluated in the same manner, only two maintained full abduction during exercise.

When performing post-sale endoscopic examination of yearlings it is not uncommon to observe full symmetrical abduction of both arytenoid cartilages following swallowing, but incomplete abduction of the left arytenoid cartilage during nasal occlusion (B.H. Anderson, unpublished observations). If the nasal occlusion maneuver was the only test applied then a false-positive result for the presence of clinically significant RLN would cause an error in decision-making on the suitability for sale.

Reflex adduction of either or both arytenoid cartilages is evoked using the "slap test". In horses with intact spinal reflexes a sharp slap to the saddle region caudal to the wither results in adduction of the contralateral arytenoid cartilage under endoscopic observation. Adduction of the contralateral arytenoid cartilage may also be detected.

Fig. 34.6. Diagram of the endoscopic appearance of the larynx in a horse with severe RLN at rest. Note the left arytenoid cartilage is positioned on or near the midline of the rima glottidis.
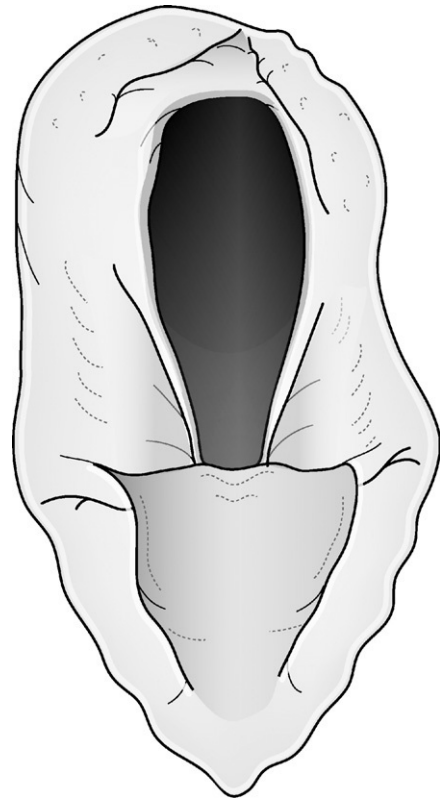
Fig. 34.7. Diagram of the endoscopic appearance of the arytenoid cartilages in a horse with incomplete abduction of the left arytenoid during deeper breathing. This can vary (see Fig. 34.8) and in this case left arytenoid cartilage abduction is only slightly more than the normal resting position.

If the reflex is absent or reduced compared to the right side RLN is suspected. The response to this test must be interpreted with respect to arytenoid abduction and by itself is not reliable because it is abolished in frightened or tense horses, weakens or disappears with continued slapping, and varies according to the force of the slap. In addition, in some horses with marked arytenoid abductor muscle dysfunction a positive adductor response might still be present (Greet et al 1980, Hawe et al 2001). In addition, positive thoracolaryngeal reflexes have been noted in horses with advanced neuropathology of the lateral cricoarytenoid muscle (Newton-Clarke et al 1994).

Increasing the respiratory rate in the resting horse by chemical means has been used to assess the efficiency of DCAM function. Doxapram hydrochloride (Archer et al 1991) (40 mg/kg intravenous) and lobeline hydrochloride (0.2 mg/kg intravenous) have both been used to stimulate hyperventilation. The degree of abduction achieved after doxapram hydrochloride administration was less than that produced after nasal occlusion in one study and doxapram administration did not cause hyperventilation in all horses (Archer et al 1991). The use of lobeline hydrochloride is more reliable and induces hyperventilation for 90–150 seconds and although tidal volumes are similar to those in horses performing strenuous exercise on a treadmill, peak inspiratory and expiratory flows are lower because the respiratory rate does not reach those obtained during exercise (Weishaupt et al 1998).

Examination immediately after exercise can also be useful and is a recommended technique for prepurchase examination of the athletic horse. If it is possible to endoscope the horse within minutes of galloping, when the effects of elevated respiratory rate and airflow on the upper airways are still present, evidence of DCAM dysfunction may be visible.

Abnormal laryngeal movements

In many horses without clinically evident respiratory abnormalities, laryngeal movements are frequently neither bilaterally symmetrical nor synchronous. Indeed figures cited from endoscopic surveys of selected or non-selected populations of mainly thoroughbred horses indicate that asynchronous and/or asymmetrical laryngeal movements (excluding obvious or marked paralysis) are present in 50–78% of all horses examined (Baker 1982, Cook 1988, Stick et al 2001, Lane 2004a). The cause(s) of these bilateral differences in arytenoid movements and their
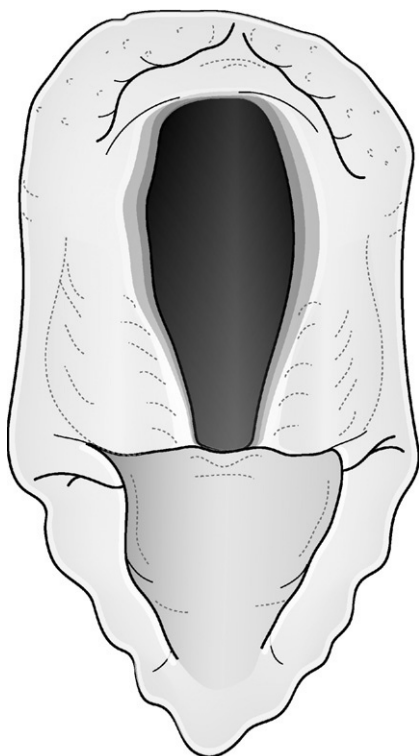
Fig. 34.8. This diagram shows the endoscopic appearance of the larynx in a horse in which the left arytenoid cartilage is incompletely abducted during deeper breathing. It is more abducted than in Fig. 34.7. Note that the abaxial margin of the corniculate process is at approximately 75° to the midline.

significance, in relation to the horses' exercise tolerance, has been the subject of much debate. Because horses with varying degrees of asymmetrical laryngeal movements have also been shown to have intrinsic laryngeal muscle damage it is generally considered that endoscopically observed abnormal laryngeal function is directly related to RLN (Duncan et al 1977, 1991b, Duncan & Baker 1987). However, a precise correlation between the two is not established and other physiological causes, e.g. the thoracolaryngeal adductory reflex, may account for some asynchronous and asymmetrical laryngeal movements. In addition, the different lengths of the left and right recurrent laryngeal nerves, the influence of excitement, time of day, duration of examination, day of examination, and the repeatability and reliability of endoscopic examinations could all cause variation in endoscopically observed laryngeal movements (Ducharme et al 1991, Anderson et al 1997, Embertson 1997).

The effect of RLN on the movement of the arytenoid cartilages can result in the following (Duncan et al 1977, 1991b, Duncan & Baker 1987):

- Loss of adductor function precedes loss of abductor function and may be demonstrated by the "grunt to the stick" test.

- Resting asymmetry of the rima glottidis – usually only significant if full and symmetrical arytenoid abduction cannot be obtained or sustained.
- Inefficient abductor muscle function, which with more severe degrees of RLN, full and symmetrical arytenoid abduction may not be achieved and even if achieved, the affected arytenoid cartilage adducts towards the midline of the rima glottidis before the opposite arytenoid cartilage following swallowing or nasal occlusion. Alternatively, abductor muscle fatigue is evident and observed as a failure to maintain full abduction and the affected cartilage slowly adducts towards the midline of the rima glottidis before the opposite arytenoid cartilage.
- Inability to obtain full arytenoid abduction.
- Total immobility of the affected arytenoid cartilage.
- Open laryngeal ventricle and "slack" vocal fold as a result of loss in muscle tone.
- Vocal fold trembling, hesitation or flutter – usually of little clinical significance.

Grading laryngeal movements

Various subjective grading systems have been used to describe laryngeal movements observed endoscopically in horses breathing quietly at rest (Cook 1988, Ducharme et al 1991, Dixon et al 2001, Lane 2004b). Using a static system to describe and categorize a dynamic process, in which an infinite range of laryngeal movements is possible, has limitations. Describing normal movements and those as a result of paralysis is simple but a wide range of potentially abnormal laryngeal movements can occur and grading can never be precise. While a uniformly accepted system for describing laryngeal function is useful, of more benefit to clinicians is one that could grade clinically significant variations in laryngeal function and which could accurately predict how the larynx will function during exercise.

Recently, a general consensus was reached amongst clinicians and researchers on an endoscopic laryngeal grading system (Table 34.2) that should be useful in clinical practice (Robinson 2004). It is an amalgamation of a number of common grading systems (Cook 1988, Ducharme et al 1991, Dixon et al 2001, Lane 2004b). The system described will be helpful in communication between clinicians on grading, and hopefully when making decisions on the necessity for surgical intervention, in prepurchase examinations, and to determine if further diagnostic information is needed using video-endoscopy of the upper respiratory tract during treadmill exercise. However, application over a large number of horses is required to assess the system's clinical accuracy. In particular, a large number of horses with each grade of laryngeal function will need to be examined on the treadmill under exercise conditions to assess the accuracy of the resting laryngeal function grades to be used as a predictor of laryngeal function during strenuous exercise.

Table 34.2. Subjective grading system of laryngeal function performed in the standing unsedated horse*

Grade	Description	Sub-grade
I	All arytenoid cartilage movements are synchronous and symmetrical and full arytenoid cartilage abduction can be achieved and maintained.	
II	Arytenoid cartilage movements are asynchronous and/or larynx is asymmetric at times but full arytenoid cartilage abduction can be achieved and maintained.	a. Transient asynchrony, flutter or delayed movements are seen. b. There is asymmetry of the rima glottidis much of the time due to reduced mobility of the affected arytenoid and vocal fold but there are occasions, typically after swallowing or nasal occlusion, when full symmetrical abduction is achieved and maintained.
III	Arytenoid cartilage movements are asynchronous and/or asymmetric. Full arytenoid cartilage abduction *cannot* be achieved and maintained.	a. There is asymmetry of the rima glottidis much of the time due to reduced mobility of the arytenoid cartilage and vocal fold but there are occasions, typically after swallowing or nasal occlusion, when full symmetrical abduction is achieved but not maintained. b. Obvious arytenoid abductor muscle deficit and arytenoid cartilage asymmetry. Full abduction is never achieved. c. Marked but not total arytenoid abductor muscle deficit and arytenoid cartilage asymmetry with little arytenoid cartilage movement. Full abduction is never achieved.
IV	Complete immobility of the arytenoid cartilage and vocal fold.	

* Description generally refers to the left arytenoid cartilage in reference to the right. However this grading system can apply to the right side (i.e. right grade IIIa)

Clinical interpretation of laryngeal movement grades and the diagnosis of RLN

As stated previously, the diagnosis of RLN requires clinical interpretation of laryngeal function in conjunction with an animal's exercise performance history, the nature of any possible abnormal exercise-related respiratory sounds present and when they occur, and palpation of the larynx for the presence of muscular atrophy. An evaluation of the larynx that depends on endoscopy alone is incomplete and the diagnosis of RLN from endoscopy alone should be made with caution. Unfortunately, in some circumstances endoscopy and laryngeal palpation findings are all that the veterinarian has to make a diagnostic decision, e.g. in the yearling racehorse at purchase time.

Based on the above grading system, the following clinical decisions can be made.

- Horses with grades IIIc and IV have significant RLN and all make a characteristic abnormal inspiratory noise at exercise. Treadmill video-endoscopy is seldom warranted. Surgical treatment is indicated (see Chapter 35).
- Horses with laryngeal movements graded I and IIa should be considered functionally normal. However, if an abnormal respiratory noise is heard at exercise, and especially if there is evidence of DCAM atrophy, treadmill video-endoscopy is warranted. A number of studies have shown that a small percentage of horses

considered to have normal laryngeal function at rest will show dynamic axial collapse of the affected arytenoid cartilage during strenuous exercise on the treadmill (Morris 1991, Kannegieter & Dore 1995, Lane 2004c). In the study by Lane (2004c), 19 of 338 horses (or 5.6%) referred for investigation of poor performance (often with a history of abnormal respiratory noise) that were graded I or IIa at rest showed dynamic collapse of the left arytenoid cartilage or vocal fold under exercise conditions.

- Horses with laryngeal movement grades IIb, IIIa, and IIIb may or may not have compromised respiratory function at exercise. In general, however, inability to achieve full abduction of the affected arytenoid cartilage during examination (grade IIIb) is likely to be associated with compromised respiratory function during exercise and poor performance (Stick et al 2001). In one study (Lane 2004c) 83% of horses with this resting laryngeal grade had axial dynamic collapse of the affected arytenoid cartilage during strenuous exercise.
- The functional significance of full arytenoid abduction that is not maintained symmetrically (grade IIIa) can be difficult to determine. Limited treadmill endoscopic examinations of horses with this laryngeal grade indicate that some 75% of such horses can maintain full symmetrical abduction under exercise conditions (Lane 2004c).

Table 34.3. Grading system of laryngeal function as assessed in the horse during exercise*

Laryngeal grade	Definition
A	Full abduction of the arytenoid cartilages during inspiration.
B	Partial abduction of the affected arytenoid cartilages (between full abduction and the resting position).
C	Abduction less than resting position including collapse into the contralateral half of the rima glottidis during inspiration.

* Description generally refers to the left arytenoid cartilage in reference to the right. However this grading system can apply to the right side (i.e. right grade IIIa)

When there is a mismatch between endoscopic, historical, and clinical findings, high-speed treadmill endoscopy is indicated to evaluate the function of the larynx during strenuous exercise. In addition, such an examination is used:

- to evaluate the efficiency of a previous surgical procedure
- in the general evaluation of poor performance
- potentially, to arbitrate on a sales dispute or to assess the likelihood of future problems in a horse to be purchased.

The endoscopic grading of laryngeal function during exercise has been reported (Rakestraw et al 1991) and is presented in Table 34.3.

Hammer et al (1998) used this exercise grading system to categorize 26 horses which all showed evidence of laryngeal hemiparesis at rest (full abduction of the left arytenoid cartilage could not be achieved or maintained – denoted grade III). Of the 26 horses, 20 (77%) had grade C laryngeal function during strenuous exercise. Of the remaining horses, five had grade B laryngeal function and one had grade A. The use of treadmill endoscopy on this group of horses was helpful in determining the need for surgical intervention. However, the success of treatment varied according to the laryngeal function observed during exercise. Horses with grade C function during exercise were treated by prosthetic laryngoplasty with reasonable results (50% success). However, when this procedure was performed on horses with grade B function the results were poor (25% success).

Clinical Progression of RLN

Historical and anecdotal evidence from experienced veterinarians indicates that some cases of RLN can be progressive. Histopathological examination of horses with RLN show that the disease is characterized by repetitive denervation and reinnervation of the intrinsic laryngeal muscles, suggesting that it is a progressive disorder. Anderson (1984) found that these pathological changes dramatically increase in prevalence and severity in thoroughbred horses during the yearling to 2-year-old period. Presumably, if asynchronous arytenoid movements are related to these pathological changes, then progression of asynchrony to hemiplegia is most likely to be found in animals of this age. In one study (Anderson et al 1997), 109 young thoroughbred and standardbred horses were endoscopically examined on two occasions, 16 months apart. At the initial examination most horses were less than 2 years old. A change in laryngeal function from that considered normal to that indicative of RLN was recorded in 12% of horses. The development of severe RLN (complete left-sided paralysis) was recorded in one horse that initially had low-grade RLN, a rate of progression of 5%. In this study it was interesting to note that 43% of the horses did not change laryngeal grades, 29% were given a "better" grade and 28% were given a "worse" grade at re-examination. While the repeatability of endoscopic laryngeal grading has limitations and could affect the results of this study, 29% of the changes in grading were of 2 RLN grades. The authors concluded that some of the apparent improvement in laryngeal function might be the result of regeneration of nerve fibers and compensatory hypertrophy of unaffected muscle fibers in the DCAM.

Changes in laryngeal function over a 12-month period in young thoroughbreds have also been reported by others (Lane 2004a). In this study, video-endoscopy was used to record the laryngeal function in 197 foals. At re-examination 12 months later, 187 yearlings were available. The video-endoscopic records were reviewed blindly. The results showed marked inconsistencies in the two age groups. The laryngeal function of 129 of the 159 foals (81%) with normal laryngeal function initially remained in this group, 20 of the 159 (13%) were recorded in the equivocal group for RLN and one of the 159 (0.63%) showed endoscopic evidence of RLN. Conversely, when nine foals with marked abductor deficiency (grade 4 of 5) on initial examination were re-examined 12 months later, one (11%) was considered normal (grade 2 of 5), five (56%) were considered equivocal (grade 3 of 5) and three (33%) remained grade 4. While it was concluded that endoscopy of foals is not reliable and decisions on whether to buy or reject horses should not be solely based upon this technique, it is possible that some of the variation in laryngeal function could be the result of reinnervation of denervated laryngeal muscles or, alternatively, of denervation of functionally normal intrinsic laryngeal musculature over this period.

Two studies have examined the possibility of progression of RLN in older horses. Baker (1982) examined 168 thoroughbred national hunt horses endoscopically, most

over 5 years old, at annual intervals on at least three occasions. Laryngeal movements were classified as normal, asynchronous, and laryngeal hemiplegia. The conclusion from the survey was that laryngeal asynchrony is not clinically significant and that horses with asynchrony do not progress to hemiplegia. More recently, Dixon et al (2002) reported on endoscopic and/or clinical progression of RLN in older National Hunt and sport horses (predominantly thoroughbreds). Fifty-two of the 351 horses examined (15%) showed evidence of progression of the degree of laryngeal dysfunction over a median period of 12 months (range 1.5–48 months) with the onset of progression occurring at a median age of 7 years.

The results of the endoscopic surveys by Anderson et al (1997) and Dixon et al (2002), both of which involve predominantly thoroughbreds, indicate that the progression of RLN with clinically significant arytenoid abductor dysfunction can occur in between 5 and 15% of horses, respectively. The age of onset of the deterioration in arytenoid function is, however, markedly different. The reason for this is unknown. In addition, Dixon et al (2002) reported that the time or rate at which progression can develop may be as short as 6 weeks. In other cases deterioration can take months to years. This has important implications for the examination of horses for sale and also for determining the most appropriate treatment for such cases.

In contrast to the above study by Anderson et al (1997), Dixon et al (2002) found no evidence of improvement in laryngeal function in the clinical cases examined.

In summary, in the majority of horses, laryngeal function remains constant over time, but in some horses, laryngeal function can deteriorate quickly over a few weeks or more slowly over some years.

REFERENCES

Anderson LJ 1984 A study of some muscles of the equine larynx and soft palate. Unpublished PhD thesis. Massey University, Palmerston North

Anderson BH 2002 Update on post-sale endoscopic examination of horses in New Zealand. Proceedings of the Annual Seminar of the Equine Branch of New Zealand Veterinary Association (Publication No 222). Massey University, Palmerston North, pp.99–105

Anderson BH, Kannegieter NJ, Goulden BE 1997 Endoscopic observations on laryngeal symmetry and movements in young racing horses. New Zealand Veterinary Journal 45: 188–192

Archer RM, Lindsay WA, Duncan ID 1991 A comparison of techniques to enhance the evaluation of equine laryngeal function. Equine Veterinary Journal 23: 104–107

Attenburrow DP 1982 Resonant frequency of the lateral ventricle and saccule and whistling In: Snow DH, Persson SGB, Rose RF (editors) Equine Exercise Physiology. Granta Publications, Cambridge, pp.27–32

Baker GJ 1982 Laryngeal asynchrony in the horse: definition and significance. In: Snow DH, Persson SGB, Rose RF (editors) Equine Exercise Physiology. Granta Publications, Cambridge, pp.46–52

Barber SM 1981 Paralaryngeal abscess with laryngeal hemiplegia and fistulation in a horse. Canadian Veterinary Journal 22: 389–392

Beard WL, Hayes HM. 1993 Risk factors for laryngeal hemiplegia in horses. Preventative Veterinary Medicine 17: 57–63

Cahill J, Goulden B 1986a Equine laryngeal hemiplegia: Part I, a light microscopic study of peripheral nerves. New Zealand Veterinary Journal 34: 161–169

Cahill J, Goulden B 1986b Equine laryngeal hemiplegia: Part II, an electron microscopic study of peripheral nerve. New Zealand Veterinary Journal 34: 170–175

Cahill J, Goulden B 1986c Equine laryngeal hemiplegia: Part III, a teased fibre study of peripheral nerves. New Zealand Veterinary Journal 34: 181–185

Cahill J, Goulden B 1986d Equine laryngeal hemiplegia: Part IV, muscle pathology. New Zealand Veterinary Journal 34: 186–190

Cahill J, Goulden B 1987 The pathogenesis of equine laryngeal hemiplegia – a review. New Zealand Veterinary Journal 35: 82–90

Christley RM, Hodgson DR, Evans DL, Rose RJ 1997 Cardio-respiratory responses to exercise in horses with different grades of idiopathic laryngeal hemiplegia. Equine Veterinary Journal 29: 6–10

Cook WR 1965 The diagnosis of respiratory unsoundness in the horse. Veterinary Record 77: 516–28

Cook WR 1970 A comparison of idiopathic laryngeal paralysis in the horse and man. Journal of Laryngology and Otology 84: 819–835

Cook WR 1988 Diagnosis and grading of hereditary recurrent laryngeal neuropathy in the horse. Journal of Equine Veterinary Science 8: 432–455

Dixon PM, McGorum BC, Railton DI, Hawe C, Tremaine WH, Pickles K, McCann J 2001 Laryngeal paralysis: a study of 375 cases in a mixed-breed population of horses Equine Veterinary Journal 33: 452–458

Dixon PM, McGorum BC, Railton DI, Hawe C et al 2002 Clinical and endoscopic evidence of progression of 152 cases of equine recurrent laryngeal neuropathy (RLN). Equine Veterinary Journal 34: 29–34

Ducharme NG, Hackett RP 1991 The value of surgical treatment of laryngeal hemiplegia in horses. Compendium on Continuing Education for the Practicing Veterinarian 13: 472–475

Ducharme NG, Hackett RP, Fubini SL, Erb HN 1991 The reliability of endoscopic examination in assessment of arytenoid cartilage movement in horses. Part II. Influence of side of examination, reexamination, and sedation. Veterinary Surgery 20: 180–184

Duncan ID 1992 Determination of the early age of onset of equine recurrent laryngeal neuropathy. 2. Nerve pathology. Acta Neuropathologica 84: 316–321

Duncan ID, Baker GJ 1987 Experimental crush of the equine recurrent laryngeal nerve: a study of normal and aberrant re-innervation. American Journal of Veterinary Research 48: 431–438

Duncan ID, Griffiths IR, McQueen A, Baker GO 1974 The pathology of equine laryngeal hemiplegia. Acta Neuropathologica (Berlin) 27: 337–348

Duncan ID, Baker GJ, Heffron CJ, Griffiths IR 1977 A correlation of the endoscopic and pathological changes in subclinical pathology of the horse's larynx. Equine Veterinary Journal 23: 22–225

Duncan ID, Griffiths IR, Madrid RE 1978 A light and electron microscopic study of the neuropathy of equine idiopathic laryngeal hemiplegia. Neuropathology and Applied Neurobiology 4: 483–501

Duncan ID, Reifenrath P, Jackson KF, Clayton M 1991a Preferential denervation of the adductor muscles of the equine larynx. II: Nerve pathology. Equine Veterinary Journal 23: 99–103

Duncan ID, Amundson J, Cuddon PA et al 1991b Preferential denervation of the adductor muscles of the equine larynx. I: Muscle pathology. Equine Veterinary Journal 23: 94–98

Ellis D, Greet TRC Lane JG 2004 Sales: problems in diagnosis of RLN – UK perspective. In: Dixon PM, Robinson NE, Wade JF (editors) Proceedings of a Workshop on Equine Recurrent Laryngeal Neuropathy, Havemeyer Foundation Monograph Series No 11. R&W Publications, Newmarket, pp.39–41

Embertson RM 1997 Evaluation of the upper respiratory tract of the immature horse. In: Rantanen NW, Hauser ML (editors) Proceedings of the Dubai International Equine Symposium. The Diagnosis and Treatment of Respiratory Disease. Matthew R Rantanen, Bonsall, pp.399–405

Gerring EL 1985 Differential diagnosis of equine respiratory noises. In Practice pp.109–117

Goulden BE, Anderson LJ 1981a Equine laryngeal hemiplegia, Part II: some clinical observations. New Zealand Veterinary Journal 29: 194–198

Goulden BE, Anderson LJ 1981b Equine laryngeal hemiplegia, Part I: Physical characteristics of affected animals. New Zealand Veterinary Journal 29: 151–154

Goulden BE, Anderson LJ, Cahill HI 1985 Roaring in Clydesdales. New Zealand Veterinary Journal 33: 73–76

Greet TR, Jeffcott LB, Whitwell KE, Cook WR 1980 The slap test for laryngeal adductory function in horses with suspected cervical spinal cord damage. Equine Veterinary Journal 12: 127–131

Gunn HM 1973 Further observations on laryngeal skeletal muscle in the horse. Equine Veterinary Journal 2: 77–80

Hammer EJ, Tulleners EP, Parente EJ, Martin BB 1998 Videoendoscopic assessment of dynamic laryngeal function during exercise in horses with grade-III laryngeal hemiparesis at rest: 26 cases (1992–1995). Journal of the American Veterinary Medical Association 212: 399–403

Harrison GD, Duncan ID, Clayton MK 1992 Determination of the early age of onset of equine recurrent laryngeal neuropathy. 1. Muscle pathology. Acta Neuropathologica (Berlin) 84: 307–315

Hawe C, Dixon PM, Mayhew IG 2001 A study of an electrodiagnostic technique for the evaluation of equine recurrent laryngeal neuropathy. Equine Veterinary Journal 5: 459–465

Holcombe SJ, Derksen FJ, Stick JA et al 1996 Effect of nasal occlusion on tracheal and pharyngeal pressures in horses. American Journal of Veterinary Research 57: 1258–1260

Kannegieter NJ, Dore ML 1995 Endoscopy of the upper respiratory tract during treadmill exercise: a clinical study of 100 horses. Australian Veterinary Journal 72: 101–107

Lane JG 1993a Fourth branchial arch defects. In: Dyke TM (editor) Proceedings of the fifteenth Bain-Fallon Memorial Lectures. Australian Equine Veterinary Association, Artarmon, NSW, pp.209–212

Lane JG 1993b Equine recurrent laryngeal neuropathy (RLN): current attitudes to aetiology, diagnosis and treatment. In: Dyke TM (editor) Proceedings of the fifteenth Bain-Fallon Memorial Lectures. Australian Equine Veterinary Association, Artarmon, NSW, pp.173–192

Lane JG 2004a Long-term longitudinal study of laryngeal function in 187 foals. In: Dixon PM, Robinson NE, Wade JF (editors) Proceedings of a Workshop on Equine Recurrent Laryngeal Neuropathy, Havemeyer Foundation Monograph Series No 11. R&W Publications, Newmarket, pp.31–32

Lane JG 2004b 5-point grading system for endoscopy of horses during quiet breathing. Proceedings of a Workshop on Equine Recurrent Laryngeal Neuropathy, Havemeyer Foundation Monograph Series No 11. Eds PM Dixon, NE Robinson JF Wade, pp.24–25

Lane JG 2004c Differences between resting and treadmill endoscopic findings in regard to RLN. In: Dixon PM, Robinson NE, Wade JF (editors) Proceedings of a Workshop on Equine Recurrent Laryngeal Neuropathy, Havemeyer Foundation Monograph Series No 11. R&W Publications, Newmarket, pp.47–48

Lane JG, Ellis DR, Greet TR 1987 Observations on the examination of Thoroughbred yearlings for idiopathic laryngeal hemiplegia. Equine Veterinary Journal 19: 531–536

Mackay-Smith MP, Marks D 1968 Clinical diagnosis of laryngeal hemiplegia in horses. Proceedings of the American Association of Equine Practitioners, pp.227–235

Morris E 1991 Dynamic evaluation of the equine upper respiratory tract. Veterinary Clinics of North America; Equine Practice 7: 403–416

Newton-Clarke MJ, Divers TJ, Valentine BA 1994 Evaluation of the thoraco-laryngeal reflex ("slap test") as an indicator of laryngeal adductor myopathy in the horse. Equine Veterinary Journal 26: 355–357

Parente E, Martin B 1995 Correlation between standing endoscopic examinations and endoscopic examination during high-speed exercise in horses: 150 cases. Proceedings of the American Association of Equine Practitioners 24: 436

Rakestraw PC, Hackett RP, Ducharme NG et al 1991 A comparison of arytenoid cartilage movement in resting and exercising horses. Veterinary Surgery 20: 122–127

Robinson NE 2004 (Chairperson) Consensus statements on equine recurrent laryngeal neuropathy: Conclusions of the Havemeyer Workshop. September 2003, Stratford-upon-Avon. Equine Veterinary Education 16: 333–336

Speirs VC 1992 Larynx. In: Auer JA (editor) Equine Surgery, 1st edn. WB Saunders, Philadelphia, pp.467–480

Stick JA, Peloso JG, Morehead JP et al 2001 Endoscopic assessment of airway function as a predictor of racing performance in Thoroughbred yearlings: 427 cases (1997–2000). Journal of the American Veterinary Medical Association 219: 962–967

Weishaupt MA, Kästner SBR, Greishaber, von Plocki K 1998 Airflow limitations in laryngeal hemiplegia: chemical versus exercise induced hyperventilation. Proceedings of the World Equine Airways Symposium 2: 40

35 Surgical Treatment of Laryngeal Hemiplegia and Hemiparesis

Christine M Adreani and Eric J Parente

Laryngoplasty: Surgical Technique

Laryngoplasty was first described by Marks et al (1970), as an alternative technique to ventriculectomy and arytenoidectomy for the treatment of recurrent laryngeal neuropathy (RLN) in athletic horses. The authors noted that although ventriculectomy was the standard technique for treating horses with RLN, this procedure was not very successful in treating race and performance horses. The technique of laryngoplasty was developed to "reconstruct the laryngeal lumen in its anatomic position of maximal inspiratory dilatation" (Marks et al 1970). To do this, they proposed replacing the atrophied cricoarytenoideus dorsalis muscle with a prosthesis that would "maintain the muscular process of the arytenoid in caudad retraction". The prosthesis, therefore, joins the dorsocaudal edge of the cricoid cartilage and the muscular process of the arytenoid cartilage.

The choice of suture material used for the prosthesis varies depending on surgeon preference. A large diameter, non-absorbable, monofilament or coated suture material is preferable. Marks et al (1970) used braided Lycra® for the prosthesis because they believed the elastic properties of this suture were necessary. Subsequent descriptions of the procedure have replaced this multifilament, inflammatory material with materials such as No. 5 coated polyester, No. 2 nylon or stainless steel wire.

Horses are positioned in lateral recumbency, with the affected side dorsally and the head and neck extended. A 3-liter bag of fluid placed under the larynx will help to elevate it towards the surgeon. It is often beneficial to position a video-endoscope in the ipsilateral nostril before the beginning of surgery to provide an image of the larynx for later use. After preparation of the skin for aseptic surgery, an 8- to 10-cm incision is made ventral and parallel to the linguofacial vein, centered at the palpable caudal edge of the cricoid cartilage. A plane of dissection is established between the linguofacial vein and the omohyoideus muscle, and a combination of sharp and blunt dissection is used to expose the caudal pharyngeal constrictor muscles (thyropharyngeus and cricopharyngeus). Careful attention should be paid to hemostasis as this surgical site is prone to large seroma formation because of the extensive dissection used for this procedure and the inability to close the large dead space created because of the many adjacent vital structures. The use of a hand-held retractor in the dorsal portion of the incision allows better exposure of the larynx. The cricopharyngeus and thyropharyngeus muscles are separated along their aponeurosis to expose the intrinsic laryngeal muscles and the muscular process of the arytenoid.

The suture prosthesis is first placed through the dorso-caudal edge of the cricoid cartilage between the cartilage and the laryngeal mucosa. The point of the needle should be carefully walked off the caudal edge of the cricoid to avoid inadvertent damage to nearby structures such as the carotid artery or esophagus, and should be placed as close to the dorsal midline as possible (at the site of the caudal cricoid "notch"). Once through the cricoid cartilage, the needle is passed under the cricopharyngeus muscle and then passed through the muscular process of the arytenoid in a caudomedial to rostrolateral direction. The trailing end of the suture is then passed under the cricopharyngeus muscle. A second prosthesis can then be placed. Intra-operative endoscopy is often used at this time to ensure that the suture has not penetrated the laryngeal lumen and to ensure that an appropriate degree of arytenoid abduction is present, as the individual sutures are tightened and knotted (Fig. 35.1). Generally it is appropriate to abduct the arytenoid into a position of 80–90% of maximal abduction in racehorses, with lesser abduction (60–80% of maximal abduction) being appropriate in non-performance horses. The degree of abduction has been semi-quantitatively graded by a number of systems including that illustrated in Fig. 35.2. The apparent degree of abduction intraoperatively is always slightly greater than that seen on resting endoscopy the following day in the standing horse. This may be because of some immediate decrease in abduction or the overestimation of abduction in the horse under anesthesia, possibly because the pharyngeal vault is relaxed onto the margin of the corniculates while the horse is under anesthesia. Once the sutures are knotted, the pharyngeal constrictor muscles are re-apposed using 2–0 absorbable sutures. Closure of the subcutaneous and skin incisions is then performed in a routine fashion.

Two variations to the standard laryngoplasty procedure have been employed by the authors. With appropriate positioning, the arytenoid muscular process can be approached
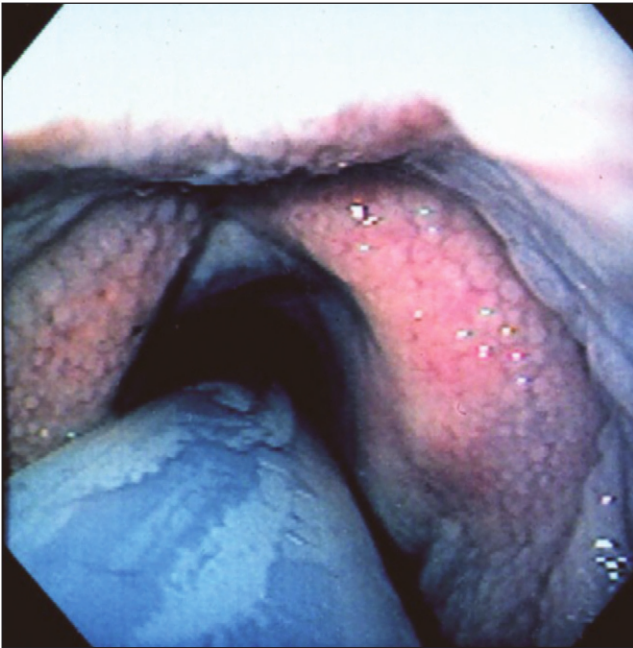
Fig. 35.1. Intraoperative endoscopic view of the larynx during laryngoplasty as the left arytenoid is being surgically abducted.

at the caudal margin of the cricopharyngeus muscle. This obviates the need for the dissection and closure between the cricopharyngeus and thyropharyngeus muscles, saving surgical time. More importantly, it minimizes the potential of subsequent suture loosening from interposed fascia. A second alternative is to approach the cricoarytenoid joint underneath the attachment of the cricoarytenoideus dorsalis to the muscular process and ablate this joint with a rotating burr. This procedure causes ankylosis of the cricoarytenoid joint and more rigid fixation of the arytenoid experimentally (Parente 2004).

Horses should be treated perioperatively with antimicrobials and anti-inflammatory medications. Sequential endoscopic evaluation of the degree of arytenoid abduction should be performed postoperatively, especially before the horse is discharged from the hospital.

Postoperative care

Most horses experience some degree of dysphagia and coughing immediately postoperatively (Speirs 1972, Hawkins et al 1997, Dixon et al 2003a). Therefore, they should be

Grade 1

Grade 2

Grade 3

Grade 4

Grade 5
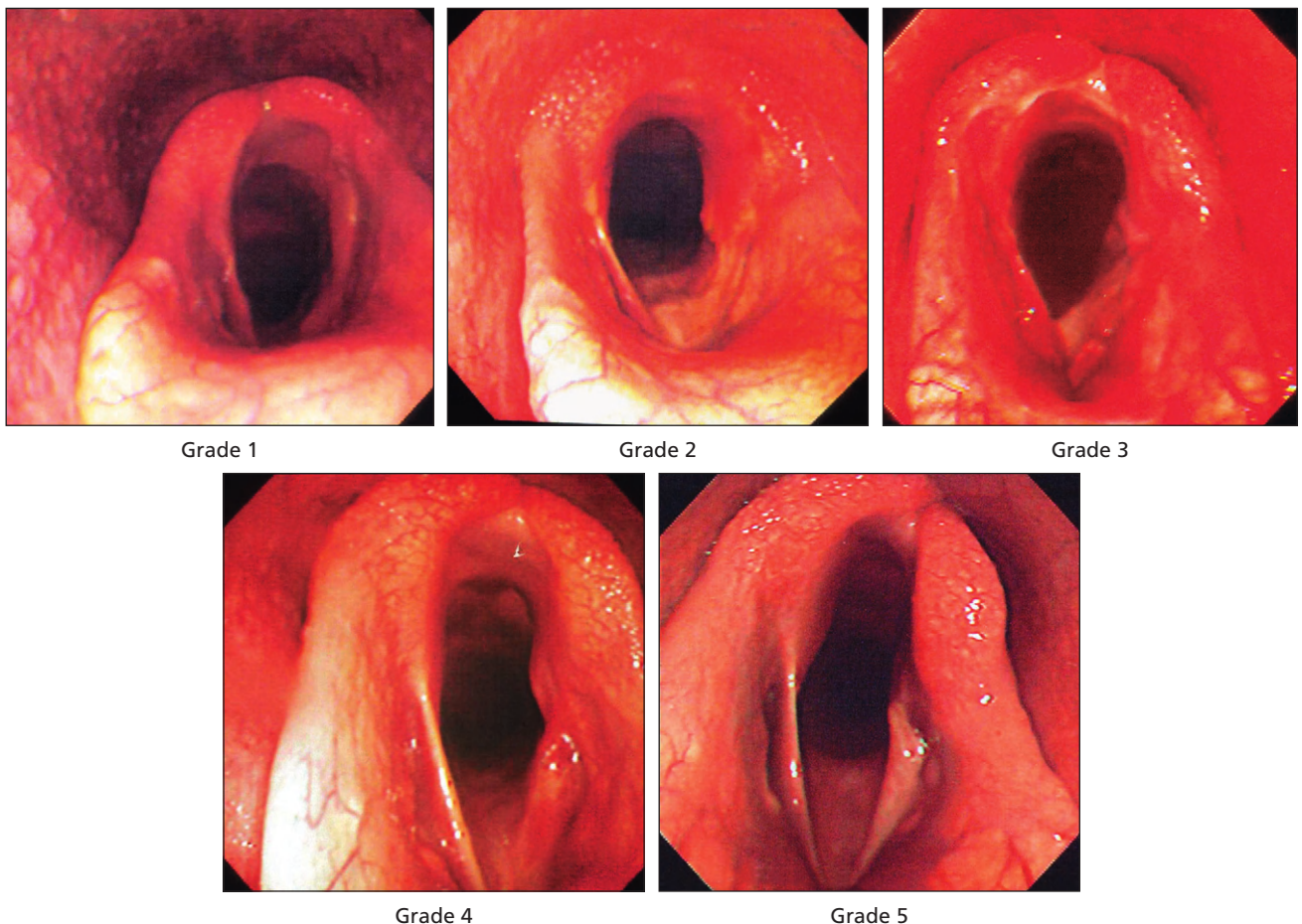
Fig. 35.2. Semi-quantitative grading of the degree of laryngoplasty abduction. Reprinted from Dixon et al, 2003a, with permission.

fed off the ground to help minimize aspiration of feed material. The incision should be carefully examined daily for signs of excessive swelling or drainage, indicative of excessive seroma formation or wound infection.

Horses should be confined to their stall with hand walking for 4–6 weeks following surgery. A further endoscopic examination should then be performed to assess healing of the vocal cordectomy and the position of the affected arytenoid before the horse resumes training (Fig. 35.3).

Efficacy of Surgical Intervention: Experimental Studies

The effects of prosthetic laryngoplasty on upper airway function and arterial blood gases and pH parameters have been examined in numerous experimental studies. Overall, prosthetic laryngoplasty has been shown to restore abnormalities in the above parameters that are present in horses with RLN or in experimental recurrent laryngeal neurectomy to values found in normal exercising horses.

Arterial blood gases measured in a horse with left RLN before and 48 h after laryngoplasty demonstrated that the hypoxemia and hypercapnia initially observed after galloping were reversed postoperatively (Bayly et al 1984). Similar results were obtained by Tate et al (1993) in a study of four horses with RLN where blood gas tensions and acid–base status were evaluated before and after corrective surgery. In that study however, although the acidosis and hypoxemia that developed during exercise

Fig. 35.3. Endoscopic examination 1 month postoperatively following left-sided laryngoplasty and vocal cordectomy. The left arytenoid is in a well-abducted position and the cordectomy site is well healed.

were significantly improved following surgical correction, arterial blood gas and pH values were not the same as in normal horses, in spite of endoscopic evidence of full arytenoid abduction postoperatively.

Upper airway flow mechanics, measured in horses exercising on a treadmill, show a predictable decrease in peak inspiratory flow and increase in inspiratory resistance following experimental recurrent laryngeal neurectomy (Derksen et al 1986). These alterations were reversed by prosthetic laryngoplasty, leading to the conclusion that laryngoplasty supported the arytenoid cartilage and so limited dynamic collapse of the laryngeal airway during inspiration. Likewise, Shappell et al (1988) showed that the increases in inspiratory impedance and inspiratory trans-upper airway pressure that occurred following experimental recurrent laryngeal neurectomy were abolished by laryngoplasty. Tetens et al (1996) tested upper airway function in horses exercising at 75 and 100% of maximal heart rate on a treadmill and found that inspiratory impedance was increased and flow was decreased following recurrent laryngeal neurectomy. Tetens et al (1996) also found that these values were restored to preneurectomy levels after laryngoplasty.

A limitation in the interpretation of these studies, however, is that horses were studied at submaximal exercise levels, and consequently airflow rates were not as high as occur in horses exercising at racing speeds. Williams et al (1990a) addressed this limitation by recording upper airway pressures in thoroughbred horses galloping on a racetrack. In this study, horses with experimental recurrent laryngeal neurectomy had a 2.5-fold increased peak inspiratory upper airway pressure as compared to preneurectomy levels. Laryngoplasty reduced the peak inspiratory pressure, but values were still significantly elevated over control (pre-neurectomy) levels. In a separate study of horses clinically affected with left RLN, Williams et al (1990b) showed that clinically affected horses had airway pressures similar to those with experimental recurrent laryngeal neurectomies. Laryngoplasty in the RLN-affected horses restored upper airway function to within the normal range.

While the effect of laryngoplasty on respiratory noise is generally evaluated in a subjective manner, recent work has shown that noise can be qualitatively and quantitatively studied using spectrogram analysis in exercising horses. Brown et al (2004) used exercising standardbreds that had received a recurrent laryngeal neurectomy, followed by laryngoplasty, to study the effect of surgery on inspiratory sound level and the three formants of inspiratory sound intensity (see Chapter 17). These authors found that sound level and intensity were increased following neurectomy, and that the first inspiratory formant was restored to its preneurectomy value by 30 days post laryngoplasty. Sound levels, as well as the second and third formants of respiratory sounds, were significantly improved by laryngoplasty but did not return to baseline levels.
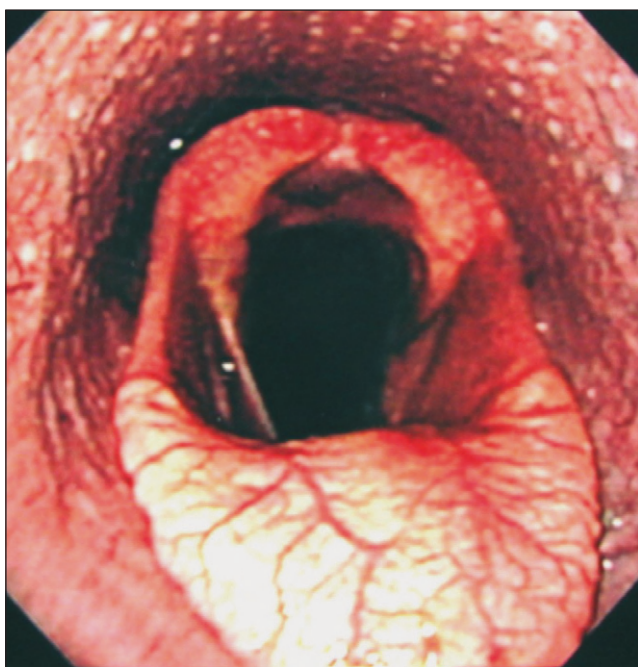
Interestingly, the degree of arytenoid abduction post-operatively was positively correlated with postoperative noise production.

Efficacy of Surgical Intervention: Outcome in Clinical Cases

The reported success rates for prosthetic laryngoplasty vary widely depending on the criteria used to define success. In general, success rates for draft and sport horses are better than those reported for racehorses, leading to the overall conclusion that even with surgical correction, there still may be respiratory limitations to high-speed exercise in some horses.

In the first published report of prosthetic laryngoplasty, Marks et al (1970) reported on 121 horses treated with this procedure. Eighty-eight per cent of these surgeries were reported to be "completely successful", with success being defined as the presence of maximal abduction of the affected arytenoid during postoperative resting endoscopy. Speirs (1972) reported on eight thoroughbreds that had been treated with prosthetic laryngoplasty. Five were reported to be in race training and free of respiratory noise. One horse was racing, but still made a noise, and the other two horses were reported to have reduced racing performance. Subsequent reports have refined the criteria for success by focusing on various aspects of functional outcome following surgery.

Outcome in racehorses

When postoperative racing performance was subjectively evaluated by owner/trainer, improvement was found in 48–69% of horses (Russell & Slone 1994, Hawkins et al 1997, Kidd & Slone 2002). Interestingly, the perceived success rate was higher in thoroughbred racehorses aged 2 years or more (70% success) when compared to those aged 3 years or above (25% success) (Russell & Slone 1994).

Several objective analyses of racing performance following laryngoplasty have been reported, with varying results depending on the method of comparison of performance before and after surgery (Speirs et al 1983, Russell & Slone 1994, Hawkins et al 1997, Strand et al 2000, Kidd & Slone 2002). A large Australian study (Speirs et al 1983) described the results of laryngoplasty in 100 racing thoroughbreds in a survival analysis which compared the postoperative racing results of these horses to those of 400 control horses of similar age, breed, sex, and occupation. The survival analysis took into account total races, wins, wins plus placings, and earnings. It showed no significant difference between the 100 horses treated with laryngoplasty and the control horses, leading the authors to conclude that laryngoplasty "restored racehorses to the standard set by their peers".

In a study of 40 racehorses, Russell & Slone (1994) found that 78% of thoroughbreds that raced before and after surgery dropped in class or distance after surgery, and that their earnings dropped 2.6-fold over this period. Hawkins et al (1997) found that 56% of thoroughbred and standardbred racehorses that raced three or more times before and after surgery had improved performance index scores postoperatively, and that 77% of horses raced at least once after surgery. In a retrospective study designed to compare the success rate of laryngoplasty in experienced and inexperienced thoroughbred racehorses, Strand et al (2000) used a performance index which was obtained from a verified regression model for standardizing the racing performance of thoroughbreds. Experienced racehorses improved their performance index following surgery compared to the period immediately preceding laryngoplasty but did not reach their previous baseline values for performance index or earnings. Nevertheless, they raced distances of similar length before and after surgery, and 45% of experienced racehorses ran a personal best time after surgery.

Age at the time of surgery does not seem to affect the outcome of laryngoplasty in racehorses. When a performance index was used to compare outcome in 2-year-old standardbred and thoroughbred racehorses to that in 3-year-old horses of the same breeds, no effect of age at the time of surgery was observed (Hawkins et al 1997). Likewise, horses that were either 2 years old or had fewer than two starts at the time of surgery had a career performance index equal to that of the older, experienced horses (Strand et al 2000). Younger, inexperienced horses require significantly more time after surgery to their first race (165 days) than older experienced horses (120 days) (Strand et al 2000).

The effect of the endoscopic degree of preoperative hemiplegia on surgical outcome has been evaluated in several retrospective studies, and the results are somewhat conflicting (Hawkins et al 1997, Hammer et al 1998, Davenport et al 2001). Hawkins et al (1997) reported on the postoperative findings of 230 thoroughbred and standardbred racehorses divided nearly equally between grades III and IV RLN, where the grading system used (Hackett et al 1991) was a four-grade system based on resting endoscopy in which a grade IV was unable to move one arytenoid, and a grade III was able to move the arytenoid but not achieve full abduction. In spite of the large number of cases of each grade of RLN, no effect of grade on postoperative performance index was observed. When horses with grade III laryngeal hemiplegia are further subdivided, based on the degree of arytenoid movement during high-speed treadmill exercise, a difference in surgical success was reported between those horses that have some ability to abduct the left arytenoid (grade IIIb) and those that have complete dynamic collapse of the arytenoid (grade IIIc) (Hammer et al 1998). Only one in four horses with grade IIIb laryngeal hemiplegia were reported to have an improved performance index postoperatively, whereas nine of 18 horses with grade IIIc

improved their racing performance postoperatively. No statistical analysis was performed and while these results appear to indicate that horses with worse preoperative function have a better outcome, it is more likely that all horses have a similar postoperative performance outcome, but those horses that are paralyzed have a greater disparity between their preoperative and postoperative performances than horses that still have partial function preoperatively. Consequently, there is no evidence to support the theory that waiting until a horse is completely paralyzed before pursuing surgery will improve the surgical outcome.

A suspected reason for the apparent lack of improvement in some horses with partial laryngeal function at the time of surgery is that they are more likely to have an unsuccessful surgery. The primary reason for laryngoplasty failure is inability of the prosthesis to maintain abduction. Some authors suspect that the major causes for prosthesis failure are repetitive contraction of the remaining muscles that results in subsequent loosening of the suture, or that the suture cuts through the cartilage. In an effort to investigate the contribution of the cricoarytenoideus dorsalis muscle movement to laryngoplasty failure, Davenport et al (2001) compared postoperative racing performance in horses with grade III laryngeal hemiplegia that were treated with laryngoplasty, ventriculocordectomy, and ipsilateral recurrent laryngeal neurectomy to horses without the neurectomy. By eliminating any movement of the existing intrinsic muscles via the neurectomy, the authors were able to determine that residual movement of the muscles caused by an intact, partially functional recurrent laryngeal nerve did not affect the postoperative performance. Therefore, a difference in success following laryngoplasty between horses with grade III and grade IV laryngeal hemiplegia would not be expected.

Outcome in non-racing breeds

Surgical success in draft and sport horses is generally higher than that reported in racehorses, regardless of the criteria used to define success (Russell & Slone 1994, Kidd & Slone 2002, Dixon et al 2003b). The perceived success rate, based on owner/trainer surveys, in non-racing breeds ranges between 86 and 93%. While exercise intolerance must be alleviated enough to allow the horse to fulfill its intended purpose, elimination of abnormal respiratory noise is often equally important for the competitive show horse. An objective comparison of performance before and after surgery is difficult in this population of horses because of the wide range of expectations on the part of the owner/trainer in terms of athletic function and presence of respiratory noise. Nevertheless, several large studies have evaluated the success of laryngoplasty in addressing the performance-limiting problems seen in the non-racehorse.

When success was defined as a significant reduction or absence of noise, Baker (1983) reported that laryngoplasty was successful in 58% of 316 horses treated with the

procedure. When combined with ventriculocordectomy, laryngoplasty resulted in 73% of horses (sports horses and racehorses) making no abnormal upper respiratory noise during exercise (Dixon et al 2003b). Similarly, respiratory noise was eliminated in 72% of draft horses treated with laryngoplasty and ventriculectomy or ventriculocordectomy (Kraus et al 2003). These results contrast somewhat with those found in racehorses, in which 25–47% of horses treated with laryngoplasty and ventriculocordectomy still make a noise while exercising at long-term follow-up (Russell & Slone 1994, Hawkins et al 1997). This difference may be the result of the higher intensity exercise and the resulting increased respiratory effort made by racehorses when compared to performance breeds.

Dixon et al (2003b) published a comprehensive evaluation of the outcome of laryngoplasty and ventriculocordectomy in an older, mixed-breed group of 200 horses. While many horses included in the study were National Hunt racehorses and therefore analogous to flat racehorses, the majority were hunters, eventers, show jumpers, draft horses, and multi-discipline sport horses. Six weeks postoperatively, 91% of the treated horses began to resume training. Interestingly, the degree of postoperative abduction at 6 weeks had little effect on whether or not the horse returned to full work, except that horses with no detectable abduction were much less likely to resume full work. Ninety-five per cent of horses with a high degree of abduction, 91% of horses with moderate abduction, and 88% of horses with slight abduction returned to full work, but only 25% of horses with no residual abduction returned to full work. When owners/trainers were asked to evaluate their horses' level of exercise performance at a mean of 12 months after surgery, 64% reported a marked increase in performance and another 11% reported a slight or moderate increase.

A report of surgical treatment for laryngeal hemiplegia in draft horses implied that laryngoplasty held no advantage over ventriculectomy alone (Bohanon et al 1990), suggesting that the additional surgery time required for laryngoplasty posed an unnecessary increased anesthetic risk to draft horses. Kraus et al (2003) described the outcome of laryngoplasty and ventriculectomy *or* ventriculocordectomy in 104 competitive draft horses, and although the incidence of anesthetic-related complications was higher than that reported for light breed horses, these complications were still relatively rare. Postanesthetic myopathy or neuropathy occurred in 7% of cases and prolonged anesthetic recoveries occurred in 4%; only one anesthetic-related death was reported. Overall, surgical correction was highly successful in this study, with 92% of horses having improved exercise tolerance postoperatively and 72% of horses being able to be used for their intended purpose of competitive driving.

Regardless of the breed, occupation, or criteria for success used, the grade of preoperative laryngeal dysfunction does not appear to have a significant effect on surgical
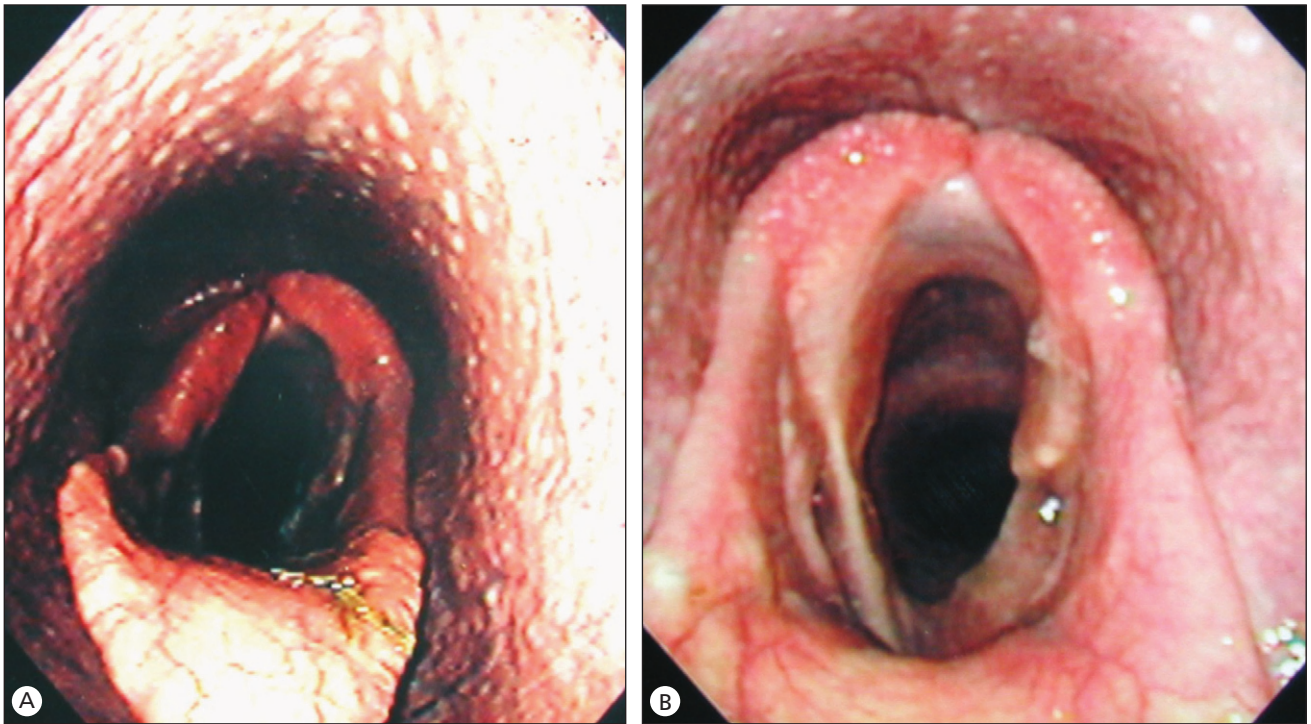
Fig. 35.4. (A) Excessive laryngoplasty abduction of this left arytenoid led to dysphagia and excessive coughing in this horse. (B) Endoscopic examination after a second surgery was performed to replace the prosthesis, with the arytenoid now in a more adducted position. The aspiration problem was resolved.

outcome in non-racehorses (Russell & Slone 1994, Dixon et al 2003a,b, Kraus et al 2003). A greater degree of post-operative surgical abduction resulted in a larger number of postoperative complications such as coughing, but in the longer term did not appear to be correlated with postoperative performance (Russell & Slone 1994, Dixon et al 2003a,b).

Postoperative Complications

Cough: short-term and chronic

Coughing, both immediately postoperatively and chronically, is a common complication of laryngoplasty surgery (Marks et al 1970, Raker 1975, Speirs et al 1983, Russell & Slone 1994, Hawkins et al 1997, Dixon et al 2003a). Although there is wide variation in the reported incidence of postoperative coughing, up to 43% of horses treated with laryngoplasty may develop coughing at some point. The cause is likely multifactorial. The presence of coughing postoperatively does not necessarily preclude improved athletic performance (Russell & Slone 1994, Hawkins et al 1997) but it is considered a common and undesirable side effect of laryngoplasty.

The presence of a maximal degree of arytenoid abduction postoperatively is significantly associated with coughing and dysphagia (Russell & Slone 1994, Dixon et al 2003a).

The horse's inability to protect its airway adequately while eating likely results in the persistent aspiration of feed material. The dorsal laryngeal wall and the trachea of humans have been shown to contain numerous irritant receptors (Guyton & Hall 2000) which, when stimulated, result in a cough reflex, although horses appear to have a less sensitive upper respiratory tract than humans and many other domestic animals. Some aspiration of feed material is likely the result of dysphagia caused by the surgically abducted arytenoid failing to protect the airway during swallowing. Dysphagia may also result from pharyngeal constrictor muscle dysfunction following surgery (Greet et al 1979). Dissection between the caudal pharyngeal constrictor muscles and inadvertent damage to the cranial laryngeal nerve may result in this dysfunction. Some surgeons have reasoned that this is why, in a minority of horses that cough postoperatively, removal of the prosthesis does not result in elimination of the clinical signs (Raker 1975, Greet et al 1979).

In some cases with maximal surgical abduction of the arytenoid and subsequent dysphagia and coughing, a second surgery is required to loosen the prosthesis (Fig. 35.4A,B). Dixon et al (2003a) reported that eight of 200 horses required a second surgery within a week of the first surgery, and another six of the remainder required a second surgery within 6 weeks of the first to alleviate persistent dysphagia and coughing; there was immediate

resolution of the dysphagia and coughing in all cases following prosthesis loosening.

Many cases of postoperative dysphagia and coughing will spontaneously resolve within a few weeks of surgery, possibly because of loosening of the prosthesis and/or adaptation of the swallowing reflex. However, if the dysphagia and coughing persist, there are several management changes that should be considered postoperatively before removing the prosthesis. Continuing to feed the horse off the ground and moistening the hay or changing the horse's diet to include a greater percentage of processed feed is often beneficial. Removing all feed several hours before exercise and rinsing out the horse's mouth with water will remove most feed stored in the mouth. Generally if these methods do not improve the horse in several weeks, suture removal or replacement is recommended. Removal of the suture alone may not be enough to resolve the coughing. To gain relaxation of the arytenoid at the time of surgery in more chronic cases, or particularly if there has been postoperative wound infection, it may be necessary to dissect the muscular process free from the fibrous adhesions that often form between the arytenoid and the adjacent wing of the thyroid cartilage, and possibly even to surgically disarticulate the cricoarytenoid joint.

Wound and prosthesis infection

Among the first reported complications of laryngoplasty were wound infection, excessive wound inflammation, and the formation of sinus tracts caused by the prosthesis (Raker 1975). These complications, which occurred in 15% of cases, were noted shortly after the laryngoplasty procedure was first described using a braided Lycra® suture material. Lycra has been associated with inflammation and delayed healing more frequently than other types of suture (Raker 1975), and the multifilament nature of this suture made it more prone to harboring infection. Currently, most surgeons use either monofilament or coated suture materials, which aids in reducing the occurrence of prosthesis infection.

The reported incidence of wound infection with laryngoplasty is between 0.5 and 6% (Hawkins et al 1997, Strand et al 2000, Davenport et al 2001, Kidd & Slone 2002, Dixon et al 2003a, Kraus et al 2003). This incidence is similar to that seen with other clean, elective equine surgical procedures (Santschi 1999). Laryngotomy incisions, used to perform ventriculectomies concurrently with laryngoplasty, are often left open to heal by second intention, and lie in close proximity to clean laryngoplasty incisions. A significant correlation between laryngotomy and laryngoplasty wound problems was observed in 200 horses that were treated with both laryngoplasty and ventriculocordectomy (Dixon et al 2003a). Nevertheless, the incidence of laryngotomy wound infections is low, regardless of whether or not the cricothyroid membrane

is sutured closed (Hawkins et al 1997), likely because of the excellent ventral drainage afforded by the location of this incision.

Meticulous postoperative local wound care aids in reducing the likelihood of soft tissue infection around unsutured laryngotomy incisions (Stick et al 1999). Laryngoplasty wound infections usually resolve with drainage, combined with antimicrobial and possibly anti-inflammatory therapy. Suture removal is rarely required (Hawkins et al 1997). Incisional seromas and minor wound swelling are more common wound complications than infection in laryngoplasty (Hawkins et al 1997, Speirs et al 1983, Dixon et al 2003a). The reported incidence for minor swelling and/or seroma formation is 7–30%. The higher number was reported in 1983 when Lycra suture material was used for laryngoplasty (Speirs et al 1983). Therefore, some reported swellings might have been the result of inflammation associated with the prosthesis. Dixon et al (2003a) found that 17% of cases had reported swellings or discharge associated with the laryngoplasty incision, and that more than half of these resolved within 2 weeks of surgery. One horse in this study developed a retrolaryngeal abscess that caused perilaryngeal swelling and loss of left arytenoid abduction. The abscess was drained, but this failed to restore arytenoid abduction. Careful attention to hemostasis and avoiding large lymphatic vessels should help avoid excessive wound swellings and seromas (Stick et al 1999).

Loss of arytenoid abduction

Failure of the prosthesis to maintain abduction of the arytenoid is another major complication of laryngoplasty, both in the immediate postoperative period and in the long term. Although two studies (Russell & Slone 1994, Kidd & Slone 2002) reported that no horses had complete loss of abduction at long-term follow-up, the incidence of laryngoplasty failure because of the loss of arytenoid abduction in other studies is 2–15% (Marks et al 1970, Raker 1975, Baker 1983, Hawkins et al 1997, Dixon et al 2003a, Kraus et al 2003). Loss of abduction can be caused by suture loosening or breakage, suture cutting through the arytenoid or cricoid cartilage, or avulsion of the muscular process of the arytenoid (Russell & Slone 1994, Kidd & Slone 2002). The majority of cases in which there is total loss of arytenoid abduction (arytenoid collapse) at the follow-up examinations are thought to be caused by sutures cutting through the muscular process of the arytenoid (Baker 1983, Johnson 1985) and *in vitro* studies have supported this conclusion (Dean et al 1990, Herde et al 2001). Dixon et al (2003a) reported that of 200 horses treated with laryngoplasty using stainless steel wire, ten required a second surgery within 7 days for excessive loss of arytenoid abduction because of prosthesis loosening/migration (five horses), broken prosthesis (three horses) or avulsion of the muscular process of the

arytenoid (two horses). An additional nine horses required a second surgery because of excessive loss of abduction within 6 weeks of the first surgery.

Most studies do not reveal the exact cause of arytenoid collapse because determining this information would require a second laryngoplasty procedure which owners may find financially unacceptable. Because the exact cause of failure is unknown in many cases, it is difficult to draw conclusions about the factors responsible for failure of surgical arytenoid abduction. Nevertheless, several *in vitro* studies on isolated equine larynges have demonstrated that the age of the horse and the type of suture material used (polyester versus polytetrafluoroethylene) have no significant effect on laryngoplasty failure.

Schumacher et al (2000) investigated the use of a stainless steel cable with stress-reducing washers as a prosthesis in cadaver larynges to minimize the risk of suture loosening or pull out. The authors concluded that the cable required significantly more force than polyester suture to result in failure. Despite the encouraging results with this system, it was not practical to use it successfully *in vivo*.

If a horse experiences a loss of abduction that limits its exercise performance, a repeat laryngoplasty should be considered. Although some clinicians advocate an arytenoidectomy rather than a repeat laryngoplasty, the success rate of repeat laryngoplasty by experienced surgeons is comparable to that of an initial laryngoplasty (Tulleners 1994), and it is likely to be more successful than a partial arytenoidectomy. If the failure is secondary to muscular process avulsion and there is no remaining cartilage to place the suture through, the partial arytenoidectomy should be considered.

Aspiration pneumonia

While chronic coughing and dysphagia are far more common following laryngoplasty than is aspiration pneumonia, this latter complication is life-threatening and requires removal of the prosthesis if the horse is to be salvaged for any purpose. The incidence of aspiration of small amounts of feed, based on postoperative endoscopic evidence of feed material in the airway, is 3–10% (Russell & Slone 1994, Hawkins et al 1997, Strand et al 2000). Relatively few of these cases, however, go on to develop fulminant bacterial pulmonary infection (Dixon et al 2003a).

Suture penetration into airway

Inadvertent penetration of the airway mucosa with the prosthesis as it is placed in the cricoid cartilage can result in an intraluminal, granulomatous inflammatory reaction or surgical wound infection (Fig. 35.5) (Raker 1975), especially with braided sutures. The inflammation associated with the intraluminal suture may serve as an irritant
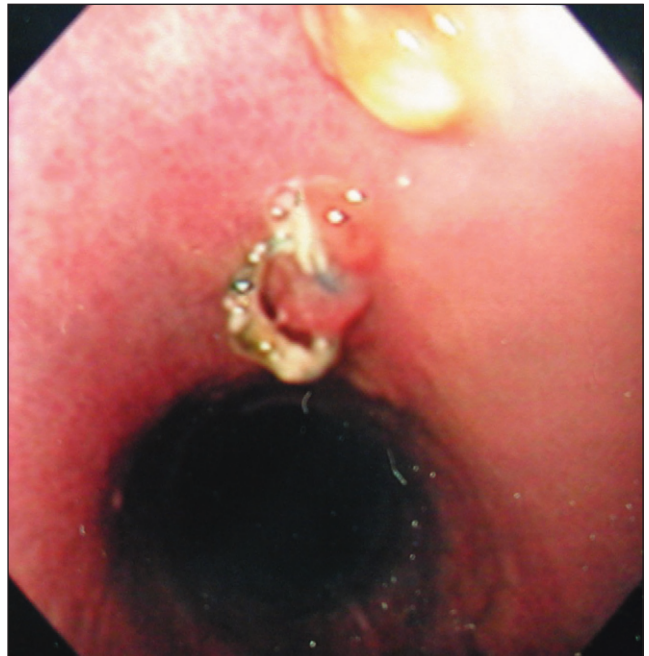
Fig. 35.5. Before the use of intraoperative endoscopy, suture penetration of the lumen was not diagnosed until postoperatively. This figure shows a granulomatous reaction around a laryngoplasty suture penetrating the laryngeal lumen.

to the airway, resulting in a chronic cough. More recent publications have cited this as a potential complication, but do not report on any cases in which this occurred. The lack of cases reported may be the result of the common use of intraoperative endoscopy to evaluate the airway lumen after suture placement, allowing removal of any that do penetrate the lumen before completing the surgery.

Postoperative changes in corniculate cartilages

Granuloma formation and chondritis are rare complications of laryngoplasty with Dixon et al (2003a) reporting a 1% incidence of such problems. Other morphological changes to the arytenoids can also occur following laryngoplasty (Figs 35.6 and 35.7). Some of these complications may be the result of trauma induced by the endotracheal tube. With concurrent use of vocal cordectomy, excision of the vocal process of the corniculate could also predispose to such lesions, as could inadvertent damage to the corniculate cartilages during laser cordectomy.

Ventriculectomy and Ventriculocordectomy: Surgical Technique

Ventriculectomy has been performed through a laryngotomy incision for many years with the horse standing and sedated, or under general anesthesia in dorsal recumbency.
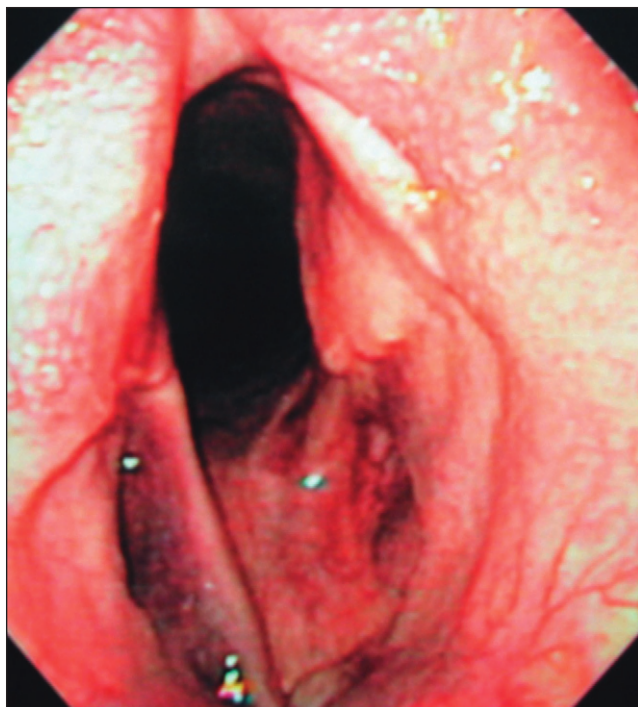
Fig. 35.6. Laryngeal endoscopy of a horse that underwent left-sided laryngoplasty 1 year earlier and raced successfully. At a revisit because of lameness, routine endoscopy now shows an area of normal corniculate cartilage that is devoid of mucosa. There is also some excessive fibrosis at the caudal aspect of the vocal cordectomy site.
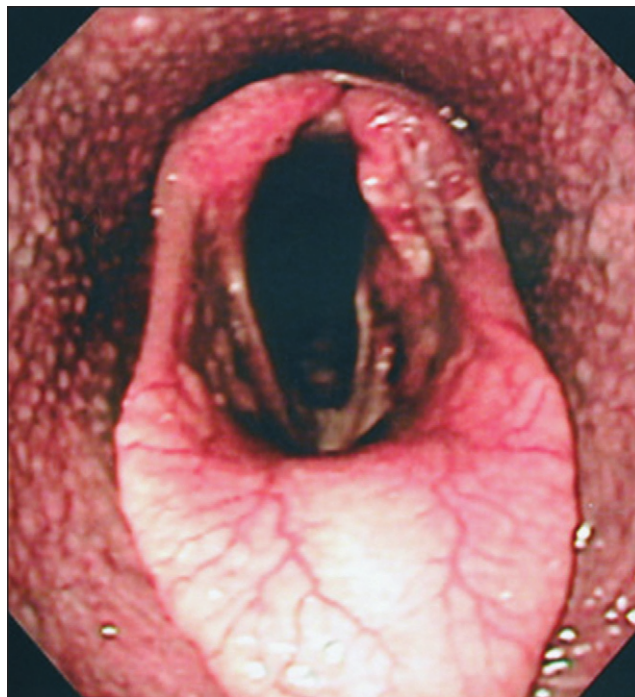
Fig. 35.7. At a routine laryngeal endoscopy of a racehorse that had received left-sided laryngoplasty several months earlier and also raced successfully, the left corniculate cartilage is atrophied and distorted and unusual ulcerations are also present on the adjacent aryepiglottic fold. This case was treated medically and continued to race for another year but eventually developed left-sided arytenoid chondritis.

As this procedure is often performed in conjunction with laryngoplasty, it is usually performed under general anesthesia on the ipsilateral lateral ventricle. There is no current evidence demonstrating the necessity to perform a bilateral ventriculectomy, but it is occasionally chosen by the surgeon to minimize the possibility of abnormal noise postoperatively. If a bilateral ventriculectomy is performed, caution should be exercised to leave an intact mucosal strip between the two cords. If not, webbing across the ventral glottis may develop.

The standard approach for ventriculectomy has been through a laryngotomy. The horse is positioned in dorsal recumbency with the head and neck extended. After skin preparation, a ventral midline incision is made between the thyroid notch and the rostral ventral edge of the cricoid cartilage. The cricothyroid membrane is then penetrated to enter the laryngeal cavity. A burr is placed deep into the ventricles and rotated to engage the membrane. It is then gently retracted up toward the laryngotomy site to evert the ventricle, which is then resected with Metzenbaum scissors. The ventricle is usually allowed to heal by second intention. The proposed mechanism of decreasing turbulence in the airway by stabilizing the vocal cord has not been supported clinically because the cord is not "lateralized" after such a procedure.

To address this, an alternative approach has been described (Brown et al 2003). The defect created by removing the ventricle is extended to include the most rostral edge of the vocal cord. This edge of the vocal cord is then sutured to the rostral lateral margin of the ventricle defect. By doing so, the cord is pulled laterally, and therefore should not be displaced into the airway during exercise and so affect airflow through the glottis.

With either procedure, the cricothyroid ligament may be either closed with interrupted absorbable suture or left open to heal by second intention. The area around and rostral to the laryngotomy should be cleaned daily and Vaseline applied until it ceases discharging to prevent skin scalding from the drainage. An open laryngotomy usually heals within 3 weeks, and those in which the cricothyroid ligament is closed will heal slightly sooner.

More recently, ventriculocordectomy has been performed using a laser via video-endoscope through the nasal or oral cavity (Chapter 39) with the horse either standing and sedated, or under general anesthesia in lateral recumbency. When performed under standing sedation, the vocal folds should be desensitized with topical local anesthetic such as Cetacaine. A laser fiber [neodymium:yttrium–aluminum–garnet (Nd:YAG) or diode] is passed through the biopsy channel of the endoscope and a transnasal grasping
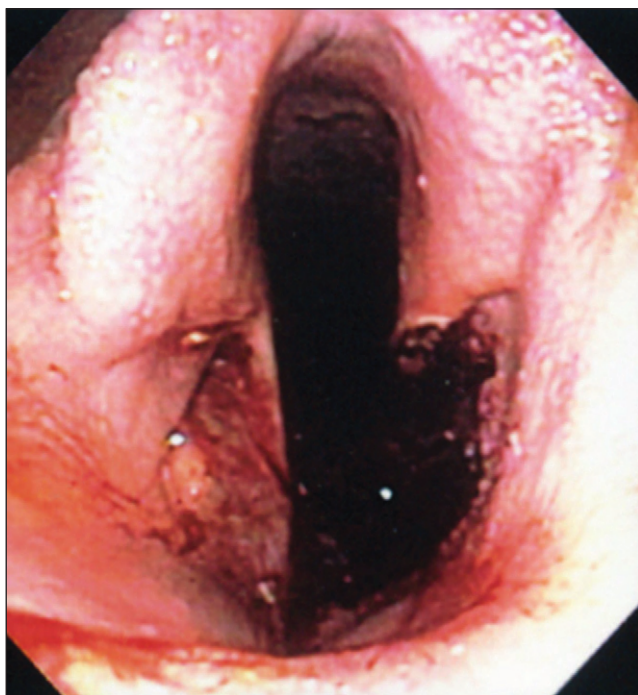
Fig. 35.8. Endoscopic view of a larynx 1 day after laryngoplasty and left-sided, laser vocal cordectomy.

forceps is used to grasp and rotate the vocal fold and part of the ventricle axially. The laser is used in contact fashion to excise the vocal fold and the everted part of the ventricular mucosa, which are removed through the nasal cavity with the grasping forceps once freed from the surrounding tissue (Fig. 35.8).

Efficacy of Ventriculectomy/ Ventriculocordectomy Surgery: Experimental and Clinical Studies

Most surgeons perform a ventriculectomy or ventriculo-cordectomy in conjunction with laryngoplasty but there is controversy regarding the value of these procedures in eliminating the clinical signs of laryngeal hemiparesis/ hemiplegia (Shappell et al 1988, Bohanon et al 1990, Tetens et al 1996, Hawkins et al 1997, Kidd & Slone 2002, Brown et al 2003). It is generally accepted that ven-triculectomy/ventriculocordectomy significantly decreases the inspiratory noise associated with laryngeal hemi-paresis/hemiplegia but most experimental and clinical evidence leads to the conclusion that these procedures must be combined with prosthetic laryngoplasty to sig-nificantly improve airway mechanics and thus exercise performance (Brown et al 2003).

The effect of ventriculectomy on upper airway flow mechanics was investigated by Shappell et al (1988) in

five horses with laryngeal hemiplegia induced by recur-rent laryngeal neurectomy. The horses were exercised on a treadmill 30 days after left ventriculectomy, at which stage inspiratory impedance, airflow, and upper airway pressure were unchanged when compared to values obtained immediately after the neurectomy. The same horses were then treated with a laryngoplasty, and exer-cised on a treadmill 14 days later. Respiratory mechanics values were now found to be restored to baseline levels, leading the authors to conclude that any adhesions formed between the vocal cord and the laryngeal wall by the ventriculocordectomy were insufficient to prevent the dynamic collapse of the affected arytenoid which had occurred during exercise. Stabilization of the arytenoid by laryngoplasty, however, was able to prevent this collapse. One limitation of this study is that the design made it impossible to compare the effects of laryngoplasty alone to laryngoplasty plus ventriculectomy. Because the two procedures were not evaluated at the same time point post ventriculectomy, the 14 days of additional healing time for the ventriculectomy may also have contributed to the improved results found after laryngoplasty.

The absence of an effect of ventriculectomy on upper airway flow mechanics was confirmed in another experi-mental study (Tetens et al 1996) in exercising standard-breds with induced left laryngeal hemiplegia. Horses were treated with laryngoplasty alone or laryngoplasty plus ventriculocordectomy. Regardless of surgical treatment, inspiratory flow mechanics were restored to preneurectomy levels 60 days postoperatively. In contrast to Shappell's study (Shappell et al 1988), a bilateral ventriculocordec-tomy was performed in this study, and the cut edge of the vocal fold was sutured to the vestibular fold. Neither experimental study addressed the issue of inspiratory noise production. Another limitation is that the treadmill speed at which the horses were exercised in both cases was below the maximal speed at which a thoroughbred race-horse performs. It is not known whether the addition of ventriculectomy/ventriculocordectomy to laryngoplasty would improve airway mechanics under more intense exercise conditions.

In another experimental study of induced left laryngeal hemiplegia in standardbreds, Brown et al (2003) performed a bilateral ventriculocordectomy, and then measured upper airway pressures and respiratory sounds during treadmill exercise at maximum heart rate. These authors found that although airway pressures were still increased over preneurectomy levels at 90 days after ventriculo-cordectomy, the pressures had significantly decreased compared to immediately postneurectomy levels. This effect of ventriculocordectomy was not observed 30 days postoperatively, leading to the conclusion that scar forma-tion between the remnant of the vocal fold and the lateral laryngeal wall required up to 90 days, which may explain why earlier studies failed to demonstrate an effect of

ventriculocordectomy on airway pressure. In addition, these authors performed a spectrogram analysis of three inspiratory sound formants 30, 90, and 120 days after ventriculocordectomy. They found that the two formants constituting much of the inspiratory sound intensity returned to baseline values by 90 days postoperatively, and that there were no detectable abnormal respiratory noises present at that time.

The addition of a ventriculectomy procedure to laryngoplasty in racehorses does not appear to improve postoperative racing performance compared to laryngoplasty alone. Hawkins et al (1997) found that the performance index in thoroughbred and standardbred racehorses was similar, whether or not the horses received a ventriculectomy in addition to laryngoplasty. Likewise, there was no statistically significant difference in postoperative respiratory noise production, according to an owner/trainer survey (Hawkins et al 1997). In this study, however, there was a strong surgeon bias toward ventriculectomy, and only 19% of horses studied received laryngoplasty alone.

When a mixed group of horses (race and performance) were treated with ventriculocordectomy at the time of laryngoplasty, only 8.7% were reported to make a respiratory noise postoperatively (Kidd & Slone 2002). This is in contrast to the 47% of horses treated by the same surgeon that made a postoperative respiratory noise when treated by ventriculectomy alone (Russell & Slone 1994). On the other hand, in draft horses treated with ventriculectomy or ventriculocordectomy in conjunction with laryngoplasty, no difference in postoperative performance score was observed, whether or not the vocal cord was resected (Kraus et al 2003).

Ventriculectomy alone has been successful in treating clinical signs of laryngeal hemiplegia in draft horses, with signs of exercise intolerance and noise production eliminated in 80% of the treated horses (Bohanon et al 1990). However, another study of draft horses used for competitive pulling found that seven of 104 horses that had been previously treated with ventriculectomy or ventriculocordectomy alone before laryngoplasty were unable to work satisfactorily until they later received a prosthetic laryngoplasty (Kraus et al 2003). These authors noted that signs of laryngeal hemiplegia were alleviated in 72% of draft horses treated with ventriculectomy or ventriculocordectomy combined with laryngoplasty.

It is logical that the ventriculocordectomy alone without a laryngoplasty may best be reserved for horses that can maintain moderate abduction of the arytenoid during exercise. It may be difficult to ascertain which horses maintain abduction without a treadmill endoscopic examination, but performing a ventriculocordectomy does not preclude a laryngoplasty at a later date if the ventriculocordectomy is not effective. In a study by Barakzai and Dixon (2004), a sutured unilateral ventriculocordectomy

improved performance in 59% of the horses that had a mild degree of paresis of the left arytenoid, and so a laryngoplasty with its potential complications were consequently avoided.

REFERENCES

Baker GJ 1983 Laryngeal hemiplegia in the horse. Compendium on Continuing Education for the Practicing Veterinarian 5: S61–S67

Barakzai SZ, Dixon PM. 2004 Ventriculo-cordectomy for treatment of recurrent laryngeal neuropathy: 75 cases in a mixed population of horses. In: Dixon PM, Robinson, NE, Wade J (editors) Proceedings of Havemeyer Foundation Monograph Series No. 11: Equine Recurrent Laryngeal Neuropathy. R & W Publications, Newmarket, pp.71–73

Bayly WM, Grant BD, Modransky PD 1984 Arterial blood gas tensions during exercise in a horse with laryngeal hemiplegia before and after corrective surgery. Research in Veterinary Science 36: 256–258

Bohanon TC, Beard WL, Robertson JT 1990 Laryngeal hemiplegia in draft horses: a review of 27 cases. Veterinary Surgery 19: 456–459

Brown JA, Derksen FJ, Stick JA et al 2003 Ventriculocordectomy reduces respiratory noise in horses with laryngeal hemiplegia. Equine Veterinary Journal 35: 570–574

Brown JA, Derksen FJ, Stick JA et al 2004 Effect of laryngoplasty on respiratory noise reduction in horses with laryngeal hemiplegia. Equine Veterinary Journal 36: 420–425

Davenport CLM, Tulleners EP, Parente EJ 2001 The effect of recurrent laryngeal neurectomy in conjunction with laryngoplasty and unilateral ventriculocordectomy in thoroughbred racehorses. Veterinary Surgery 30: 417–421

Dean PW, Nelson JK, Schumacher J 1990 Effects of age and prosthesis material on in vitro cartilage retention of laryngoplasty prostheses in horses. American Journal of Veterinary Research 51: 114–117

Derksen FJ, Stick JA, Scott EA et al 1986 Effect of laryngeal hemiplegia and laryngoplasty on airway flow mechanics in exercising horses. American Journal of Veterinary Research 47: 16–20

Dixon PM, McGorum BC, Railton DI et al 2003a. Long-term survey of laryngoplasty and ventriculocordectomy in an older, mixed-breed population of 200 horses. Part 1: Maintenance of surgical arytenoid abduction and complications of surgery. Equine Veterinary Journal 35: 389–396

Dixon PM, McGorum BC, Railton DI et al 2003b. Long-term survey of laryngoplasty and ventriculocordectomy in an older, mixed-breed population of 200 horses. Part 2: Owners' assessment of the value of surgery. Equine Veterinary Journal 35: 397–401

Greet TRC, Baker GJ, Lee R 1979 The effect of laryngoplasty on pharyngeal function in the horse. Equine Veterinary Journal 11: 153–158

Guyton AC, Hall JE 2000 Pulmonary ventilation. In: Guyton AC, Hall JE (editors) Textbook of Medical Physiology, 10th edn. Saunders, Philadelphia, pp.441–442

Hackett RP, Ducharme NG, Fubini SL et al 1991 The reliability of endoscopic examination in assessment of arytenoid cartilage movement in horses. I. Subjective and objective laryngeal evaluation. Veterinary Surgery 20: 174–179

Hammer EJ, Tulleners EP, Parente EJ et al 1998 Video-endoscopic assessment of dynamic laryngeal function during exercise in horses with grade-III left laryngeal hemiparesis at rest: 26 cases (1992–1995). Journal of the American Veterinary Medical Association 212: 399–403

Hawkins JF, Tulleners EP, Ross MW et al 1997 Laryngoplasty with or without ventriculectomy for treatment of left laryngeal hemiplegia in 230 racehorses. Veterinary Surgery 26: 484–491

Herde I, Boening KJ, Sasse HHL 2001 Arytenoid cartilage retention of laryngoplasty in horses – in vitro assessment of effect of age, placement site, and implantation technique. Proceedings of the American Association of Equine Practitioners 47: 115–119

Johnson JH 1985 Laryngeal surgery: techniques and results. Proceedings of the American College of Veterinary Surgeons 13: 60

Kidd JA, Slone DE 2002 Treatment of laryngeal hemiplegia in horses by prosthetic laryngoplasty, ventriculectomy, and vocal cordectomy. Veterinary Record 150: 481–484

Kraus BM, Parente EJ, Tulleners EP 2003 Laryngoplasty with ventriculectomy or ventriculocordectomy in 104 draft horses. Veterinary Surgery 32: 530–538

Marks D, Mackay-Smith MP, Cushing LS et al 1970 Use of a prosthetic device for surgical correction of laryngeal hemiplegia in horses. Journal of the American Veterinary Medical Association 57: 157–163

Parente EJ 2004 Improvements in laryngoplasty. In: Dixon PM, Robinson, NE, Wade J (editors) Proceedings Havemeyer Foundation Monograph Series No. 11: Equine Recurrent Laryngeal Neuropathy. R & W Publications, Newmarket, pp.66–67

Raker CW 1975 Complications related to the insertion of a suture to retract the arytenoid cartilage to correct laryngeal hemiplegia in the horse. Archives of Veterinary Surgery 4: 64–66

Russell AP, Slone DE 1994 Performance analysis after prosthetic laryngoplasty and bilateral ventriculectomy for laryngeal hemiplegia in horses: 70 cases (1986–1991) Journal of the American Veterinary Medical Association 204: 1235–1241

Santschi EM 1999 Diagnosis and management of surgical site infection and antimicrobial prophylaxis. In: Auer JA, Stick JA (editors) Equine Surgery, 2nd edn. Saunders, Philadelphia, pp.55–60

Schumacher J, Wilson AM, Pardoe C et al 2000 In vitro evaluation of a novel prosthesis for laryngoplasty of horses with recurrent laryngeal neuropathy. Equine Veterinary Journal 32: 43–46

Shappell KK, Derksen FJ, Stick JA et al 1988 Effects of ventriculectomy, prosthetic laryngoplasty, and exercise on upper airway function in horses with induced left laryngeal hemiplegia. American Journal of Veterinary Research 49: 1760–1765

Speirs VC 1972 Abductor muscle prosthesis in the treatment of laryngeal hemiplegia in the horse. Australian Veterinary Journal 48: 251–254

Speirs VC, Bourke JM, Anderson GA 1983 Assessment of the efficacy of an abductor muscle prosthesis for treatment of laryngeal hemiplegia in horses. Australian Veterinary Journal 60: 294–299

Stick JA, Tulleners EP, Robertson JT et al 1999 The larynx. In: Auer JA, Stick JA (editors) Equine Surgery, 2nd edn. Saunders, Philadelphia, pp.355–360

Strand E, Martin GS, Haynes PF et al 2000 Career racing performance in Thoroughbreds treated with prosthetic laryngoplasty for laryngeal neuropathy: 52 cases (1981–1989). Journal of the American Veterinary Medical Association 217: 1689–1696

Tate LP, Corbett WT, Bishop BJ et al 1993 Blood gas tensions, acid–base status, heart rates, and venous profiles in exercising horses with laryngeal hemiplegia before and after corrective surgery. Veterinary Surgery 22: 177–183

Tetens J, Derksen FJ, Stick JA et al 1996 Efficacy of laryngoplasty with and without bilateral ventriculocordectomy as treatments for laryngeal hemiplegia in horses. American Journal of Veterinary Research 57: 1668–1673

Tulleners EP 1994 Management of failed laryngoplasty: 25 horses (1988–1993). Veterinary Surgery 23: 419

Williams JW, Pascoe JR, Meagher DM et al 1990a Effects of left recurrent laryngeal neurectomy, prosthetic laryngoplasty, and subtotal arytenoidectomy on upper airway pressure during maximal exertion. Veterinary Surgery 19: 136–141

Williams JW, Meagher DM, Pascoe JR et al 1990b Upper airway function during maximal exercise in horses with obstructive upper airway lesions: effect of surgical treatment. Veterinary Surgery 19: 142–147

36

Equine Laryngeal Reinnervation

Ian C Fulton

Introduction

Experiments with laryngeal reinnervation in animals were reported as early as 1946 (McCall & Hoerr 1946) and the nerve–muscle pedicle (NMP) graft technique of laryngeal reinnervation was first demonstrated successfully in dogs in 1973 (Hengerer & Tucker 1973). Laryngeal muscles that had been denervated for 6 months could be reinnervated using the NMP graft technique, with success of the reinnervation determined by visual and histological assessment (Lyons & Tucker 1974). Clinical use of the NMP graft method of laryngeal reinnervation in humans was first reported in 1976 in five patients with bilateral laryngeal paralysis (Tucker 1976). Success rates up to 90% with unilateral laryngeal paralysis and 80% for bilateral laryngeal paralysis have since been reported in humans (Tucker & Rusnov 1981).

Initial attempts at equine laryngeal reinnervation were conducted in ponies with experimentally induced left laryngeal hemiplegia by Ducharme et al (1989a,b,c). Using the first or second cervical nerve (Fig. 36.1), with

or without some attached omohyoideus muscle, as the donor, three techniques of laryngeal reinnervation were investigated: the NMP graft (Ducharme et al 1989a), nerve implantation (Ducharme et al 1989b) and nerve anastomosis (Ducharme et al 1989c). All three methods produced histological evidence of reinnervation. However, only the nerve anastomosis technique, joining the first cervical nerve to the distal segment of the left recurrent laryngeal nerve, produced significant clonic movement of the left arytenoid cartilage, although this was believed to be insufficient to allow maximal exercise to be performed.

Further research into equine laryngeal reinnervation focused on the NMP graft technique using standardbred horses with experimentally induced left laryngeal hemiplegia (Fulton et al 1991). That study not only identified successful reinnervation of the cricoarytenoideus dorsalis (CAD) muscle by histological examination (Fulton et al 1992), but also found that laryngeal reinnervation allowed upper airway function in horses with experimentally induced laryngeal hemiplegia to return to normal.
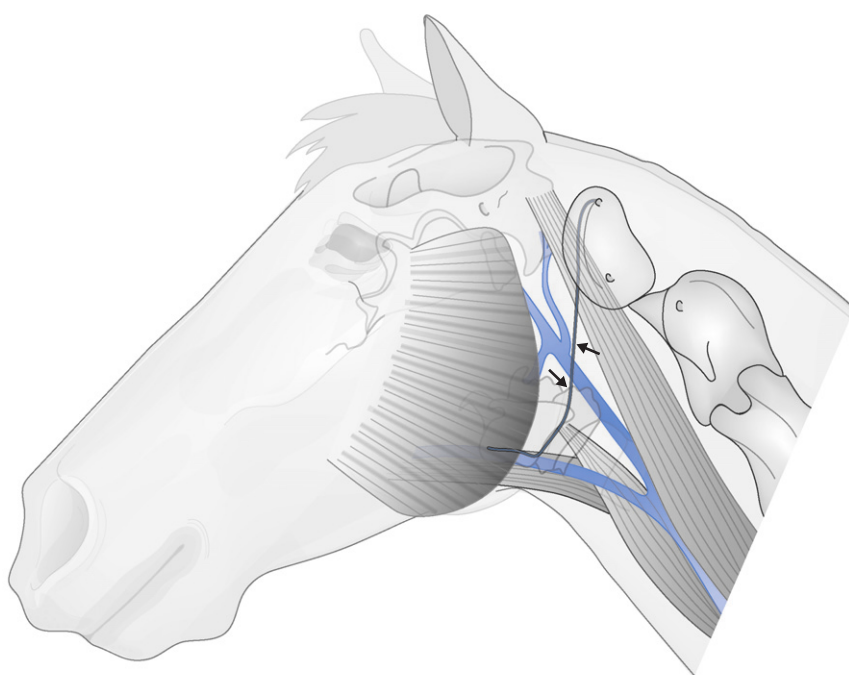
Fig. 36.1. Path of the first cervical nerve (arrows) as it travels from the alar foramen of the atlas and a common branching pattern before implanting into the omohyoideus muscle

While endoscopic evidence of CAD muscle reinnervation can be detected as early as 4 months after reinnervation surgery, return of full upper airway function during exercise can take up to 6–12 months. The major advantage of laryngeal reinnervation is the absence of permanent alteration to the laryngeal architecture resulting in minimal postoperative complications compared with prosthetic laryngoplasty.

Since 1991, the NMP graft technique has been used by the author in horses with grade 3 or grade 4 recurrent laryngeal neuropathy (RLN) (Hackett et al 1991). The author has operated on 182 horses in total, including 164 thoroughbreds, 11 standardbred and 7 warmblood horses.

Surgical Technique

Laryngeal reinnervation using the NMP graft technique is performed with the horse under general anesthesia in right lateral recumbency for cases of left RLN. A 15-cm skin incision is made along the ventral border of the linguofacial vein, with the center of this incision being in a direct line with the middle of the left wing of the atlas. The first cervical nerve exits from a foramen in the wing of the atlas and passes over the lateral aspect of the larynx before branching and entering the omohyoideus muscle (Fig. 36.1). Careful dissection of the subcutaneous tissues is performed, sometimes requiring an incision through the (subcutaneous) panniculus muscle to expose the linguofacial vein. A ventral branch of the linguofacial vein is often present near the midpoint of the exposed vein (Fig. 36.2). Double ligation of this branch is essential to allow further dissection to expose the body of the first cervical nerve. Once ligated, elevation of the linguofacial vein provides an easily identifiable plane of dissection between the omohyoideus muscle and the skin/subcutaneous fascia and linguofacial vein. Elevation of the skin/subcutaneous fascia and linguofacial vein can be maintained with a broad, malleable retractor.

The first cervical nerve is a 2–4 mm wide, white, band-like structure lying over the lateral aspect of the larynx. In 1- to 2-year-old horses, lymph nodes frequently overlie the nerve, increasing the difficulty of dissection of the distal nerve and its branches. Accurate dissection of the distal segment of the first cervical nerve from its loose connective tissue allows identification of two or three distal branches, which can be followed to their point of entry into the omohyoideus muscle. Elevation of the proximal aspect of the first cervical nerve with a small hooked retractor allows for fine dissection of the nerve from the surrounding connective tissue, which allows easier repositioning of the nerve over the dorsal aspect of the larynx, after the muscle pedicles have been created. When the sites of muscle insertion of the distal nerve branches have been exposed, the surgical field is flooded with local anesthetic. This reduces
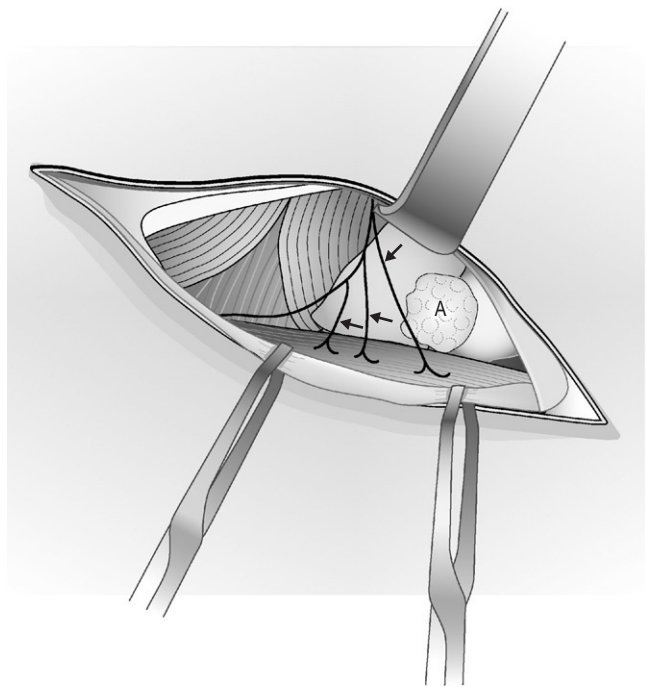
Fig. 36.2. The surgical approach with the linguofacial vein retracted dorsally, lymph nodes (A) which are often enlarged in yearlings, and the distal branches of the first cervical nerve (arrows).

markedly the vigorous contraction of the omohyoideus muscle that occurs when the nerve–muscle pedicle is formed, thus avoiding trauma to the nerve–muscle interface.

A small block of muscle, approximately 3 mm^3, is dissected from the omohyoideus muscle with the branch of the first cervical nerve attached. An abundance of motor endplates exists in the muscle fibers around the point of entry of the distal nerve branches (Fulton et al 2003). As many as five pedicles can be harvested for transplantation, depending on the number of nerve branches identified. A single cruciate suture of 2–0 polydioxanone is used to reduce hemorrhage from each omohyoideus muscle pedicle site. Branches of the first cervical nerve that need to be transected for dorsal repositioning of the nerve are cut as long as possible so that they can be directly used as donor nerves also (without muscle pedicle). Implantation of transected nerve ends into atrophied skeletal muscles can result in reinnervation via axonal sprouting (Hall et al 1988).

The bundle of nerve–muscle pedicles and transected nerve branches are carefully placed within the caudal aspect of the surgical field while the existing plane of dissection is continued proximally to expose the dorsal aspect of the larynx. Access to the left CAD muscle is improved by rotating the larynx laterally. A narrow, hooked retractor is placed through the septum between the left cricothyroideus and cricopharyngeus muscles and
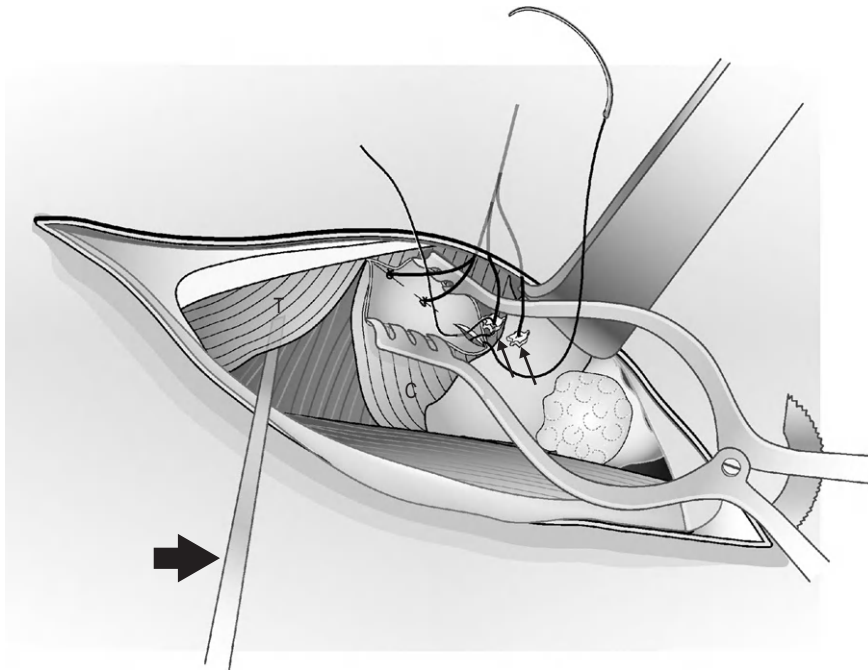
Fig. 36.3. A hooked retractor (large arrow) placed through the septum between the thyropharyngeus (T) and cricopharyngeus (C) muscles, is placed over the lateral wing of the thyroid cartilage to rotate the larynx exposing the dorsal aspect of the larynx. The Weitlaner retractor is placed with one arm in the dorsal border of the cricopharyngeus muscle and the other in the connective tissue overlying the larynx to expose the cricoarytenoideus dorsalis muscle. The nerve muscle pedicle grafts (small arrows) are sutured into slits created parallel with the atrophied fibers of the cricoarytenoideus dorsalis muscle.

is hooked over the wing of the left thyroid cartilage to help maintain this rotation (Fig. 36.3). Using a 20-mm endotracheal tube for anesthesia makes rotation of the larynx easier. Dissection through either the caudal portion of the cricopharyngeus muscle or the fascia overlying the CAD muscle will allow placement of a blunt tipped Weitlaner retractor to maintain access to the CAD muscle (Fig. 36.3). A plexus of vessels commonly overlies the CAD muscle and hemorrhage from these vessels reduces visibility of the CAD muscle and so of the implantation site. Control of such hemorrhage is often more easily achieved by pressure, using gauze swabs, rather than by ligation. The left CAD muscle in RLN horses is usually pale in color and shows various degrees of atrophy, with atrophy in grade 4 RLN horses more obvious than in grade 3 horses. In grade 3 RLN horses, the CAD often has a striped appearance, with pale denervated muscle fibers lying next to more normal appearing muscle fibers.

A long vascular forcep is used to create a deep, slit-like opening in the CAD muscle belly, parallel with the fibers of the CAD muscle, and the pedicle grafts are inserted into these individual pockets. A polydioxanone suture (4–0) is used to suture the pedicle graft into the pocket of the CAD muscle with minimal tension applied to the knot. The needle is passed from the muscle surface into the base of

the pockets and then retrieved. The needle is then passed through the muscle fibers of the pedicle graft (or through the perineurium of transected nerve branches) and then passed back down into the base of the pockets to exit through the muscle surface, next to the original insertion point. The pedicle grafts and nerve implants are distributed as evenly as possible in the muscle belly. However, the length of the nerve branches will dictate how far apart pedicles can be placed in relation to each other. After suturing the pedicles and nerve implants in place, the laryngeal retractor is removed to allow the larynx to rotate back to its natural position and an examination is made to ensure that there is not excessive tension on the first cervical nerve.

No attempt is made to close the potential dead space over the dorsal and lateral aspects of the larynx. The subcutaneous and subcuticular layers are closed using 2–0 polydioxanone and a simple interrupted 2–0 monofilament non-absorbable suture is used for the skin. During wound closure, examination for leaking lymphatic vessels (commonly seen in younger horses) should be made, especially along the border of the omohyoideus muscle. Failure to ligate these will lead to significant postoperative seroma formation. A stent bandage is sutured over the incision and a firm adhesive elastic bandage is placed around the neck,

in front of and behind the ears, to apply pressure over the incision area, and so help minimize seroma formation.

Ventriculectomy via laryngotomy has not been performed by the author immediately following the NMP graft, primarily to reduce possible disruption of the fine NMP grafts by further surgical manipulation of the larynx. Prior to 2000, ventriculectomy was only performed in horses deemed to be making excessive noise after surgery. Now, all horses undergo standing, unilateral laser vocal cordectomy the day following laryngeal reinnervation surgery.

Postoperative Management

Immediate postoperative care includes confinement for 2 weeks in stalls where horses cannot easily rub the neck incision. Procaine penicillin and gentamicin are routinely given for 4 days after surgery. These prophylactic broadspectrum antibiotics are used because of the loss of the protective mucosal tissue associated with the laser cordectomy. Phenylbutazone is administered for 7 days postoperatively. The neck bandage is removed after 48–72 h. Sutures are removed after 14 days.

After 2 weeks of stall rest, a further 2 weeks of confinement in a yard, followed by paddock turnout for 12 weeks is recommended. Training resumes 16 weeks postoperatively. This timing is based on the results of reinnervation studies in dogs (Hengerer & Tucker 1973), humans (Tucker 1976) and horses (Fulton et al 1991), where evidence of reinnervation has been identified 12–16 weeks postoperatively. When horses resume training, short episodes of fast exercise are introduced as early and as frequently as possible. Since the omohyoideus muscle is an accessory muscle of respiration, considerable respiratory effort must be induced to activate the first cervical nerve, hence the recommendation that horses gallop over 300–400 m every second day of training to help promote physiotherapy of the reinnervated muscle.

After 6 weeks of training we advise endoscopic assessment of the larynx. At rest, the function of the left arytenoid cartilage commonly appears identical to before the surgery; this is because the first cervical nerve is inactive at rest, resulting in absence of CAD muscle contraction. Two diagnostic reflexes have been identified to stimulate contraction of the omohyoideus muscle and also the newly innervated CAD in the standing horse at rest. The first requires stretching the head and neck upwards as high as possible while observing the larynx with the endoscope. If reinnervation has been successful there is often a spontaneous abductory movement of the left arytenoid cartilage as the head is elevated. The second reflex involves pulling back rapidly and firmly with a finger on the commissure of the lips. Again, a sudden abduction of the left arytenoid cartilage occurs if reinnervation has been successful. This reflex can be stimulated from the left or right side of the head. Move-

ment of the left arytenoid cartilage may only be seen once or twice during several attempts at stimulating either of these reflexes.

An abductory movement of the left arytenoid cartilage is a positive indicator of reinnervation of the CAD muscle. Once these movements have been identified, it is recommended that training continue as normal. If no evidence of movement is seen at the first revisit, turning the horse out again for another 8 weeks is advised. Another 6 weeks of the previously described training program is performed before further laryngeal assessment, usually at least 9 months following surgery. Some horses may take up to 12 months to show evidence of successful reinnervation. Information from direct stimulation of the first cervical nerve in horses suggests that reinnervation probably occurs in most horses by 4–5 months after surgery. The problem appears to be not whether reinnervation has occurred or not, but whether sufficient regeneration of the CAD muscle has occurred to provide stable abduction of the left arytenoid cartilage during exercise.

The best evidence of successful reinnervation in an individual horse is derived from treadmill endoscopy or treadmill upper airway flow mechanics studies but only a small number of research cases and a few clinical cases have undergone this evaluation. In treadmill studies, horses with movement of the left arytenoid cartilage visible at rest following NMP graft surgery can maintain a level of arytenoid abduction during vigorous exercise (Fulton et al 1991, Dart et al 2001). If movement of the affected arytenoid could not be demonstrated in the standing horse using the reflexes described earlier, dynamic collapse of the arytenoid cartilage would be expected to occur during exercise.

Surgical Complications

Complications are uncommon following laryngeal reinnervation surgery. The most frequent has been seroma formation 3–5 days following surgery, no doubt partly as a result of the potential dead space that exists dorsal and lateral to the larynx following an NMP graft procedure (also following laryngoplasty). The use of a sutured stent bandage and compressive neck bandage, described earlier, markedly reduces this complication. Seroma formation may also be predisposed to by inadvertently cutting lymphatic vessels during surgery. Many seromas resolve without intervention but some require open drainage and daily lavage and these horses are also given a short course of procaine penicillin. A small number of seromas become infected necessitating antibiotic treatment based on culture and sensitivity results. Based on the results of both clinical and research cases, these complications do not appear to compromise the success of the NMP graft (Fulton 1990).

Other infrequent complications include a single case of laryngeal obstruction, which occurred at recovery from

anesthesia and was successfully treated by passage of a nasotracheal tube. One horse developed a large hematoma 2 h after surgery that required the incision to be reopened in the standing horse and a blood vessel in the omohyoideus muscle to be ligated. Three horses that apparently had successful reinnervation have subsequently begun making noise and performing poorly again. One horse that had competed in high-level 3-day event competitions for 5 years suddenly began making a loud inspiratory noise and showing evidence of marked exercise intolerance as a result of sudden-onset deterioration in left laryngeal function.

Results of Clinical Cases

Thoroughbreds

To date, 164 thoroughbred horses have been operated on by the author using the NMP graft. Ninety-six of these horses had grade 4 RLN and 68 had grade 3 RLN. While many of the horses had been presented for poor performance associated with noise, a number of unraced horses had been presented after diagnosis of RLN following yearling sale endoscopy. To appraise success of the surgery technique, horses were placed into one of two groups.

- **group A** – raced prior to surgery – 76 horses
- **group B** – unraced prior to surgery – 88 horses.

Group A
To date 65 of the 76 horses aged 2–6 years (mean 3.2 years) have had long-term assessment and 86% had evidence of reinnervation as described earlier. Ninety-five per cent started in one or more races and Group A horses raced a mean of 12.5 times each. Fifty-four per cent (35 of 65) won one or more races after surgery. The average length of time from surgery to their first race was 7.5 months for grade 3 (Hackett at al 1991; i.e. laryngeal asymmetry) horses, and 8.6 months for grade 4 (Hackett et al 1991; i.e. hemiplegic) horses.

Objective assessment of the success of the NMP graft was undertaken by calculating four parameters before and after surgery. A performance ranking for both total starts and per start was calculated by allocating points, dependent on race position at finish as well as total prize money and prize money per start. Fifty-eight per cent (38 of 65) of horses had an improved total performance ranking and performance ranking per start after surgery. Fifty-one per cent (33 of 65) of horses had higher total prize money after surgery while 57% (37 of 65) earned more money per start after surgery. Twenty-two horses raced and won over the same distance before and after surgery allowing comparison of race times. Eighty-six per cent (19 of 22) of these horses had better race times over the same distance after surgery.

Group B
Of the 88 horses operated on, 65, aged 1–3 years (mean 1.8 years) were available for long-term follow-up. Twenty of these unraced horses were considered failures based on their inability to start a race. Seventy-six per cent (43 of 65) had evidence of laryngeal reinnervation based on endoscopic assessment. Sixty-six per cent also started in at least one race, with these horses racing a mean of 10.6 times. Of the 43 horses that raced, 27 (63%) won at least one race. Since the Group B horses had no prior performance level to allow for performance comparisons after surgery, the prize money earned per start was compared with the national average for the same age group. Those horses treated with an NMP graft for left RLN earned a similar amount of prize money per start in all age groups except as 3-year-olds, when NMP graft horses earned considerably more per start than the national average.

After laryngeal reinnervation many horses will make an audible inspiratory "noise" early in training. However, in horses with successful reinnervation this "noise" often reduces progressively as training continues. This is believed to be the result of a time-related progressive increase in muscle regeneration of the reinnervated CAD. Recent information suggests that respiratory noise during exercise cannot be easily equated to degree of airway obstruction (Brown et al 2004) so trainers are encouraged to continue training even if noise is heard early in the retraining period.

Standardbreds

A total of 11 standardbred horses, all with grade 4 LRN, have been treated using an NMP graft. Six of the horses raced following surgery, three were retired with unrelated problems, one died and one was considered a failure. Three of the six horses earned more money in total after surgery than before (mean of nine races presurgery and of 15 races postsurgery). The trainers indicated that in five of these six horses they were satisfied with the decrease in noise and improvement in racing performance.

Warmbloods

Seven horses with grade 4 LRN have been treated with the NMP graft, without vocal cordectomy being performed. Five of these seven horses have performed at a higher level after surgery. A horse that was 12 years old at the time of surgery and had been affected with left RLN for 4 years, had endoscopic evidence of reinnervation 5 months after surgery. All five horses have been reported to have a markedly reduced noise level during exercise.

In another report, 18 eventing and hunt horses received an NMP graft and 14 were available for follow-up. Six horses (43%) were considered failures and eight (57%) were subjectively judged by their owners to have improved performance (Tyler 2000).

Overview

The NMP graft technique can be used to successfully reinnervate the CAD muscle of horses with RLN. Seventy-six per cent of unraced horses and 84% of raced horses demonstrated endoscopic evidence of CAD reinnervation. Assessment of results indicates that the NMP graft technique is as efficacious as prosthetic laryngoplasty when racing performance is measured. In one study, 77% of horses raced after prosthetic laryngoplasty with 56% racing at an improved level (Hawkins et al 1997). Another study found that 94% of horses raced after prosthetic laryngoplasty, with 45% demonstrating improved performance (Strand et al 2000). Following treatment of previously raced horses with the NMP graft, 95% went on to race one or more times; 58% had improved performance scores and 57% earned more prize money per start after surgery. The time period from surgery to first race is longer following NMP graft than prosthetic laryngoplasty. The age, breed, and use of the horse will determine if sufficient time is available to wait for muscle regeneration following laryngeal reinnervation. The primary advantage of the NMP graft technique is the absence of potentially chronically debilitating complications that can occur after prosthetic laryngoplasty.

The Future

Based on results from a pilot study (Fulton et al 2003), external nerve stimulation has the potential to decrease time from reinnervation surgery to the first race and more importantly, to increase the strength of the reinnervated CAD muscle by more effective physiotherapy via nerve stimulation. Other research is currently investigating the use of growth factors that may increase the speed of reinnervation of the CAD muscle. In people, nerve anastomosis is now considered by some surgeons to be the method of choice for unilateral vocal cord paralysis (Crumley 1991). Given the results in a small group of ponies (Ducharme et al 1989c), nerve anastomosis may be worthy of further research.

REFERENCES

Brown JA, Derksen FJ, Stick JA et al 2004 Effect of laryngoplasty on respiratory noise reduction in horses with laryngeal hemiplegia. Equine Veterinary Journal 36: 420–425

Crumley RL 1991 Update: ansa cervicalis to recurrent laryngeal nerve anastomosis for unilateral laryngeal paralysis. Laryngoscope 101: 384–388

Dart AJ, Dowling BA, Hodgson DR et al 2001 Evaluation of high-speed treadmill videoendoscopy for diagnosis of upper respiratory tract dysfunction in horses. Australian Veterinary Journal 79: 109–112

Ducharme NG, Horney FD, Partlow GD et al 1989a Attempts to restore abduction of the paralysed equine arytenoid cartilage I. Nerve-muscle pedicle transplants. Canadian Journal of Veterinary Research 53: 202–210

Ducharme NG, Horney FD, Hullard TJ et al 1989b Attempts to restore abduction of the paralysed equine arytenoid cartilage II. Nerve implantation (Pilot study). Canadian Journal of Veterinary Research 53: 210–215

Ducharme NG, Viel L, Partlow GD et al 1989c Attempts to restore abduction of the paralysed equine arytenoid cartilage III. Nerve anastomosis. Canadian Journal of Veterinary Research 53: 216–223

Fulton IC 1990 The effectiveness of a nerve muscle pedicle graft for the treatment of equine left laryngeal hemiplegia. Thesis, Michigan State University

Fulton IC, Derksen FJ, Stick JA et al 1991 Treatment of left laryngeal hemiplegia in standardbreds using a nerve muscle pedicle graft. American Journal of Veterinary Research 52: 1461–1467

Fulton IC, Derksen FJ, Stick JA et al 1992 Histologic evaluation of nerve muscle pedicle graft as a treatment for left laryngeal hemiplegia in Standardbreds. American Journal of Veterinary Research 53: 592–595

Fulton IC, Stick JA, Derksen FJ 2003 Laryngeal reinnervation in the horse. Veterinary Clinics of North America; Equine Practice 19: 189–208

Hackett R, Ducharme N, Fubini S et al 1991 The reliability of endoscopic examination in assessment of arytenoid cartilage movement in horses. Part 1. Subjective and objective laryngeal evaluation. Veterinary Surgery 20: 174–179

Hall SJ, Trachy RE, Cummings CW 1988 Facial muscle reinnervation: A comparison of neuromuscular pedicle with direct implant. Annals of Otology, Rhinology and Laryngology 97: 229–233

Hawkins JF, Tulleners EP, Ross MW et al 1997 Laryngoplasty with or without ventriculocordectomy for treatment of left laryngeal hemiplegia in 230 racehorses. Veterinary Surgery 26: 484–491

Hengerer AS, Tucker HM 1973 Restoration of abduction in the paralysed canine vocal fold. Archives of Otolaryngology 97: 247–250

Lyons RM, Tucker HM 1974 Delayed restoration of abduction in the paralyzed canine larynx. Archives of Otolaryngology 100: 176–179

McCall JW, Hoerr NL 1946 Reinnervation of a paralysed vocal cord. Laryngoscope 56: 527–535

Strand E, Martin GS, Haynes PF et al 2000 Career racing performance in Thoroughbreds treated with prosthetic laryngoplasty for laryngeal neuropathy: 52 cases (1981–1989). Journal of the American Veterinary Medical Association 217: 1689–1696

Tucker HM 1976 Human laryngeal reinnervation. Laryngoscope 86: 769–779

Tucker HM, Rusnov M 1981 Laryngeal reinnervation for unilateral vocal cord paralysis: long term results. Annals of Otolaryngology 90: 457–459

Tyler R 2000 Experiences with the nerve muscle pedicle graft. Proceedings of the British Equine Veterinary Association Congress. Equine Veterinary Journal Ltd, Newmarket pp.86–87

37

Arytenoid Chondropathy

Eric J Parente

Arytenoid chondropathy is a disease process affecting one or both arytenoid laryngeal cartilages that may be secondary to inflammation and swelling of the arytenoid cartilage. This disorder can cause decreased mobility and varying degrees of respiratory obstruction. The chondropathy is likely the consequence of ascending inflammation and/or infection into the body of the arytenoid cartilage through a mucosal disruption on the axial side of the cartilage at the level of the glottis. Potential causes of this disorder are discussed in Chapter 38. Arytenoid chondropathy is more commonly seen in the racing thoroughbred but can be observed in all breeds and ages.

Diagnosis

Clinical history, palpation of the larynx, and resting endoscopy are used to diagnose arytenoid chondropathy. The findings will vary slightly, depending on whether the chondropathy is diagnosed in the acute, subacute, or chronic stage. In the acute stage, dramatic laryngeal and perilaryngeal inflammation and edema are seen on resting endoscopy. Infrequently there will be an associated cellulitis in the perilaryngeal region and palpation of the larynx may be difficult. Horses may be presented as emergencies because of severe respiratory distress and stridor, and they often have a history of recent fast galloping before the flare-up. Mucosal abnormalities may have been observed at previous endoscopic examinations but may not have caused any clinical abnormalities until the acute stage. The severity of the mucosal swelling during the acute phase prohibits accurate assessment of the more long-term conformational changes to the cartilage and may even make it difficult to determine which arytenoid is affected. The final shape and function of the arytenoid cannot be determined until after the completion of aggressive medical treatment.

While it is simple to make a diagnosis of arytenoid chondrosis in the acute phase, it is sometimes more difficult to do so in chronic cases. In the chronic stages, there is no laryngeal edema but abnormalities of the shape of the corniculate process can be seen endoscopically. Cartilaginous protrusions are often observed endoscopically on the axial aspect of the arytenoid, just dorsal to the vocal process. There are often coexisting superficial ulcerated ("kissing") lesions, or granulation tissue, on the axial aspect of the apposing arytenoid, that may appear more significant than the lesion on the affected arytenoid. Rarely, lesions are absent at the axial aspect of the arytenoid but more caudally positioned ulcerative lesions are present within the laryngeal lumen that are difficult to observe because of swallowing (and subsequent glottic closure) during the examination. Affected horses may have a history of poor performance and exercise-induced respiratory noise similar to that of a "roarer" but horses usually make no abnormal noises at rest.

There is usually some degree of concurrent compromised abduction of the affected arytenoid. Laryngeal hemiplegia may have preceded the chondrosis, further contributing to the reduced abduction. The concurrent swelling of the arytenoid cartilage results in mechanical restriction of the movement of the arytenoid because it is laterally limited by the wing of the thyroid cartilage. Observation of an immobile cartilage may lead to an erroneous diagnosis of a typical case of recurrent laryngeal neuropathy (RLN); consequently it is essential to differentiate laryngeal paralysis from a structurally abnormal chondritic arytenoid or cricopharyngeal laryngeal dysplasia (fourth brachial arch defect) before considering treatments. These disorders should be differentiated by resting endoscopy and palpation of the larynx. An immobile hemiplegic arytenoid will normally have no space between it and the palatopharyngeal arch, making the rim of the arch difficult to visualize on resting endoscopy. If there is a space present lateral to the corniculate process, and the palatopharyngeal arch can be clearly seen, this indicates that a structural enlargement of the arytenoid is pushing the palatopharyngeal arch laterally (Fig. 37.1). External laryngeal palpation may also help diagnose arytenoid chondropathy. While a horse with RLN should have a very prominent muscular process, the horse with arytenoid chondropathy will have a less prominent and less defined muscular process. Obtaining an accurate diagnosis is critical to ensure that a laryngoplasty is not mistakenly attempted on a horse with arytenoid chondropathy.
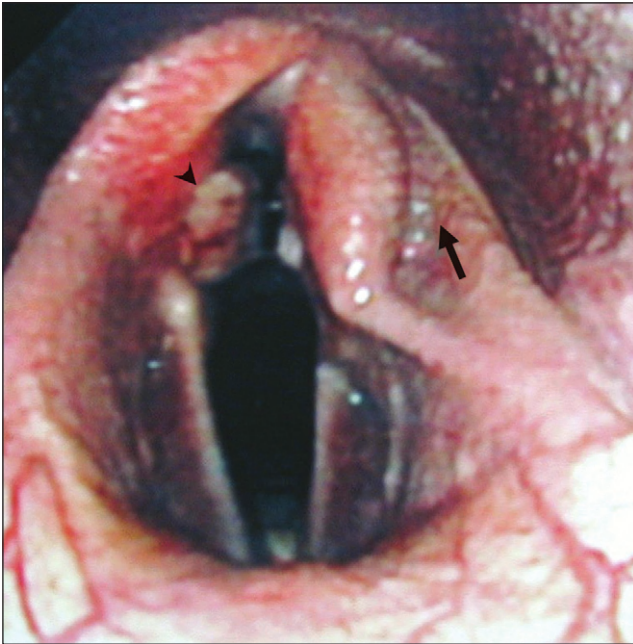
Fig. 37.1. Chondropathy of the left arytenoid. Note the abnormal corniculate shape, cartilaginous projection on left arytenoid, abnormal appearance of palatopharyngeal arch behind left arytenoid (large arrow), and kissing granulation tissue on right arytenoid (arrowhead).

Fig. 37.2. Arytenoid chondritis in the acute stage with excessive edema limiting the ability to assess eventual arytenoid structure and function.

Treatment

Medical treatment

In the acute inflammatory stage, arytenoid chondropathy should be treated aggressively with intravenous antimicrobial and anti-inflammatory drugs (Fig. 37.2). It is difficult to obtain a representative bacterial culture to target treatment so broad-spectrum antimicrobials are commonly used, such as potassium penicillin (22,000 IU/kg four times a day) and gentamicin (6.6 mg/kg once a day), in addition to phenylbutazone (4.4 mg/kg twice a day), and dexamethasone (0.025–0.05 mg/kg once a day) intravenously. It is uncommon to have to perform an emergency tracheostomy but the horse should be kept in a quiet environment and monitored closely because respiratory distress can be induced with excitement. An emergency tracheotomy kit should be available by the stall. Tracheotomy is reserved for horses that cannot be maintained in a quiet environment and those which have respiratory stridor at rest.

Within a few days of initiating treatment there is usually dramatic improvement, with a decrease in the laryngeal soft tissue swelling observed endoscopically (Fig. 37.3). However, surgical treatment should be delayed, because many horses will continue to improve for 30 days with further rest and oral antimicrobial treatment. There are occasional cases that improve so dramatically that they never require surgery. Following 1 month of antimicrobial

Fig. 37.3. The larynx of the same horse as in Fig. 37.2 after a 1-week course of intravenous antimicrobial and anti-inflammatory drugs.

treatment and stall rest, endoscopic re-evaluation and decisions for possible further treatment can be made. Surgical treatment may be initiated earlier in those horses which do not show dramatic improvement within the first few days of intravenous antimicrobial treatment, those

with gross purulent material exiting their arytenoid, and those with swelling of the laryngeal saccule (which indicates accumulation of purulent material abaxial to the arytenoid).

The decision to pursue surgery after intensive medical management is dependent on the degree of response to therapy and the intended use of the horse. Several horses have gone back to racing after medical treatment alone, despite the corniculate processes of their arytenoids having a slightly abnormal endoscopic appearance. However, all of these horses could maintain good abduction bilaterally (E. Parente, personal observation). Horses that have granulation tissue persisting on their arytenoid following the above medical management are best treated by laser excision of this tissue followed by rest. Several weeks are required for the mucosa to cover the defect before resuming exercise. If laryngeal function is still compromised beyond what is necessary for the horse's athletic purpose, a partial arytenoidectomy should be considered. Before this surgery is pursued for an athletic horse, it is essential to determine that the normal-looking arytenoid has full abductory function. The prognosis is significantly worse for bilateral disease even after unilateral partial arytenoidectomy (Tulleners 1988a, Parente 2003).

Surgical treatment

Different forms of arytenoidectomies have been described (Belknap et al 1990, Tulleners 1990, Hay et al 1993), but partial arytenoidectomy has been shown to provide the least postoperative obstruction (Belknap et al 1990, Williams et al 1990, Lumsden et al 1994). A temporary tracheotomy is required to administer the inhalant anesthesia for a partial arytenoidectomy, because the surgery is performed through a laryngotomy. Usually the glottis is wide enough to permit passage of an endotracheal tube, so the tracheotomy can be performed under general anesthesia. The endotracheal tube is then switched to the tracheotomy site so it does not pass through the larynx. A cleaner, smaller tracheotomy can be performed in this manner. However, if there is any risk that endotracheal intubation may be problematic, then the tracheostomy should be performed before induction of general anesthesia. When performing a tracheotomy under general anesthesia, care should be taken to avoid placing the tracheotomy too far cranially because the relative position is deceiving when the horse is under anesthesia with the head extended. If the tracheotomy is placed too far cranially it may become obstructed during recovery.

To perform an arytenoidectomy, a standard laryngotomy approach (Chapter 35) is first made. A headlamp is useful for illumination while working within the larynx, and placing an endoscope transnasally with its tip placed in front of the larynx can also provide supplemental light. It is arguable whether it is worthwhile attempting to salvage a mucosal flap on the axial side of the arytenoid to achieve primary mucosal closure after the arytenoid is removed (Tulleners et al 1988b, Barnes et al 2004) but it is recommended by this author because it reduces the postoperative development of granulation tissue.

To form the mucosal flap, dorsoventral mucosal incisions are made at the caudal border of the arytenoid, and at its rostral border just caudal to the corniculate. These incisions are connected with a horizontal incision along the ventral border of the arytenoid. The mucosa is slowly dissected free from the arytenoid and is left attached dorsally. The abaxial border of the arytenoid is then freed of its muscular attachments using primarily blunt dissection to minimize hemorrhage. The muscular process is isolated and transected. The arytenoid is then elevated with Allis forceps, and freed completely by cutting the remaining corniculate mucosa rostrally and sectioning any remaining dorsal attachments and the cricoarytenoid joint capsule caudally. Mucosae are positioned together to plan their closure, and excess mucosa is excised. The caudal edge of the mucosal flap is reapposed to the laryngeal mucosa in a simple continuous pattern with 3–0 absorbable suture, in a dorsal to ventral direction. The rostral edge of the mucosal flap is apposed similarly to the remaining mucosa lying abaxial to the corniculate, in a line parallel with the caudal edge. The most difficult part to suture is the dorsal aspect of the vertical incisions, and closure of this area is important to prevent the formation of postoperative granulation tissue. The ventral aspect of the mucosal wound is left open for drainage. Bleeding should be minimal once the mucosal edges are apposed. After closing the mucosal flap the vocal cord and ventricle are removed. This leaves an opening at the ventral aspect of the arytenoidectomy site for drainage of submucosal hemorrhage or blood clots lying abaxial to the final mucosal flap. Granulating "kissing" lesions on the opposite arytenoid should be debrided at this time. If extensive purulent material is present abaxial to the arytenoid, or if there is excessive loss of mucosa, a primary closure is not performed. At the conclusion of surgery, the endotracheal tube is replaced with an equivalent sized tracheotomy tube for recovery from general anesthesia.

An endoscopic examination should be performed the morning following surgery (Fig. 37.4). A moderate opening to the glottis should be seen, and blocking the tracheotomy tube while watching the horse's respiratory effort can be used to assess if the horse can breathe easily through its larynx. The tracheotomy tube can usually be removed at this time. The horse should be maintained on perioperative antimicrobials and anti-inflammatories for 1 week and maintained in a stall for 1 month with only hand grazing. The tracheotomy and laryngotomy sites are left open to heal by second intention. All feeding should be from the ground to minimize the risk of aspiration.
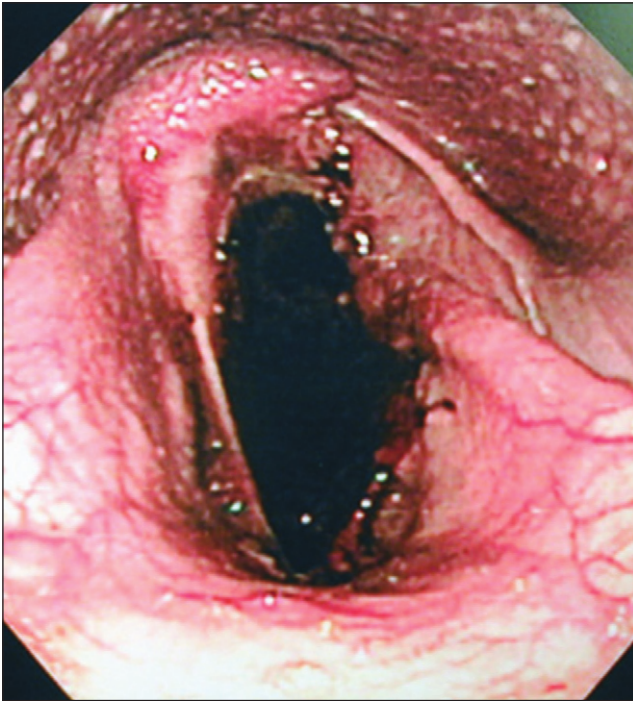
Fig. 37.4. Endoscopic examination the day following left partial arytenoidectomy. The mucosal flap is sutured in this case and there is ample glottic space enabling removal of the tracheotomy tube.

Fig. 37.5. The final appearance of a successful left partial arytenoidectomy.

Endoscopy should be performed 1 month following surgery to examine for the presence of intralaryngeal granulation tissue. If present, it can be transendoscopically removed with a laser on an outpatient basis. Once there is complete mucosal healing, the horse should receive one further month of rest before resuming exercise (Fig. 37.5).

Prognosis

The reported prognosis for arytenoid chondropathy following treatment is extremely variable, and is dependent upon the extent and duration of the disease before treatment, and to the actual type of treatment performed (Speirs 1986, Tulleners 1988a, Dean & Cohen 1990, Parente 2003, Barnes et al 2004). Horses that have a chronic, non-active chondropathy can function quite adequately at low levels of exercise without surgical intervention. Horses with concurrent severe hemiplegia or those with more severe chondropathy will likely require surgical intervention to provide an adequate airway for any athletic function. After unilateral partial arytenoidectomy, most racehorses will return to athletic function and have significant earnings (Parente 2003). In contrast, horses with severe bilateral disease are unlikely to return to any significant athletic function (Parente 2003).

Complications

There are several potential complications of this surgery. The most common complication after arytenoidectomy is the presence of aspiration and coughing. The risk may be dramatically decreased by less traumatic dissection of the arytenoid from the lateral musculature at the time of surgery, because many of these adductor muscle bellies play a protective role by narrowing the glottis during swallowing, even in the absence of the arytenoid body. Somewhat surprisingly, a minor degree of aspiration does not preclude a successful racing career. Another complication is the development of postoperative granulation tissue, which occurs in approximately 15% of the horses, or the presence of excessive residual mucosa within the glottis (Parente 2003). Granulation tissue, or excessive mucosa, should be removed using a transendoscopic laser at 1 month after arytenoidectomy and the surgical site will then often resolve without further complications. If not removed in its early stages, intraluminal granulation tissue may mineralize and make subsequent excision much more difficult.

Postoperative respiratory noise during exercise can also occur following arytenoidectomy. The presence of postoperative respiratory noise is most likely from residual arytenoid/corniculate mucosa impinging on the glottis during fast work, provided the affected cartilage has been removed and no concurrent disorders (e.g. RLN) are

present. There appear to be no reports on results of partial arytenoidectomy in show horses where noise would be a fault, but generally, the prognosis is considered worse for abnormal noise production than for exercise performance. A treadmill examination may be beneficial to determine the source of any noise. Inspiratory axial deviation of the adjacent aryepiglottic fold that is no longer held abaxial by the corniculate process of the arytenoid is another likely cause of abnormal noise after partial arytenoidectomy. This tissue, or any excessive residual arytenoid mucosa impinging on the airway, can be identified by high-speed treadmill endoscopy and the offending tissue removed as needed.

REFERENCES

Barnes AJ, Slone DE, Lynch TM 2004 Performance after partial arytenoidectomy without mucosal closure in 27 thoroughbred racehorses. Veterinary Surgery 33: 398–403

Belknap JK, Derksen FJ, Nickels FA et al 1990 Failure of subtotal arytenoidectomy to improve upper airway flow mechanics in exercising standardbreds with induced laryngeal hemiplegia. American Journal of Veterinary Research 51: 1481–1487

Dean PW, Cohen ND 1990 Arytenoidectomy for advanced unilateral chondropathy with accompanying lesions. Veterinary Surgery 19: 364–370

Hay WP, Tulleners EP, Ducharme NG 1993 Partial arytenoidectomy in the horse using an extralaryngeal approach. Veterinary Surgery 22: 50–56

Lumsden JM, Derksen FJ, Stick JA et al 1994 An evaluation of partial arytenoidectomy as a treatment for equine laryngeal hemiplegia. Equine Veterinary Journal 26: 125–129

Parente EJ 2003 Partial arytenoidectomy for treatment of failed laryngoplasty or arytenoid chondritis in racehorses. Proceedings of the 49th Annual Convention of American Association of Equine Practitioners, pp.373–376

Speirs VC 1986 Partial arytenoidectomy in horses. Veterinary Surgery 15: 316–320

Tulleners EP, Harrison IW, Raker CW 1988a Management of arytenoid chondropathy and failed laryngoplasty in horses: 75 cases (1979–1985). Journal of the American Veterinary Medical Association 192: 670–675

Tulleners EP, Harrison IW, Mann P et al 1988b Partial arytenoidectomy in the horse with and without mucosal closure. Veterinary Surgery 17: 252–257

Tulleners EP 1990 Arytenoidectomy. In: White NA, Moore JN (editors) Current Practice of Equine Surgery. Lippincott, Philadelphia, pp.255–261

Williams JW, Pascoe JR, Meagher DM et al 1990 Effects of left recurrent laryngeal neurectomy, prosthetic laryngoplasty, and subtotal arytenoidectomy on upper airway pressure during maximal exertion. Veterinary Surgery 19: 136–141

Miscellaneous Disorders of the Larynx and Epiglottis

Padraic M Dixon

Congenital Defects of the Larynx

A number of congenital defects of the equine larynx (including the epiglottis) have been described, with cricopharyngeal–laryngeal dysplasia (fourth branchial arch defect) being most frequently recorded. This disorder is described in detail in Chapter 31. Subepiglottic cysts are also relatively common in standardbreds (Stick & Boles 1980) and are described in Chapter 29. Hypoplasia of the epiglottis has also been recorded in horses (Rooney & Robertson 1996). This can be of varying degrees from aplasia, or gross hypoplasia, as illustrated by Lane (1997), to slight shortening of the epiglottis as determined by lateral pharyngeal radiography (Linford et al 1983, Tulleners 1991a). Horses with an abnormally shortened epiglottis are prone to dorsal displacement of the soft palate, as described in Chapter 29. Whitton & Kannegieter (1995) described four cases of epiglottic deformity in adult horses, two of which appeared to have a developmental deformity of the mid-epiglottic region, with a deep unilateral infolding in one, and axial folding of both epiglottic borders in the other. Yarborough et al (1999) described persistent frenulum of the epiglottis in four foals.

Lees et al (1987) recorded a developmental laryngeal web (stenosis) in a 10-day-old quarterhorse foal with respiratory distress and dysphagia. A thick band of fibrous tissue adjoined both vocal folds, occluding the ventral aspect of the rima glottidis. The foal also had a short, vertically angulated epiglottis, permanent soft palate displacement that caused dysphagia, and abnormally shaped arytenoids and lateral ventricles. The foal was euthanased and necropsy showed a hypoplastic larynx with arytenoids that could not abduct because of a thick, mucosa-covered, fibrous web. Byars (2004) described a neonatal foal with marked laryngeal obstruction caused by a congenital cyst on the right aryepiglottic fold that was successfully excised using a diode laser. A further congenital laryngeal deformity recorded in the horse is paralaryngeal accessory bronchial cyst (Baxter et al 1992), a deformity that has been well described in humans. Whilst performing laryngoplasty in a 3-year-old thoroughbred with left-sided recurrent laryngeal neuropathy, Baxter et al (1992) found a fluid-filled cyst, 3–4 cm in diameter, lying between the medial aspect of a laterally displaced thyroid cartilage and the lateral aspect of deformed cricoid and arytenoid cartilages. Following surgical excision of the cyst and laryngoplasty, the horse remained asymptomatic. Histology of the excised cyst showed it to have a mucosal lining with features of a paralaryngeal accessory bronchial cyst (i.e. lined with tracheal/bronchial type mucosa).

A wide range of human congenital laryngeal defects has been described. Laryngomalacia, a disorder in which the laryngeal inlet collapses on inspiration, accounts for 60–70% of all congenital laryngeal defects (Lusk 1991), but this defect does not appear to have been described in horses. Other common human congenital laryngeal defects include absent or bifid epiglottis, laryngeal cysts, laryngeal clefts (deficient separation from the esophagus) and laryngeal paralysis as a result of congenital central nervous system disorders, including hydrocephalus, meningocele and nucleus ambiguus dysgenesis (Lusk 1991), none of which have been reported in the horse.

Bilateral Arytenoid Cartilage and Vocal Fold Collapse

Bilateral arytenoid cartilage and vocal fold collapse have been recorded in Norwegian coldblooded trotter racehorses that have no evidence of neurological laryngeal disease (Strand et al 2004). This phenomenon could also account for some of the undiagnosed abnormal noises that are recorded in other breeds that are exercised with head flexion. Diagnosis of this disorder is by high-speed treadmill video-endoscopy, while the horse is wearing appropriate tack (as discussed in Chapter 16).

Hyperkalemic periodic paresis, an inherited generalized myasthenic disorder of quarterhorses, can also cause intermittent upper airway obstruction, attributed to laryngeal spasm and nasopharyngeal muscle dysfunction (Carr et al 1996). More generalized skeletal muscle dysfunction may also be present, and the disorder may respond to appropriate medical treatment.

External Laryngeal Trauma

The equine larynx is anatomically well protected from trauma and consequently severe external laryngeal trauma rarely occurs in this species, as compared to humans where laryngeal damage is common in car accidents and with assaults such as knife wounds and blows (Miles-Foxen 1980, Sanders & Billers 1991). Laryngeal trauma is also well recorded in dogs, usually as a result of choke chain abuse and gunshot wounds (Manus 1965, Nelson & Wykes 1985). Occasionally a horse may damage its larynx after trapping its head, such as when trying to feed from sheep or cattle feeders. If a horse escapes from its rider and gets its bridle or reins caught in a fence or other fixed structure, it can severely traumatize its neck and larynx trying to free itself.

External laryngeal trauma can cause inflammation and edema of the larynx, which can result in fatal airway obstruction. Cartilage fractures and submucosal hemorrhage can follow direct laryngeal trauma (Miles-Foxen 1980), which can also lead to acute respiratory obstruction. In such cases, an immediate temporary tracheotomy should be performed below the level of any intercurrently damaged trachea. Non-steroidal anti-inflammatory drugs and/or corticosteroids should be administered, along with antibiotics if there are open wounds. Damage to the overlying skin or internally to the laryngeal mucosa caused by cartilage fractures will invariably lead to emphysema of the laryngeal region that may spread extensively.

Of greatest long-term concern with laryngeal trauma is the presence of laryngeal mucosal damage. Extensive mucosal loss, particularly in the presence of concurrent fractures of the laryngeal cartilages, may lead to extensive granulation tissue formation in the larynx and possibly to permanent fibrotic webs (laryngeal stenosis) (Dixon et al 1994), as described later in this chapter.

If fractures of the laryngeal cartilages are non-displaced and the overlying mucosa and skin are intact, no long-term laryngeal obstruction should ensue. However, if the cricoid cartilage is fractured and displaced, some degree of permanent laryngeal obstruction is likely, because this ring-shaped cartilage is the scaffold of the larynx. Accurate assessment of traumatic laryngeal cartilage damage is difficult, especially when extensive perilaryngeal swelling and emphysema are present. Assessment is best performed by computed tomography or magnetic resonance imaging scans, but if these imaging modalities are unavailable, assessment by clinical, radiographic, ultrasonographic and even surgical exploration may be necessary. Bearing in mind the need to preserve the laryngeal mucosa, displaced cartilage fragments can be realigned and stabilized with steel sutures, whilst loose and necrotic tissue should be resected. Following penetrating wounds of the larynx, chronic chondropathy with cartilage necrosis are common sequelae in humans, and secondary fibrotic obstruction of the laryngeal lumen can also occur. For this reason, intensive antibiotic therapy and cartilage debridement are indicated with open laryngeal wounds (Miles-Foxen 1980).

Laryngeal and Epiglottic Inflammation

The tight junction between the laryngeal submucosa and underlying fibrocartilage limits the degree of mucosal inflammation and swelling that can occur in the larynx. This evolutionary feature is of obvious benefit to the host by preventing glottic obstruction. Even in horses with viral respiratory infections or strangles, which may have gross inflammation of the surrounding nasopharyngeal mucosa, marked swelling of the laryngeal, and in particular of the epiglottic, mucosa is rare (Dixon 1995).

Inflammation of the larynx, mainly of the medial aspects of the arytenoids, often occurs following intubation for general anesthesia, and similar inflammation also occurs in the trachea (Holland et al 1986). This inflammation can occur within 1 h of intubation and lesions can include focal mucosal hyperemia, submucosal hemorrhage or ulceration. This inflammation invariably begins to regress within 24–48 h (Holland et al 1986). More marked bruising, and even arytenoid mucosal tears and hemorrhage can occur with difficult intubations, such as in horses with laryngeal paralysis. Serial endoscopic examinations of such cases following surgery have shown this inflammation to be self-resolving within days in virtually all cases (P.M. Dixon, personal observations). Similar arytenoid bruising and even ulceration are also well recorded in humans following prolonged intubation (Bradley 1997).

Gross inflammation of the larynx including the epiglottis can occur with smoke inhalation (from both thermal and chemical insults) and severe concurrent damage to other upper respiratory tract sites and also to the lungs can contribute to the respiratory embarrassment in such cases. The treatment of horses with smoke inhalation pulmonary injury is covered in Chapter 43.

Occasionally horses will develop severe edema of the head that can also involve the larynx; causes include insect and snake bites to the head and upper neck, urticarial reactions of the head region, angioedema, and purpura hemorrhagica. Focal laryngeal inflammation can occur with purpura hemorrhagica following strangles vaccination (Byars 2004). Generalized head swelling can also occur as the result of adverse reactions to drugs (e.g. penicillin) or vaccines. Obstruction to venous and lymphatic drainage of the head, including that caused by head or neck neoplasia (Fig. 38.1), or bilateral jugular phlebitis may also cause gross swelling of the head. Some degree of head edema can develop in horses under general anesthesia, particularly if their head is maintained in a dependent position for prolonged periods, such as in horses having prolonged esophageal lavage for esophageal choke.

Fig. 38.2. Laryngeal endoscopy of a pony with marked inspiratory stridor. Both arytenoids are swollen and erythematous, with edema and distortion of the aryepiglottic folds, especially on the right side (AE). There appear to be adhesions between the mucosa of the right arytenoid and the palatopharyngeal arch (arrow). These laryngeal changes were caused by inflammation associated with an adjacent thyroid neoplasm.

Fig. 38.1. This pony has gross bilateral swelling of the head region secondary to neoplasia of the mandibular region. The pony is unable to dilate its grossly swollen nostrils and marked edema of the eyelids is also present, preventing full eye opening. A temporary tracheostomy tube is in place because of life-threatening nasal and laryngeal edema.

labored breathing, neck extension, and distress. As previously noted, concurrent edema of the nasal cavity and nostrils may exacerbate the respiratory obstruction in such cases. Horses with laryngeal obstruction should be carefully clinically evaluated, and unless they are in severe respiratory distress, they should also be endoscopically examined to confirm the site and extent of the airway obstruction. Intranasal oxygen therapy (if tolerated) and an emergency temporary tracheotomy (Fig. 38.1) (see Chapter 40) are indicated in cases with severe dyspnea. For less severe cases of laryngeal inflammation, rest and non-steroidal anti-inflammatories can be employed, with regular 24-h monitoring for evidence of increasing airway obstruction. An emergency tracheostomy tube and appropriate surgical equipment should be immediately available by the stall.

Epiglottic Inflammation

Inflammation of the epiglottis alone can develop (primarily in racehorses) in a number of situations, including some horses with epiglottic entrapment (Chapter 30) and in most horses following surgical correction of epiglottic entrapment (even if minimal epiglottic inflammation was present pre-surgery). Some horses may consequently

Marked swelling of the nasal mucosa is also a feature of some of these cases with postanesthetic head edema. As noted elsewhere (Chapter 33), some horses develop dyspnea following anesthesia because of bilateral laryngeal paralysis following general anesthesia.

Horses with acute head edema may develop significant perilaryngeal and laryngeal edema (Fig. 38.2) and can therefore develop inspiratory dyspnea and stridor, with

develop a permanent deformity of the epiglottis following its entrapment and surgical correction (Whitton & Kannegieter 1995, Dixon & Collins 2004). Severe epiglottic inflammation can also develop following resection of sub-epiglottic mucosa to treat dorsal displacement of the soft palate (DDSP) and in particular following subepiglottic injections of polytetrafluoroethylene (Teflon) paste or collagen to stiffen a "hypoplastic" epiglottis to help prevent DDSP (Tulleners et al 1997, Stick et al 1999). Trauma from a nasopharyngeal foreign body (usually a twig) entrapped on the lateral aspect of the larynx can also cause laryngeal, and especially epiglottic, trauma and inflammation (Dixon 1995).

Less severe degrees of idiopathic epiglottic inflammation have been described in racehorses, especially thoroughbreds showing poor exercise performance (Hawkins & Tulleners 1993, Davenport-Goodall & Parente 2003). This inflammation may be related to abnormal epiglottic–soft palate contact, as can occur in horses with DDSP, and some such cases may respond to rest. Tulleners (1997) also proposed that epiglottic inflammation in American racehorses might be caused by the inhalation of dirt from racetracks or be the result of the stresses of race training. Ulceration of the subepiglottic area (glossoepiglottic fold), which is detectable by elevating the epiglottis with a malleable rod *per nasum*, is also increasingly recognized in some racehorses with similar histories, and concurrent ulceration of the caudal aspect of the soft palate is also apparent in some of these cases (Blea & Arthur 2003). Marked inflammation of these areas with sudden-onset permanent DDSP can occur rarely in any breed of horse (Fig. 38.3).

In humans, primary bacterial infection with *Haemophilus influenzae* type B, and less commonly with *Streptococcus* spp. and *Staphylococcus* spp., can cause acute inflammation of both the nasopharynx and larynx. Of most clinical importance is peracute epiglottitis as a result of severe cellulitis of the epiglottic mucosa and submucosa that can prove fatal within hours of onset (Bastian 1991). A mortality of 6.1% was recorded in human epiglottitis cases managed conservatively (antibiotics, with or without corticosteroid therapy) as compared to 0.9% in cases that additionally received a tracheotomy or intubation to bypass the obstructed glottis (Cantrell et al 1978).

In contrast to humans, acute epiglottitis is rare in horses. Barclay et al (1982) recorded acute epiglottitis in a 3-year-old thoroughbred that presented with acute-onset stridor and dysphagia. Endoscopy revealed marked swelling of the epiglottis, with ulceration of its tip. The epiglottitis partly resolved following 2 days of penicillin and phenylbutazone therapy, and fully resolved after 5 days of systemic corticosteroid therapy. A peracute case of epiglottitis in an 8-day-old foal was described by Dacre et al (2004), which presented with dyspnea, coughing, and dysphagia of 2 days' duration. Endoscopy showed marked inflammation of an almost vertically positioned epiglottis to be causing
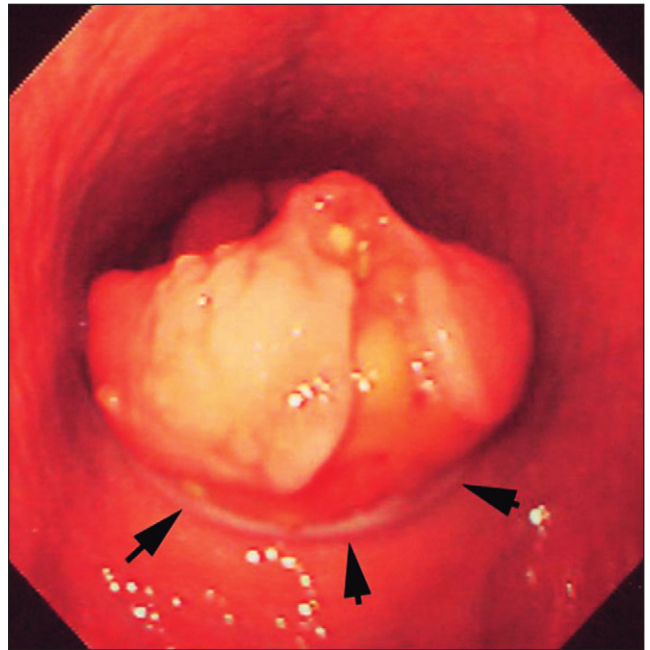
Fig. 38.3. Swollen epiglottis of a 12-year-old pony that suddenly developed exercise intolerance and near permanent dorsal displacement of the soft palate. The ventral aspect of the epiglottis and caudal aspect of the soft palate (arrows) are inflamed and ulcerated. This epiglottic inflammation did not respond to 9 months of rest along with prolonged phenylbutazone therapy. The initial cause of this epiglottitis may have been nasopharyngeal foreign body damage or a self-resolving epiglottic entrapment.

marked nasopharyngeal and laryngeal obstruction that resulted in dyspnea, dysphagia, and aspiration (Fig. 38.4). The cause of the underlying epiglottitis was not determined and the inflammation resolved fully following crystalline penicillin, gentamicin, and flunixin therapy.

A sequel to human epiglottitis is the development of an epiglottic abscess (Bastian 1991). In horses, dorsal epiglottic abscessation is a rare idiopathic disorder, presenting endoscopically as well-circumscribed, smooth swellings of the dorsal surface of the epiglottis. Treatment is by drainage, using a transendoscopic needle or biopsy forceps, or using transendoscopic laser as described by Tulleners (1991b).

Idiopathic Laryngeal Mucosal Ulceration and Granulomas

Mucosal ulceration and granulomas are commonly present on the arytenoids of horses affected with chondropathy, as described in detail in Chapter 37. Arytenoid granulomas, without obvious underlying chondropathy, can also occasionally occur following laryngoplasty (Dixon et al 2003). More commonly, granulomas can form following intraluminal surgery of the larynx (e.g. following partial arytenoidectomy or at the dorsal aspect of a vocal
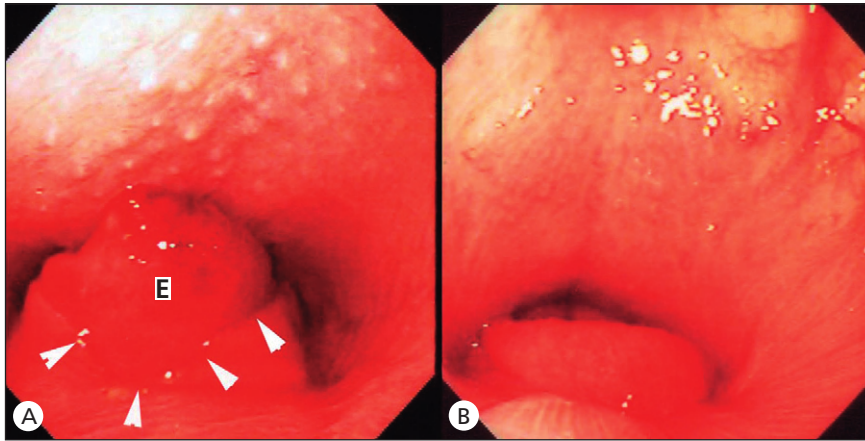
Fig. 38.4. (A) Nasopharyngeal endoscopy of a dyspneic foal that has gross inflammation and swelling of its epiglottis (E) that is dorsally deviated and whose tip is touching the nasopharyngeal roof. This deviation is the result of the swollen epiglottis becoming trapped by the caudal aspect of the soft palate (arrowheads). (B) On occasions, the soft palate could relocate beneath the epiglottis, and the latter then resumed a more normal, horizontal position. Reproduced with the permission of Dr K. Dacre.

cordectomy site) but these are usually self-resolving (Haynes 1978, Dixon et al 1994).

Focal, bilateral areas of arytenoid erythema often occur in horses after tracheal endoscopy (tracheoscopy) or bronchoscopy, especially in the presence of normal laryngeal function, where forceful glottic closure on an intralaryngeal endoscope induces local mucosal bruising, usually on the ventromedial aspects of the corniculate cartilages, i.e. the vocal processes. Laryngospasm, which can be life-threatening in other species when intralaryngeal endoscopy is performed without sedation or local anesthesia, seldom if ever occurs in horses, probably because they have a relatively insensitive larynx. As noted earlier, endotracheal intubation can cause inflammation of the equine glottis in addition to more severe laryngeal bruising if intubation was difficult. Mucosal ulcers can also occasionally occur in horses with bilateral laryngeal paralysis as a result of abnormal contact between the arytenoids.

In addition to the aforementioned types of laryngeal inflammation, idiopathic mucosal ulceration or granuloma formation has been reported in horses in which the underlying arytenoid cartilages appear normal and in which normal laryngeal function is present (Hay & Tulleners 1993, Embertson 1998, Stick et al 1999, Kelly et al 2003, Anderson 2004, Byars 2004). Such focal arytenoid mucosal lesions usually occur medially (axially) on the vocal processes, just above the dorsal insertion of the vocal folds. These mucosal lesions are sometimes erroneously referred to as arytenoid chondritis, despite the underlying arytenoids being of normal shape and size, unlike in most cases of arytenoid chondropathy (Figs 38.5 and 38.6). Unilateral arytenoid granulomas often have a corresponding ("kissing") mucosal ulcer on the medial aspect on the opposite arytenoid, which is believed to be caused by contact. These equine mucosal and granulomatous arytenoid lesions are likely to be related, as is the case in humans where arytenoid granulomas are believed to begin as mucosal ulcers, with continued inflam-
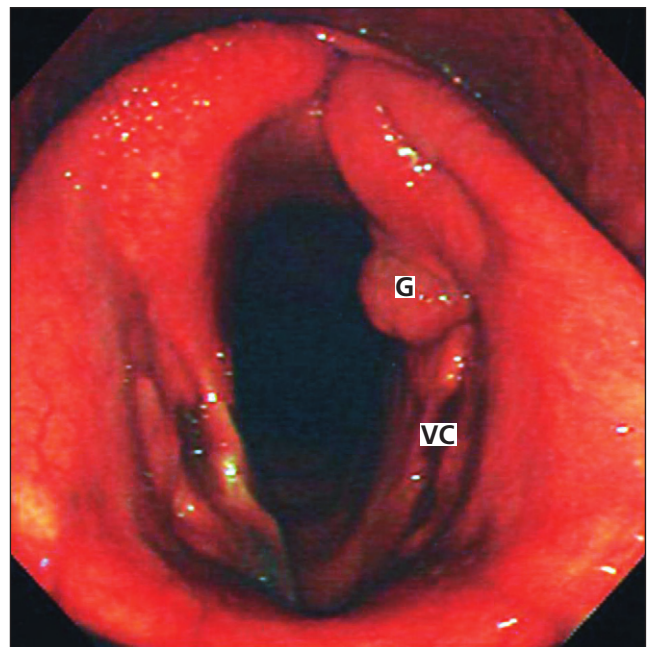
Fig. 38.5. Laryngeal endoscopy of a 3-year-old thoroughbred racehorse that had laryngoplasty, left vocal cordectomy (VC) and right-sided ventriculectomy for recurrent laryngeal neuropathy circa 6 months earlier. More recently, an arytenoid granuloma (G) has developed, dorsal to the left vocal process, a common site for idiopathic arytenoid granuloma formation. There is no distortion of the arytenoids, indicating that arytenoid chondropathy is not present.

mation and repetitive injury later inducing granulation tissue formation (Bradley 1997). Whilst usually small (< 3 cm in diameter), larger granulomas have been illustrated that can cause marked laryngeal obstruction even at rest (Byars 2004).

Embertson (1998) described the occurrence of idiopathic, small, reddened areas of the corniculate process or vocal process of unknown origin in thoroughbred yearlings in Kentucky. Intercurrent respiratory disease with
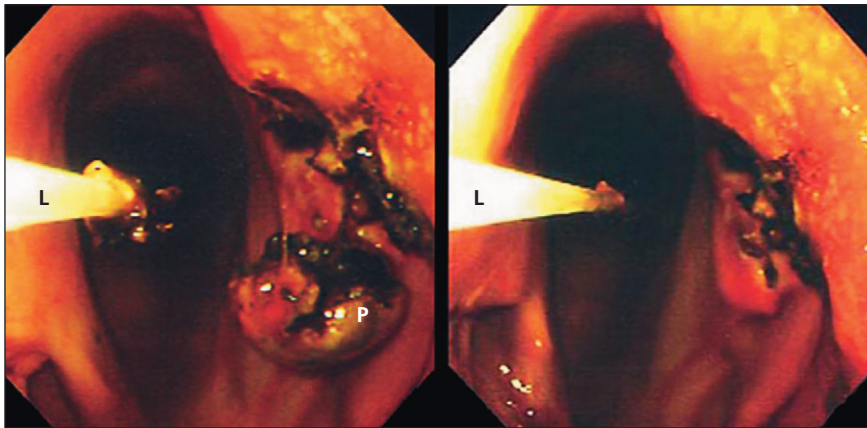
Fig. 38.6. On the left, the arytenoid granuloma shown in Fig. 38.5 is almost completely dissected free and hanging into the glottis, following diode laser (L) resection. The right image shows the larynx immediately following removal of the granuloma.

laryngeal mucosal swelling and arytenoid mucosal trauma caused by endoscopy, coughing or exercise testing at sales could account for these mucosal lesions.

Kelly et al (2003) reported 21 cases of arytenoid mucosal lesions in 3,312 thoroughbred yearlings in Australia (0.63% prevalence), this disorder being the most common upper airway abnormality found in these horses. Nineteen of the 21 cases had mucosal ulceration (13 bilateral, six unilateral), one horse had bilateral arytenoid granulomas (with concurrent total left recurrent laryngeal neuropathy) and one had unilateral arytenoid granulomas and ulceration. The mucosal ulcers were 2–6 mm in diameter and were usually located at the rostroventral aspect of the arytenoids, just above the vocal process. Of 18 yearlings that were followed up, 15 recovered fully following antimicrobial and anti-inflammatory therapy combined with box rest, and the other three later developed mucosal granulomas, with arytenoid chondropathy developing subsequently in one. It was hypothesized that the cause of these lesions was a respiratory mucosal infection with secondary traumatic damage to focal areas of the arytenoids by abnormally forceful contact with the contralateral arytenoid during coughing or fast exercise. Kelly et al (2003) dismissed endoscopic trauma as a cause of these lesions because they expected endoscopically induced lesions to be more rostrally positioned. However, as noted above, closure of the larynx can induce mucosal bruising on the medial aspects of the arytenoids. It is also likely that such horses would have undergone frequent laryngeal endoscopy before and during yearling sales.

Anderson (2004) reported that, in addition to endoscopically recording arytenoid chondropathy in thoroughbred yearlings in New Zealand, arytenoid mucosal ulceration without apparent concurrent chondropathy was also observed. These usually bilateral, idiopathic arytenoid ulcers were present on the medial aspect of the arytenoid near the vocal process. These lesions appear identical to those described in Australia by Kelly et al (2003). Booth

et al (2000) described acute laryngeal obstruction caused by a large unilateral arytenoid granuloma in a pregnant mare that was successfully removed using a transendoscopic electrosurgical snare.

Hay & Tulleners (1993) described arytenoid granulomas in 25 horses, with an underlying arytenoid chondropathy present in 17 of these cases (following partial arytenoidectomies in three cases). However, minimal or no underlying arytenoid swellings, or laryngeal abductory deficits were present in the other eight cases. Interestingly, 18 of the 22 (82%) "primary" granulomas were right-sided, and all originated axially on the arytenoid between the vocal and corniculate processes. Using a neodymium:yttrium–aluminum–garnet (Nd:YAG) laser, the base of the lesion was excised circumferentially creating a 1- to 2-mm deep concave defect. The granuloma was grasped with bronchoesophageal forceps before it was fully removed. All eight cases without significant arytenoid swelling responded to this contact laser excision. Stick et al (1999) have also noted that removal of larger laryngeal granulomas can help the healing of underlying mucosal lesions. It is unclear if the eight cases of Hay and Tulleners (1993) described above were early stage arytenoid chondropathy cases or simply idiopathic mucosal ulceration with later granuloma formation.

Arytenoid contact ulcers are common in feedlot cattle, with an incidence of 13% in one large abattoir study (Jensen et al 1980). Respiratory infection with laryngeal mucosal inflammation and excessive coughing (Jensen et al 1980) or focal infarct (Dillman 1972) has been proposed as the cause of these lesions. Excessive vocalization during the breeding season is believed to be the cause of laryngeal inflammation in male sheep (especially in Texel and Suffolk breeds) in Britain (C. Penny, personal communication 2004). Likewise, excessive vocalization associated with transport and mixing with new horses could also be a potential cause of focal trauma to the arytenoid mucosa in young horses, for example at times of sales. The fact that these obvious lesions are rarely recorded in other

countries with large, well-evaluated equine populations, such as Britain and Ireland, is interesting, and suggests that some of the predisposing factors for the development of idiopathic arytenoid ulceration are absent in these two countries.

In humans, arytenoid granulomas can occur following mucosal injury caused by endoscopy or endotracheal intubation, especially if intubation is prolonged, as can occur in patients maintained on ventilators and also following laryngeal surgery (Bradley 1997). The majority of endotracheally induced human laryngeal granulomas resolve spontaneously within 8–14 weeks after extubation. A second type of contact ulcer of the human arytenoid (usually of the vocal process) is caused by excessively forceful apposition of the two arytenoids (Sanders & Billers 1991, Bradley 1997) and this type of lesion may be unilateral or bilateral. The lesions tend to occur in older males, particularly in those that use forceful speech in their work (e.g. lawyers, clergy). Chronic coughing, gastro-esophageal reflux, and smoking are additional risk factors (Bradley 1997). This type of human laryngeal contact ulcer usually responds to voice rest, and later voice re-education. Failure to respond usually indicates the presence of underlying arytenoid inflammation. Human arytenoid granulomas are commonly excised by sharp dissection and laser application to the mucosal wound to control hemorrhage. These arytenoid mucosal lesions differ from the vocal cord nodules that can develop, especially in young children ("screamer's nodules") or older female singers ("singers nodules") (Sanders & Billers 1991), as the result of excessive vocalization.

In conclusion, it is likely that continued inflammation of idiopathic arytenoid ulceration in horses leads to arytenoid granuloma formation as has been recorded in humans (Bradley 1997). It also appears likely that some arytenoid granulomas will progress to arytenoid chondropathy, as has also been reported in humans (Sanders & Billers 1991).

Perilaryngeal Infections

Barber (1981) described a 3-year-old thoroughbred filly that developed bilateral nasal discharge and swelling of the left laryngeal area. Following an emergency tracheostomy and 2 weeks of antibiotic therapy, endoscopy showed left-sided laryngeal paralysis, a swollen left arytenoid and drainage of pus into the laryngeal lumen. Radiography showed a gas-capped abscess superimposed over the larynx and rostral trachea indicating a communicating abscess. Surgical drainage via a left-sided paramedian ventral approach was successful in resolving the abscess. The author has also treated a horse with a dorsal laryngeal abscess caused by an ingested piece of wire that involved a common carotid artery, and which resolved with foreign body removal and drainage.

Miscellaneous Arytenoid Swellings

Shapiro et al (1979) described laryngeal obstruction and stridor in an aged horse that were caused by bilateral, irregular enlargements of all of the laryngeal cartilages, in particular by medial swellings of the thyroid cartilage, that prevented arytenoid abduction. The lesions were histologically described as hypertrophic ossification of the laryngeal cartilages, with osseous metaplasia that extended well beyond the normal borders of the cartilages. The marked inflammatory nature of the cartilaginous changes, in addition to the reported cartilage calcification in this case, differs greatly from the normal ossification of equine laryngeal cartilages, which can develop in horses as young as 2 years (P.M. Dixon, personal observations) but more commonly develops in older horses. This normal ossification first appears in the thyroid cartilage, then the dorsal lamina of the cricoid and the arytenoid muscular processes (Rooney & Robertson 1996).

Parasitic Laryngeal Inflammation

Lane et al (1986) described a parasitic infection of the larynx in a horse imported from Central America that was caused by *Besnoitia bennetti* (a coccidian type organism). The affected horse had multiple papillomatous lesions (ranging from 0.3 to 2 cm in diameter) on its arytenoids, epiglottis, and aryepiglottic and vocal folds, although normal laryngeal movement was present. Histology showed the lesions to be composed of subepithelial connective tissue containing the parasite, covered by acanthotic epithelium.

Laryngeal Foreign Bodies

The height of the horse makes it unlikely that inhalation of plant material will occur whilst running, in contrast to shorter mammals such as dogs in which inhalation of ears (awns) of grass and corn are commonly described. Nevertheless, 10- to 15-cm long, branched twigs will occasionally become inhaled and may lodge in the nasal concha or, more commonly, in the laryngopharynx lateral to the arytenoids, laryngeal lumen, lateral ventricles, or in the trachea. Horses are very selective eaters but occasionally shorter twigs (e.g. hedge clippings) will be ingested, masticated, and partially swallowed before becoming entrapped in the oropharynx, laryngopharynx or larynx (T. Greet, personal communications 2005). Pieces of wire in forage may also be ingested and also may become entrapped in the oropharynx, laryngopharynx or larynx. If sharp and pointed, such ingested twigs or wire may become deeply embedded in the nasopharyngeal wall during swallowing or as the result of spasm of the nasopharyngeal muscles. Likewise, similar objects may become embedded in the larynx as a result of laryngospasm.
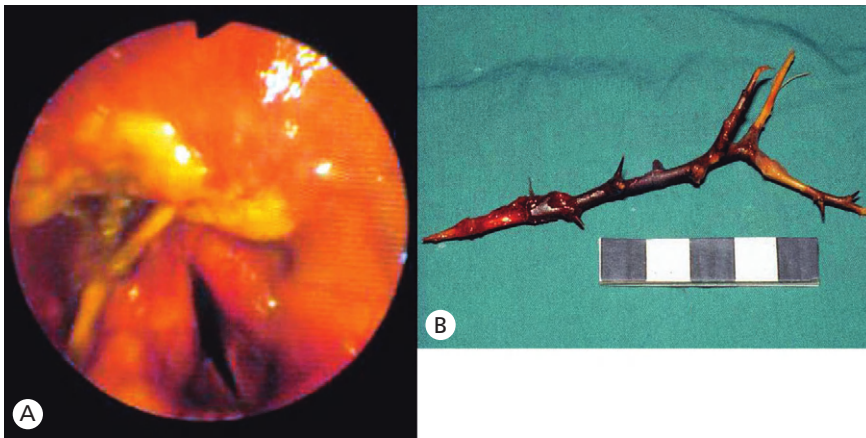
Fig. 38.7. (A) Nasopharyngeal endoscopic image of a horse with sudden-onset dysphagia and distress which shows a Y-shaped twig embedded in the roof of the pharynx, just lateral to right arytenoid. The foreign body caused gross, fibrinous inflammation of the punctured nasopharynx and inflammation of the adjacent arytenoids and epiglottis. (B) The twig that was removed *per os*. The structural damage caused to the epiglottis in this horse caused long-term intermittent soft palate displacement.
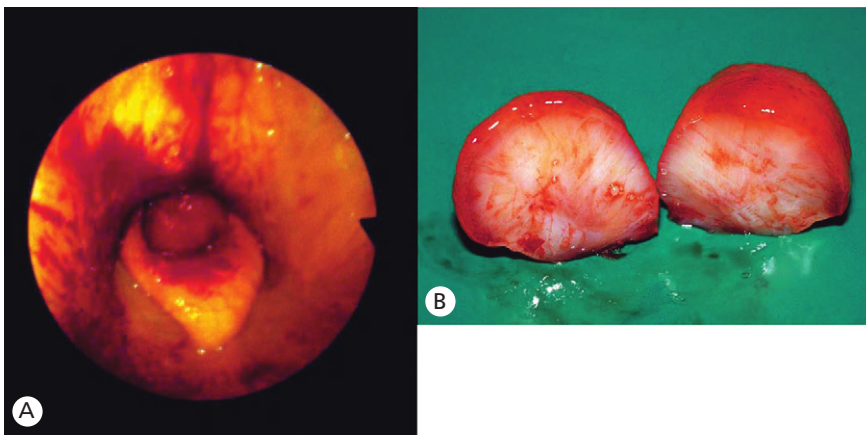
Fig. 38.8. (A) Endoscopic image of the caudal nasopharyngeal and laryngeal area of a pony with severe dyspnea and stridor. There is severe bruising of the nasopharyngeal area and epiglottis, and the laryngeal lumen appears to be almost fully occluded by a spherical mass. Following emergency tracheostomy, a laryngotomy showed a polyp-like mass protruding from the right lateral ventricle, which was surgically excised at its base. (B) The excised polyp (transected).

Laryngeal or perilaryngeal foreign bodies may become dislodged and then swallowed or coughed out. Consequently, laryngeal (especially epiglottic) trauma of unknown origin can reasonably be attributed to such foreign body damage. When embedded at the side of the larynx or in its lumen, foreign bodies will cause sudden-onset paroxysmal coughing, dysphagia, and anorexia accompanied by malodorous breath. Depending on the degree of laryngeal inflammation that occurs, dyspnea and stridor may also develop. Clinical examination will usually be unrewarding in early cases, unless malodorous breath and/or dysphagia (as manifested by nasal discharge containing food) are present. Later, with the onset of marked laryngeal inflammation, stridor may be present even at rest, and deep palpation of the laryngeal area may be resented and may also induce coughing.

Diagnosis is best made by nasopharyngeal endoscopy, when the foreign body, possibly covered by food, mucus or fibrin can be visualized, in addition to swollen, reddened, and possibly fibrinous inflammation of the larynx and laryngopharynx or nasopharynx (Fig. 38.7). The epiglottis in particular can be extensively inflamed and torn. Smaller foreign bodies may become entrapped in the lateral

ventricles and cause a granulomatous reaction at this site, with the granuloma protruding into the laryngeal lumen (Fig. 38.8). In the case shown in Fig. 38.8 the lateral ventricle granuloma was removed via a laryngotomy but clinical signs recurred some months later as the result of regrowth of a similar-sized polyp. Radiography showed a piece of wire in the ventricle. At a second surgery, the polyp was removed and exploration of the ventricle revealed the embedded wire that had caused the granuloma formation (Fig. 38.9). The wire was removed and there was permanent resolution of the laryngeal inflammation. In contrast, Ordridge (1988) reported a horse with a 5-cm long twig in a laryngeal lateral ventricle for an estimated 3 weeks, where minimal laryngeal inflammation occurred. The twig was successfully removed using a snare of thick nylon fishing line inserted transendoscopically.

Although radiography can only detect radio-opaque foreign bodies, it is nevertheless a worthwhile technique to use in cases of pharyngeal or laryngeal swelling, and in horses with sudden-onset dysphagia or stridor of unknown etiology. Perilaryngeal or peripharyngeal metallic foreign bodies may be detected radiographically in some of these
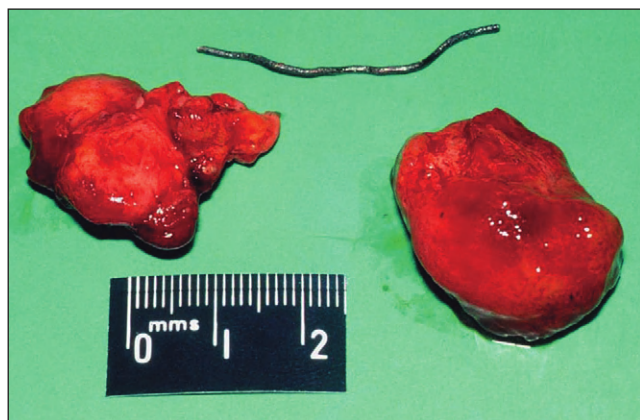
Fig. 38.9. The upper airway obstruction in the pony described in Fig. 38.8 recurred after several months and endoscopy showed regrowth of a polyp from the right lateral ventricle. Radiography at that stage showed a piece of wire embedded in the lateral ventricle. The polyp and the wire fragment (embedded in granulation tissue) were removed, leading to permanent resolution of the laryngeal obstruction.
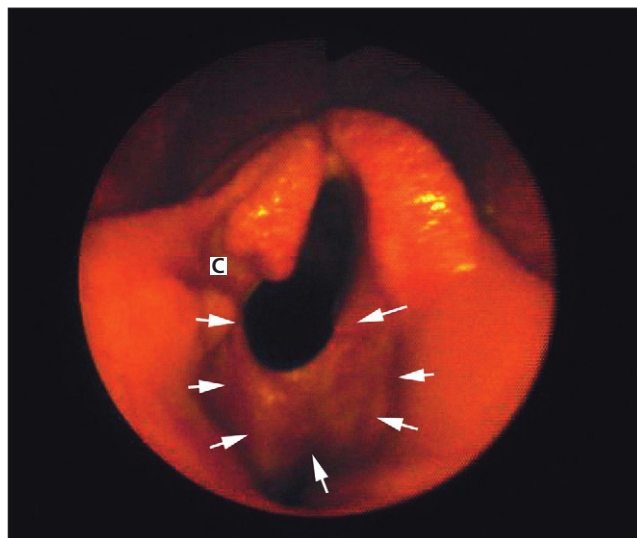
Fig. 38.10. Endoscopic view of the larynx of a horse with marked ventral glottic stenosis, which occludes most of the laryngeal lumen (arrows). The ventral aspect of the right corniculate process (C) has been resected. This laryngeal web developed following bilateral vocal cordectomy, and the web became taller and thicker following surgical resection.

cases, as may perilaryngeal abscesses and gas pockets. The latter lesions may be caused by non-opaque foreign bodies, hematogenous infections or trauma.

A horse with an inhaled foreign body is obviously treated by removal of the foreign body. As these objects are often large and also deeply embedded into the pharynx or larynx, attempted removal with transendoscopic biopsy or grasping forceps may be unsuccessful or may break the foreign body, possibly leaving some of it embedded *in situ*. The use of long, rigid grasping forceps (such as bronchoesophageal forceps) *per nasum* can be successful in suitably sedated horses with use of topical local anesthesia. If a perilaryngeal or laryngeal foreign body cannot be retrieved *per nasum* in adult horses, the use of heavy sedation, transendoscopic lavage of the nasopharynx and larynx with local anesthetic, and the use of a full mouth speculum will usually allow their manual removal *per os*. To perform such procedures, the medial (palatal) aspect of any sharp upper cheek teeth should be rasped, to protect the operator's hands. The wet hand should be pushed caudally into the oropharynx, and then directed dorso-caudally, displacing the soft palate and elevating the epiglottis. The laryngeal structures can then be palpated and a caudally positioned nasopharyngeal, laryngopharyngeal or laryngeal foreign body can be grasped and removed via the oral cavity. Alternatively, *per os* retrieval can be performed with the horse under general anesthesia (Greet 2003), possibly using rigid instruments passed *per nasum* to assist removal.

A laryngotomy can be performed to remove deeply embedded laryngeal foreign bodies, such as those trapped in the lateral ventricles. Intralaryngeal foreign bodies can also be removed via a high cervical tracheostomy by inserting grasping forceps retrograde to the larynx under nasopharyngeal endoscopic guidance (Voss & Seahorn 2004), but the postoperative risks are greater for tracheostomy than laryngotomy when used for this purpose.

Laryngeal Stenosis

Laryngeal stenosis (laryngeal webbing or cicatrix) of horses can be congenital, as described earlier. More commonly in the horse these webs are acquired following extensive endolaryngeal mucosal injury, when granulation tissue protrudes so extensively from denuded mucosa on opposite sides of the larynx that it prevents normal mucosal healing. This granulation tissue then contacts granulation tissue on an opposite surface and the two sides join together in the laryngeal lumen. This bridge of granulation tissue then develops into a fibrous fold across the larynx, which later becomes covered with mucosa (Fig. 38.10) (Langman et al 1989, Bastian 1991, Dixon et al 1994). Laryngeal stenosis is common in humans, where it usually involves the narrowest intralaryngeal areas (i.e. glottic stenosis) or areas just caudal to the larynx (i.e. subglottic stenosis) and is congenital in about 40% of cases (Flexon et al 1989, Lusk 1991). Acquired glottic stenosis in humans most commonly develops following extensive mucosal injury caused by prolonged nasotracheal intubation (Langman et al 1989), or as a sequel to serious trauma, e.g. from steering wheel trauma in car accidents, knife and gunshot wounds (Sanders & Billers 1991) or

A further case with a thin "V"-shaped, ventral web that occurred following severe external trauma (which is rare in horses because the larynx is anatomically well protected from external trauma) responded to resection of the larger side of this web and some mobilization and suturing of the underlying mucosal deficit, with long-term total absence of respiratory noise and the development of normal exercise tolerance. Lane (1997) also reported a case of ventral glottic stenosis following bilateral vocal cordectomy via a ventral laryngotomy to treat laryngeal paralysis.

Laryngeal Tumors

Neoplasia of the equine larynx is rare (Cotchin 1977, Rooney & Robertson 1996) with only occasional reports of laryngeal tumors, mainly squamous cell carcinomas (Cotchin 1977) and lymphosarcomas (Lane 1985, Dixon 1995) in the literature. Lane (1997) described a 9-year-old horse with stridor and exercise intolerance as the result of a pedunculated laryngeal lymphosarcoma attached near the right ventricle. The tumor recurred within 3 months of surgical removal. Most cases of laryngeal lymphosarcoma are not treated because of the inaccessibility of lesions and the presence of concurrent tumors elsewhere in the upper respiratory tract. Trotter et al (1990) described a 5-year-old American quarterhorse with a 2-week history of respiratory distress caused by a 3-cm diameter chondroma on the medial aspect of the left arytenoid that was successfully surgically removed. Sarli et al (2001) described a hemangioma protruding submucosally into the laryngeal lumen that caused airflow obstruction with exercise intolerance and abnormal noise production. The lesion was successfully removed surgically.

REFERENCES

Anderson BH 2004 Non-RLN URT disorders identified during post sale endoscopic examinations of 5559 TB yearlings (1997–2002) in New Zealand. In: Dixon PM, Robinson NE, Wade JF (editors) Equine Recurrent Laryngeal Neuropathy, Havemeyer Foundation Monograph Series No. 11. R & W Publications (Newmarket), Newmarket, pp.51–54

Barber SM 1981 Paralaryngeal abscess with laryngeal hemiplegia and fistulation in a horse. Canadian Veterinary Journal 22: 389–392

Barclay WP, Phillips TN, Foerner JJ 1982 Acute epiglottidis in a horse. Journal of the American Veterinary Medical Association 181: 925

Bastian RW 1991 Acute inflammatory diseases of the larynx. In: Ballenger JJ (editor) Diseases of the Nose, Throat, Ear, Head and Neck, 14th edn. Lea and Febiger, Philadelphia, pp.605–615

Baxter GM, Allen D, Farrell RL 1992 Paralaryngeal accessory bronchial cyst as a cause of laryngeal paralysis in a horse. Equine Veterinary Journal 24: 67–69

Blea JA, Arthur RM 2003 How to evaluate and treat ulceration of the glosso-epiglottic fold (subepiglottic area) in the standing horse. Proceedings of the American Association of Equine Practitioners 49: 295–300

Booth TM, Hainisch EK, Knottenbelt DC et al 2000 Endoscopic electrosurgical excision of laryngeal granulation tissue in a standing pregnant mare. Veterinary Record 147: 418–420

Bradley PJ 1997 Arytenoid granuloma – Editorial review. Journal of Laryngology and Otology 111: 801–803

Byars TD 2004 Pharyngoscopy and laryngoscopy In: Slovis NM (editor) Atlas of Equine Endoscopy. Mosby, St Louis, MO, pp.55–81

Cantrell RW, Bell RA, Morioka WT 1978 Acute epiglottitis: intubation versus tracheostomy. Laryngoscope 88: 994

Carr EA, Spier SJ, Kortz GD et al 1996 Laryngeal and pharyngeal dysfunction in horses homozygous for hyperkalemic periodic paralysis. Journal of the American Veterinary Medical Association 209: 798–803

Cotchin 1977 A general survey of tumours in the horse. Equine Veterinary Journal 9: 16–21

Davenport-Goodall CLM, Parente EJ 2003 Disorders of the larynx. Veterinary Clinics of North America; Equine Practice 19: 169–187

Dacre KJ, Pirie RS, Prince DR 2004 Primary epiglottitis with associated dysphagia in a foal. Equine Veterinary Education 16: 296–301

Dillman RC 1972 Laryngitis in feedlot calves and its relationship to *Haemophilus somnus* complex. Proceedings of the United States Animal Health Association 66: 498–501

Dixon PM 1995 A review of the role of the epiglottis in equine upper airway obstruction. Equine Veterinary Education 7: 131–139

Dixon PM, Collins N 2004 The equine epiglottis. Equine Veterinary Education 16: 299–301

Dixon PM, Railton DI, McGorum BC 1994 Ventral glottic stenosis in 3 horses. Equine Veterinary Journal 26: 166–170

Dixon PM, McGorum BC, Railton DI et al 2003 A long-term survey of laryngoplasty in an older, mixed-breed population of 200 horses. 1: Maintenance of surgical arytenoid abduction and complications of surgery. Equine Veterinary Journal 35: 389–396

Embertson RM 1998 Evaluation of the upper respiratory tract in weanlings and yearlings. In: White NA, Moore JN (editors) Current Techniques in Equine Surgery and Lameness, 2nd edn. WB Saunders, Philadelphia, pp.122–127

Flexon PB, Cheney ML, Montgomery WW et al 1989 Management of patients with glottic and subglottic stenosis resulting from thermal burns. Annals of Rhinology and Laryngology 98: 27–30

Greet TRC 2003 Endoscopic retrieval of foreign bodies. Equine Veterinary Education 15: 232

Harrison IW, Raker CW 1988 Dorsal glottic stenosis after bilateral arytenoidectomy in two horses. Journal of the American Veterinary Medical Association 192: 202–204

Harvey CE 1983 Partial laryngectomy in the dog. I. Healing and swallowing function in normal dogs. Veterinary Surgery 12: 192–196

Hawkins JF, Tulleners EPI 1993 Epiglottitis in the horse: 20 cases (1998–1993). Veterinary Surgery 22: 383

Hay WP, Tulleners E 1993 Excision of intralaryngeal granulation tissue in 25 horses using a neodymium:YAG laser (1986–1991). Veterinary Surgery 22: 129–134

Haynes PF 1978 Surgical failures in upper airway respiratory surgery. Proceedings of the American Association of Equine Practitioners 24: 223–249

Holland M, Snyder JR, Steffey EP et al 1986 Laryngotracheal injuries associated with nasotracheal intubation in the horse. Journal of the American Veterinary Medical Association 189: 1447–1450

Jensen R, Lauerman LH, Braddy PM et al 1980 Laryngeal contact ulcers in feedlot cattle. Veterinary Pathology 17: 667–671

Kelly G, Lumsden JM, Dunkerly G et al 2003 Idiopathic mucosal lesions of the arytenoid cartilages of 21 Thoroughbred yearlings: (1997–2001). Equine Veterinary Journal 35: 276–281

Koterba AM, Drummond WH, Kosch P 1975 Intensive care of the neonatal foal; Symposium on neonatal equine disease. Veterinary Clinics of North America; Equine Practice 1: 3–34

Koufman JA, Branch ME, Ryu JH 1988 A comparison of the carbon dioxide and neodymium: YAG lasers in a canine model of acquired subglottic stenosis. Journal of Otolaryngology 17: 223–226

Lane JG 1982 Ear, Nose and Throat and Oral Surgery of the Dog and Cat. Wright PSG, Bristol, pp.112–113

Lane JG 1985 Palatine lymphosarcoma in two horses. Equine Veterinary Journal 17: 465–467

Lane JG 1997 Larynx. In: Traub-Dargatz JL, Brown CM (editors) Equine Endoscopy. Mosby, St Louis MO, pp.74–96

Lane JG, Lucke VM, Wright AI 1986 Parasitic laryngeal papillomatosis in a horse. Veterinary Record 119: 591–593

Langman AW, Lee KC, Dedo HH 1989 The endoscopic Teflon keel for posterior and total glottic stenosis. Laryngoscope 99: 571–577

Lees MJ, Scuh JCL, Barber SM et al 1987 A congenital laryngeal web in a Quarterhorse filly. Equine Veterinary Journal 19: 561–563

Linford RL, O'Brien TR, Wheat JD et al 1983 Radiographic assessment of the epiglottic length and pharyngeal and laryngeal diameters in the Thoroughbred. American Journal of Veterinary Research 44: 1660–1666

Lusk RP 1991 Congenital malformations of the larynx. In: Ballenger JJ (editor) Diseases of the Nose, Throat, Ear, Head and Neck. Lea and Febiger, Philadelphia, pp.570–588

Manus AG 1965 Canine epihyoid fractures. Journal of the American Veterinary Medical Association 147: 129–132

Matushek KJ, Bjorling DE 1988 A mucosal flap technique for correction of laryngeal webbing: results in four dogs. Veterinary Surgery 17: 318–320

Miles-Foxen EH 1980 Injury of the larynx and trachea. In: Miles-Foxen EH (editor) Diseases of the Ear, Nose and Throat. Blackwell Scientific Publications, Oxford, pp.159–160

Nelson AW, Wykes PM 1985 Upper respiratory system. In: Slatter DH (editor) Textbook of Small Animal Surgery, Vol. I. WB Saunders, Philadelphia, pp.979–985

Ordridge RM 1988 Twig removal. Veterinary Record 123: 608

Peterson SL, Smith MM, Senders CW 1987 Evaluation of a stented laryngoplasty for cranial glottic stenosis in four dogs. Journal of the American Veterinary Medical Association 191: 1582–1584

Rooney JR, Robertson JL 1996 The respiratory system. Equine Pathology, 1st edn. Iowa State University Press, Ames, IA, pp.24–56

Sanders I, Billers H 1991 Trauma to the larynx. In: Ballenger JJ (editor) Diseases of the Nose, Throat, Ear, Head and Neck. Lea and Febiger, Philadelphia, pp.602–604

Sarli G, Torre F, Simoni P et al 2001 Diagnosis, surgical treatment and histological characterisation of a laryngeal haemangioma in a Standardbred horse. Ippologia 12: 7–12

Shapiro J, White NA, Schlafer DH et al 1979 Hypertrophic ossification of the laryngeal cartilages of a horse. Journal of Equine Medicine and Surgery 3: 370–374

Stick J, Boles C 1980 Subepiglottic cyst in three foals. Journal of the American Veterinary Medical Association 177: 62–64

Stick JA, Tulleners EP, Robertson JT et al 1999 The larynx. In: Auer J, Stick JA (editors) Equine Surgery, 2nd edn. WB Saunders, Philadelphia, pp.349–375

Strand E, Hanche-Olsen S, Gronvold AMR et al 2004 Dynamic bilateral and vocal fold collapse associated with head flexion in 5 Norwegian Coldblooded Trotter racehorses. Equine Veterinary Education 36: 235–241

Trotter GW, Aanes WA, Snyder SP 1990 Laryngeal chondroma in a horse. Journal of the American Veterinary Medical Association 178: 829–830

Tulleners E 1991a Correlation of performance with endoscopic and radiographic assessment of epiglottic hypoplasia in racehorses with epiglottic entrapment corrected by use of contact neodymium:yttrium aluminium garnet laser. Journal of the American Veterinary Medical Association 198: 621–626

Tulleners E 1991b Use of transendoscopic contact neodymium:yttrium aluminum garnet laser to drain dorsal epiglottic abscesses in two horses. Journal of the American Veterinary Medical Association 198: 1765–1767

Tulleners E 1997 Epiglottitis. In: Robinson NE (editor) Current Therapy in Equine Medicine 4. WB Saunders, Philadelphia, pp.407–409

Tulleners TP, Stick JS, Leitch M et al 1997 Epiglottic augmentation for treating dorsal displacement of the soft palate in racehorses: 59 cases (1985–1994). Journal of the American Veterinary Medical Association 211: 1022–1028

Voss E, Seahorn T 2004 Tracheobronchoscopy. In: Slovis NM (editor) Atlas of Equine Endoscopy. Mosby, St Louis, pp.97–118

Whitton RC, Kannegieter NJ 1995 Deformity of the epiglottis in 4 horses. Equine Veterinary Education 7: 127–130

Yarborough TB, Voss E, Herrgesell EJ et al 1999 Persistent frenulum of the epiglottis in four foals. Veterinary Surgery 28: 287–291.

39 Laser Surgery of the Upper Respiratory Tract

Eric J Parente

Introduction

Lasers have been used in the biomedical field for over 40 years, but their use in equine respiratory surgery began in the mid-1980s when it was found that laser light could be transmitted down a small diameter fiber that could be passed through the biopsy channel of an endoscope (Tate 1991). The first such laser was the neodymium:yttrium–aluminum–garnet (Nd:YAG) laser and with its use, equine transendoscopic laser surgery was born.

Before the advent of laser surgery, many procedures performed on the larynx or pharynx of the horse required an open surgical approach under general anesthesia. A distinct advantage of transendoscopic laser surgery was that the surgery could be performed without a skin incision and without general anesthesia. Additional features were increased visualization of the surgical field, increased surgical precision, better hemostasis, and ultimately shorter hospitalization and convalescence periods for the patient.

The Principles of Lasers

Laser is an acronym for "light amplification of stimulated emission of radiation". The light emitted by lasers works according to the basic properties of light and electromagnetic radiation, and like white light, laser light consists of particles (photons) traveling through space in unique waveforms. Visible light has an electromagnetic spectrum of wavelength of approximately 400–700 nm and each color of visible light has its own characteristic wavelength. Laser light is very different from the light produced by more common white light sources, such as incandescent bulbs, fluorescent lamps, or sunlight, for several different reasons.

Laser light can be within the visible spectrum of light but differs significantly from white light because of its monochromicity, direction, and coherence. Laser light consists of a single wavelength, or an extremely narrow range of wavelengths and is therefore considered "monochromatic", while white light is a mixture of different wavelengths. Light emitted from bulbs or headlights diverges rapidly, while laser light has a very narrow cone of divergence. Finally, light waves can travel through space

without any fixed relationship to each other, meaning that they are incoherent. If all waves are lined up together so their peaks and valleys match, they are said to be in phase, or coherent. Laser light is coherent.

To truly understand lasers, basic atomic physics must be reviewed. An atom consists of a positive nucleus surrounded by negative electrons. A neutral atom of a given element has the same number of positive charges (protons) in its nucleus and negative charges (electrons) around the nucleus. When electrons are in orbits close to the nucleus, the atom is in its lowest energy level, which is termed the ground or resting state. If an electron absorbs energy in the form of heat or light, the electrons will jump or make a quantum leap into a higher orbit (i.e. further away from the nucleus) and assume an excited state. Atoms in an excited state tend to return to the resting or ground state and in so doing, they spontaneously emit energy in the form of photons or light. In 1917, Einstein proposed that there was a difference between spontaneous and stimulated emission of light. His theory was eventually applied in the invention of the laser. He predicted that when electrons in higher orbits or energy levels were bombarded by particular types of photons, they would decay to a lower energy level, emitting a photon in the process. The stimulating photon, instead of being absorbed, would continue to be propagated. The end result is two photons or quanta of energy with identical wavelengths. A chain reaction ensues, resulting in stimulated emission of photons.

Since atoms tend to be in a ground state, they are more likely to absorb an encountered photon than to stimulate the emission of a second one. Therefore to reach a state of producing a sustained stimulated emission, more atoms need to be in higher energy levels than in the ground state. This condition is known as "population inversion" and is created by exposing atoms to a source of energy, a process that is termed "pumping". The energy source used to create the population inversion can be heat, light or electricity. Once the population inversion occurs, atoms decay to their lower energy levels and release photons. The spontaneously emitted photons collide with atoms in the higher energy states, resulting in stimulated emission, which begins the chain reaction.

There is a general four-grade (classes I–IV) classification system for laser power and safety. Classes I and II are low

risk lasers with < 1 milliwatts (mW) of power. Class III lasers are the "cold" or therapeutic lasers. All surgical lasers are class IV (> 0.5 W). While the power is measured in watts, the power density is termed "irradiance" and refers to the amount of power per unit surface area. Irradiance = laser power output/laser beam size; therefore a larger beam size will have a smaller irradiance. The total energy applied is measured in joules, i.e. laser output × exposure time. The "energy fluence" is equal to Joules/laser beam size, and measures the total amount of energy directed to the tissue during a treatment.

Components of Surgical Lasers and Tissue Interactions

The components of a laser system are an active medium, a power source, an optical resonator, and an output coupler (partially transmitting mirror). The active medium is the material that determines the wavelength of the laser. The medium can be a gas, a liquid, a solid material, or a junction between two slabs of semiconductor materials. The power source is the pump that puts the atoms into a population inversion state and the particular type is determined by the lasing medium that is being used. The optical resonator can be thought of as mirrors on either side of the medium, which reflect the light back into the medium for "amplification." The output coupler allows a portion of the laser light between the two mirrors to leave the laser resonator in the form of a beam. The fraction of the coherent light allowed to escape varies greatly from one laser to another.

Laser light interacts with tissue in several ways. It can be absorbed, transmitted, or reflected. The reflected light can be specular (like a mirror) or scattered (diffuse). It can be transmitted through the tissue without having any effect, or it can be absorbed and transformed into heat energy. The amount of absorption is dependent upon the wavelength of the light and the chromophore content of the tissue (such as hemoglobin, keratin, protein, water, and melanin content). Each chromophore has its own absorption spectrum for different wavelengths of light. The thermal energy created through absorption can result in coagulation, cutting, or ablation of tissue. The primary colors of lasers (blue, green and red) are different to pigment colors (blue, green, and yellow) and light of one primary color will be absorbed by the other two colors. Therefore, the red pigment of hemoglobin readily absorbs the blue-green frequencies of the argon laser, making the argon laser effective for coagulation and ablation of superficial vascular lesions.

Whether lasers incise, coagulate, or vaporize tissue is both dependent upon factors that can be controlled (such as power density, duration of application, wavelength, and use of a contact versus non-contact technique), and also on factors that cannot be controlled but which influence which laser is used and also how it is used (such as absorption spectrum, scatter, thermal conductivity, and local circulation). The interaction of laser energy with tissue is by a thermal effect. If tissue is heated to over 60°C, protein is coagulated, and if tissue is heated to greater than 100°C, it begins to be vaporized. When the laser is used in contact fashion there is more focused energy application, less loss of power and less lateral thermal damage. With non-contact use of lasers, there will be a greater margin of ablation and coagulation. Therefore, a contact technique where the fiber touches the tissue will provide a more precise cut with less lateral damage, while a non-contact technique is more successful at ablating highly vascular tissue, such as ethmoid hematomas. Furthermore, a non-contact laser can be used focused or unfocused. The unfocused beam will have a decreased irradiance but an increased treated area, leading to a greater margin of coagulation. To cut tissues precisely, the use of a contact technique and keeping tension on the tissue will minimize lateral thermal damage. Also some lasers can be used in a continuous, pulsed or superpulsed mode. The pulsed modes will again minimize the extent of lateral thermal energy but in most cases a continuous technique is effective without excessive collateral damage.

Several different laser fibers are available with diameters of 400–1,000 μm. Most often, a 600–800 μm fiber (e.g. quartz or silica) is used. While special tips are also available for laser fibers, using a bare fiber is satisfactory. The cladding surrounding the laser fiber will burn back with use and the exposed fiber will soon become brittle. The fiber should periodically be broken off back to the level of the cladding to help prevent fibers breaking in the airway.

Laser Systems

Lasers are often referred to by the medium they contain, which determines the laser wavelength that is generated. Two lasers are presently used commonly in the equine upper respiratory tract, namely the diode laser and the Nd:YAG. These lasers have similar wavelengths of around 1,000 nm and the primary reason for their use is because they can be transmitted through a silica or quartz fiber that can be passed through the biopsy channel of a video-endoscope. A narrow diameter hollow fiber is currently being developed so that the CO_2 laser can be used trans-endoscopically, but this fiber has had limited clinical use to date (Anastassiou et al 2004).

The diode laser is created by producing a charge across two slabs of semiconductor materials and it has a wavelength of 800–1,100 nm. It is a solid-state system that does not require a cooling system, requires only a standard US household electrical current (110 V), is small (under 7 kg), and is less expensive than the Nd:YAG laser. A disadvantage is that the maximum power is usually less than 30 W. A bare fiber technique as previously described is most
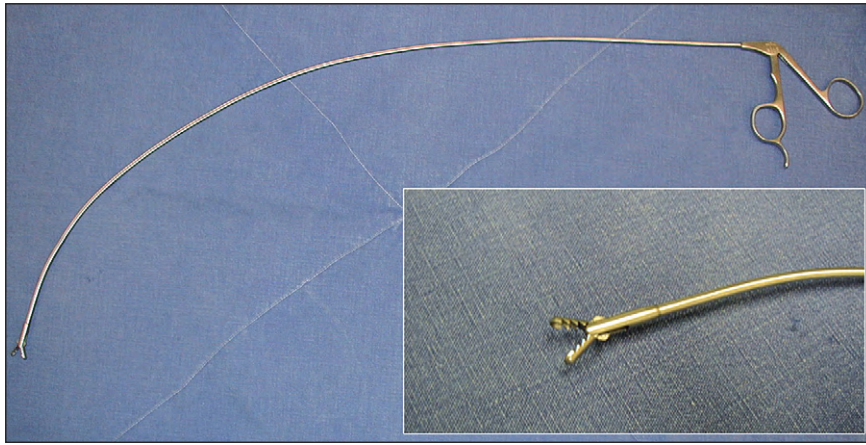
Fig. 39.1. The bronchoesophageal grasping forceps that have been bent to the contour of the nasal passage. The insert is a close-up of the jaws.

commonly used and the fiber can be cleaved (extended tip broken off) as the cladding is burned back during use.

The Nd:YAG laser is yttrium–aluminum–garnet (fake diamond) doped with neodymium (medium), which uses a xenon flash lamp or tungsten filament lamp for excitation of the medium; it is a larger machine that usually requires 240 V. It has a wavelength of 1,064 nm. While it is slightly more difficult to operate than the diode laser, the Nd:YAG can operate at a higher power setting of up to 100 W. Since the wavelength is similar, the coagulation and penetration properties of the Nd:YAG are very similar to those of the diode laser.

Preoperative Preparation

There are three major issues to consider before performing laser surgery, namely patient safety, personnel safety, and having appropriate instrumentation for the envisaged procedure. Most patient safety issues will be addressed by appropriate use of the laser and by minimizing the use of excessive energy to perform the procedure. Appropriate restraint can be achieved easily with intravenous sedation (xylazine 0.44 mg/kg, or detomidine 0.006–0.01 mg/kg) and topical anesthetic administered by a transendoscopic catheter. Depending on the length of the procedure, additional sedation may be required later. In the author's opinion, opioids such as butorphanol should be avoided because some horses will develop head twitching following their administration. It is not recommended that the horse's head be restrained with cross ties because alteration of the horse's head position is frequently required during laser surgery of the upper airways. If the laser has to be used while the animal is under general anesthesia, there is risk of spontaneous ignition of the oxygen in the endotracheal tube. Rather than attempting to shield the tube, the inhalant gas mixture can be changed to a

mixture of helium and oxygen, with less than 40% oxygen ($F_1O_2 < 0.4$). This gaseous mixture significantly diminishes the risk of spontaneous ignition without compromising the patient (Driessen et al 2002).

To prevent injury to personnel, several precautions should be taken. The surgery should be performed in a closed room with signs posted to prevent unauthorized personnel from entering the room during laser procedures. Ocular injury is the most significant risk and specialized glasses to block the specific wavelength of laser light being used should be worn by all personnel in the room. Noxious fumes as well as microbes may be released into the local environment if a large amount of tissue is being ablated in a non-contact fashion and therefore an adequate smoke evacuation system may be necessary. Smoke evacuation may be necessary for visualization while performing laser procedures in horses under general anesthesia. If the surgery is being performed in a standing sedated horse, the horse's breathing acts to ventilate the airway and remove most laser-created smoke or laser plume.

Finally, appropriate equipment must be available. As mentioned, it is uncommon that suction for smoke evacuation or hemorrhage is necessary, particularly in procedures performed in standing sedated horses but it is often required if working with horses under general anesthesia. If suction is needed, modifications should be made to the suction tubing to atraumatically place it into the upper respiratory tract. A 600-mm long bronchoesophageal grasping forceps (Richard Wolfe Medical Instruments, Vernon Hills, IL) is very useful for manipulation of tissues during upper respiratory laser surgery. These forceps are bent to the curve of the upper respiratory tract (Fig. 39.1) and can be passed up the contralateral nostril from the video-endoscope. It is beneficial to apply topical anesthetic to the nasal passage or perform an infraorbital nerve block on the side that the forceps will be passed.

Postoperative Treatment

Depending on the specific procedure performed, little postoperative medication or rest may be required before returning the horse to training. The horse should initially be maintained in a stall with hand walking only. Following simple laryngeal procedures, a topical anti-inflammatory medication applied via a nasal catheter can be used twice daily for 1 week. Systemic anti-inflammatory drugs (phenylbutazone 4.4 mg/kg and dexamethasone 0.04 mg/kg) are usually given only once perioperatively. Most horses should have a further endoscopic examination to ensure there are no remaining abnormalities, before returning to exercise at 2–3 weeks postoperatively.

For more complex laser procedures, or procedures performed on very fibrotic tissues, postoperative management can be more complex. Usually more rest time will be required before returning the horse to exercise and postoperative antimicrobial treatment is often administered, although it is uncommon for infection to develop at a laser surgery site within the respiratory tract. While the surgical site is essentially sterile at the time of surgery and generally has an excellent blood supply, it is an open wound that will become contaminated quickly. Furthermore, some chronic ulcerated tissues become fibrotic with a compromised blood supply and have the potential for foreign material (food) to become lodged within them. Complications most commonly occur following laser treatment of chronic epiglottic entrapment.

Procedures

Epiglottic entrapment

Epiglottic entrapments can be simple, with just a thin entrapping membrane, or more complicated, with thicker, ulcerated entrapping tissue and possibly the tip of the epiglottis rolled up in the entrapment. These variations do not affect the initial approach of an axial division of the membrane that is performed in the standing sedated horse, but may affect the treatment at the conclusion of the division (Parente 2002). A power setting of 16–18 W is sufficient when a laser is used in contact fashion. After sedation of the horse, local anesthetic is applied and the video-endoscope is positioned with its tip just 1–2 cm rostral to the tip of the epiglottis. The laser fiber is then advanced out of the endoscope for 2–3 cm. Before enabling the laser, the surgeon should ensure that the fiber can be dragged from the caudal edge of the entrapping membrane to the tip of the epiglottis, while keeping light pressure on the membrane with the tip of the fiber, as this will be the cutting stroke used for the division. If this stroke cannot be made, the horse's head position should be adjusted or the endoscope should be withdrawn and re-inserted via the ventral meatus, and not more dorsally.

Multiple thin cuts should be repeated in the same plane, extending from the center of the caudal margin of the entrapping membrane towards the tip of the epiglottis to perform the axial division. It is wise to make one or two axial strokes from the ventral surface of the entrapped epiglottis towards its tip in the early part of the division, before this part of the entrapping membrane moves ventrally under the soft palate. The strokes should be made in smooth, slow motions of 1–2 seconds duration, by withdrawing the laser fiber toward the biopsy channel. As each stroke is made, the membrane will peel back, exposing its deeper layers, and the entire membrane will begin to slide rostrally and ventrally.

Swallowing should be induced to encourage the membrane to move under the epiglottis and thereby maintain tension on the tissue. If the membrane recedes completely under the epiglottis, the horse is induced to swallow again to ensure the entrapment does not return. The procedure can be considered completed if the entrapment does not return after multiple swallows. Usually between 1,000 and 3,000 joules of energy are sufficient to accomplish the entire procedure. If after the division is complete, the membranes do not recede under the epiglottis, they can be initially treated medically and should resolve within 7–10 days. Alternatively, the sectioned entrapping folds can be resected by grasping them with a 600-mm long bronchoesophageal grasping forceps and then using the laser in contact fashion. If the epiglottis is normal in size, recurrence of entrapment is minimal. If the epiglottis is hypoplastic, up to 10% of affected horses may later have problems with dorsal displacement of the soft palate (Tulleners 1990).

Axial deviation of the aryepiglottic folds

Axial deviation of the aryepiglottic folds (ADAF) has been recognized as a cause of dynamic upper respiratory obstruction in horses since high-speed treadmill exercise testing was introduced for the evaluation of poor performance. The membranous portions of the aryepiglottic folds collapse axially, giving an hourglass appearance in front of the laryngeal airway, and they can occlude the glottis during the deep inspirations of fast work. The problem can be unilateral or bilateral and the folds may have a varying degree of axial collapse. Horses affected with significant ADAF not only finish races poorly but make an abnormal noise during inspiration at exercise, which may sound similar to the noise associated with recurrent laryngeal neuropathy. Resting endoscopy is normal, and the cause of the stridor and poor exercise performance cannot be determined without treadmill endoscopy.

Transendoscopic laser excision of the aryepiglottic fold tissue that impinges on the glottis is best performed in the standing sedated horse utilizing the laser in contact fashion. After topical anesthetic is applied, the video-endoscope is
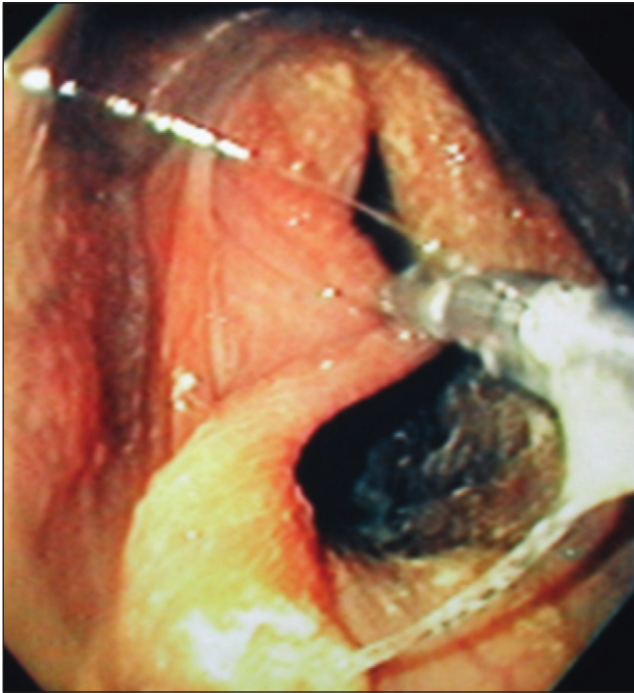
Fig. 39.2. The aryepiglottic fold being grasped and manipulated with the forceps to simulate axial deviation of the aryepiglottic fold before resection of the tissue.

inserted via the nasal passage ipsilateral to the target aryepiglottic fold. The tip of the endoscope is placed a few centimeters rostral to the surgical site and held in place there by an assistant. Bronchoesophageal forceps are passed via the nasal passage contralateral to the target aryepiglottic fold and are controlled by a second assistant. The free margin of the membranous portion of the aryepiglottic fold is grasped midway between the arytenoid and the epiglottis and manipulated medially to reproduce the ADAF deviation and to determine the margins of tissue to be resected (Fig. 39.2). The first cut is directed

horizontally through the membrane by sweeping the fiber from side to side and cutting the tissue in a rostral to caudal direction, immediately adjacent to its epiglottic attachment. The grasping forceps are then rotated to apply traction to the aryepiglottic fold in a rostromedial direction. A vertical incision is then made from dorsal to ventral, incising the tissue adjacent to its attachments on the corniculate process. The vertical incision is extended ventrally to intersect the initial horizontal incision and the resected tissue is then removed with the grasping forceps. The video-endoscope and forceps are positioned in the opposite sides for excision of the contralateral aryepiglottic fold when performing a bilateral excision.

No complications have been documented following this surgical procedure, and in particular, no adverse effects on deglutition or laryngeal or pharyngeal function have been reported. In a retrospective study of racehorses affected with ADAF, 75% of horses treated by laser excision showed improved performance (King et al 2001).

Pharyngeal cysts/masses

Cysts can develop anywhere on the wall of the nasopharynx, but most commonly occur on its dorsal surface. Pharyngeal cysts usually contain a yellow viscous fluid and often rupture during their surgical resection, and it is therefore important to ablate the entire cyst lining to prevent recurrence. The cyst can be grasped with bronchoesophageal forceps inserted through the opposite nostril and traction can then be applied to the cyst while it is resected. The cyst is easily resected using the laser in contact fashion, by stroking the fiber across the junction of the cyst with the pharyngeal wall. Other pharyngeal masses that would be extremely difficult to resect using standard surgical approaches can be approached similarly with the laser (Fig. 39.3).

An alternative approach is to ablate the cyst with the laser in non-contact fashion. Initially, the cyst is blanched
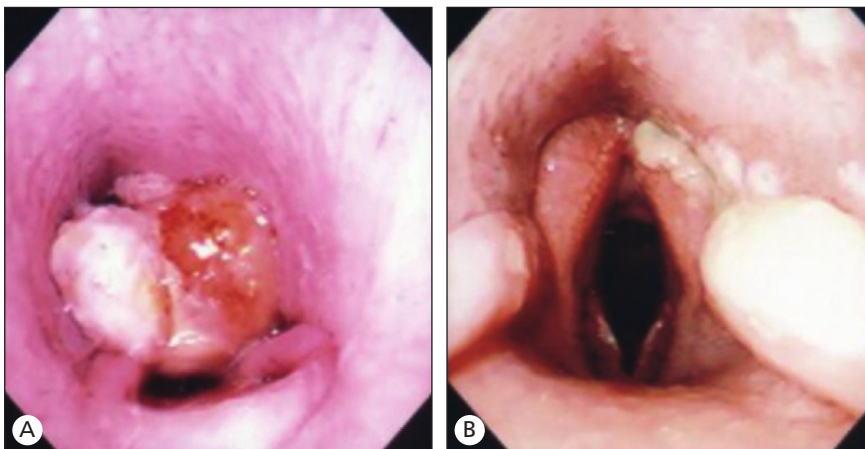
Fig. 39.3. (A) A granulomatous mass extending from the lateral pharyngeal wall in pony causing laryngeal obstruction. The inciting cause was unknown. (B) The same pony 2 days after laser excision of the mass performed with standing sedation and local anesthetic.

at a power setting of 40 W to prevent puncture, with the fiber held perpendicular to, and just millimeters away from, the cyst. With a sweeping motion of the fiber, the cyst is slowly blanched. The laser is then set to a higher setting of 100 W and the cyst is vaporized (Tate 1991).

Subepiglottic cysts/granulation tissue

Laser surgery of subepiglottic cysts can be challenging to perform. When the cyst is endoscopically visible above the soft palate, the horse can be sedated and the area anesthetized as previously described. Care should be taken not to cause the horse to swallow after the nasopharynx is anesthetized, until the cyst is firmly grasped with the grasping forceps. Once the cyst is grasped, it can be manipulated using tension, repulsion, and rotation so that its base can be visualized and then it can be resected with the laser fiber in contact fashion. If the cyst is accidentally punctured during the procedure, the lining membrane can still be visualized and resected. The subepiglottic tissue is highly elastic and care should be taken to avoid removing too much adjacent tissue with the cyst. If the cyst cannot be displaced above the soft palate for resection, the horse should be placed under general anesthesia and a similar procedure can be performed through the horse's oral cavity (Tulleners 1991). As noted, suction is required for smoke evacuation during laser procedures under general anesthesia.

Infrequently, horses will develop exuberant subepiglottic granulation tissue at the site of subepiglottic cyst resection. This granulation tissue is presumed to be a non-healing, contaminated wound and resection or vaporization of the abnormal tissue is required. Resection can be performed in the standing sedated animal, but, because of the friable nature of the tissue, the resection is often incomplete. Any residual granulation tissue should be vaporized in non-contact fashion at a power setting of 40 W. Again, care should be taken to avoid causing excessive collateral thermal damage to adjacent normal tissues, in particular the epiglottic cartilage.

Intralaryngeal granulation tissue

Removal of intralaryngeal granulation tissue with a laser can be an effective treatment, provided there is no significant underlying pathology of the arytenoid cartilage or continued trauma to the arytenoid secondary to hemiplegia. Laser excision is also a very effective way to remove any granulation tissue present after partial arytenoidectomy.

The horse is sedated and locally anesthetized as previously described. Non-contact laser ablation would likely result in excessive and unappreciated thermal damage to the larynx, and so contact laser excision is recommended. The video-endoscope is passed up the ipsi-

lateral nostril to the lesion and the laser fiber is dragged across the base of the granulation tissue in short strokes until it is almost completely loose, before attempting to grasp it. The grasping forceps are then passed up the contralateral nostril and the tissue is grasped under video-endoscopic guidance. Once grasped, just one or two more passes with the laser fiber should release the tissue for removal. Grasping the granulation tissue early in the procedure will just shred it, and will also stimulate the horse to swallow frequently. Recurrence is uncommon and the prognosis is good if there is no significant underlying laryngeal cartilage pathology (Hay & Tulleners 1993).

An alternative approach has been described in which the laser fiber is passed through a cannula inserted through the thyroid notch, i.e. the ventral aspect of the larynx. Again this is performed under video-endoscopic guidance. This technique has the advantage of facilitating resection of intracartilagenous tracts or abscesses that could not be treated via the standard *per nasum* trans-endoscopic approach (Sullins 2002).

Vocal cordectomy

Vocal cordectomy (cordectomy) is often performed as an adjunctive procedure along with a laryngoplasty, or is the sole treatment for cases of milder recurrent laryngeal neuropathy in non-racehorses. It can be performed in contact fashion, under general anesthesia or in the standing horse, based on the clinician's preference (Hawkins & Andrews-Jones 2001, Ducharme et al 2002, Parente 2002). Disadvantages of performing the procedure under anesthesia include the requirement of using heliox (to diminish the risk of spontaneous ignition of the oxygen within the endotracheal tube) and also the increased technical difficulty of having to perform surgery in the restricted nasopharyngeal space of anesthetized horses. Yet, with experience, a complete resection can be performed under general anesthesia in less than 10 min, with less lateral thermal damage than occurs with other techniques.

The horse is placed under general anesthesia with the vocal cord to be resected uppermost, and the horse is nasotracheally intubated via the ventrally positioned nostril. Oxygen concentration is decreased with helium to preclude the risk of ignition of the gases within the endotracheal tube. A speculum is placed in the horse's mouth and the horse's palate is manually displaced; suction tubing is placed just rostral to the more ventral vocal cord. The video-endoscope and the bronchoesophageal forceps are inserted through the mouth, and the targeted vocal cord is grasped. If it cannot be grasped because of the vertical orientation of the cord and the forceps jaws, an initial cut is made through the cord just below the vocal process with the laser. This results in a free horizontal plane of tissue that can be grasped. The forceps are rotated clockwise and used to move the cord away from the

saccule. The laser fiber is used to cut the vocal fold in contact fashion, in a dorsal to ventral direction. The most ventral and rostral aspect of the cord has a significant blood vessel that can obstruct vision of the medial surface of the cord if it hemorrhages; thus this portion of the cord is not cut until beginning the medial cut. The forceps are derotated to separate the two vocal cords slightly and the targeted cord is repelled caudally. This allows visualization and application of tension on the medial side of the cord so it can be transected. This is performed in a rostral to caudal direction, freeing the entire cord, so it can be removed with the forceps. A 4 × 4 gauze sponge tied to 40 cm of umbilical tape can be placed in the defect with the forceps to provide hemostasis while proceeding with a possible laryngoplasty. The gauze is removed just before the horse enters the recovery stall.

If the procedure is performed standing, a contact (Ducharme et al 2002) or non-contact (Hawkins & Andrews-Jones 2001) laser resection technique can be used. While there are no histology reports for postsurgical cordectomy sites of horses undergoing contact cordectomy, these wounds are presumed to heal quicker than the reported 47 days following non-contact surgery. The risk of complications is minimal with only four of 106 horses having complications associated with non-contact ventriculectomy in a study by Bristol et al (1994). Horses that have a contact laser vocal cordectomy can resume work within 1 month of surgery.

Guttural pouch tympany

A standard method of treatment of unilateral guttural pouch tympany is to establish a fenestration through the median septum or create an opening from the auditory tube into the nasopharynx (salpingopharyngeal fistula). Both of these procedures are more readily performed with the laser than other techniques (Tetens et al 1994, Tate et al 1995) in the anesthetized or standing sedated animal.

The septal fenestration is performed transendoscopically with the laser in contact mode at 16–18 W. A Chambers' catheter should be passed up the contrateral nostril and directed into the unaffected guttural pouch under video-endoscopic guidance. The endoscope is then passed into the affected guttural pouch. The Chamber's catheter is used to tent the median septum and then a 2- to 3-cm diameter defect is made in the septum with the laser. Care should be taken to create the defect rostrodorsally in the septum to minimize the risk of any inadvertent nerve or vascular damage (Fig. 39.4).

If the disease is bilateral, a salpingopharyngeal fistula should be created. Again, the catheter is used to enter the guttural pouch but this time the catheter is used to tent the auditory tube so it can be seen within the nasopharynx. A hole is then cut from the nasopharynx into the guttural pouch using the laser. The hole should be

dorsal and caudal to the normal guttural pouch opening. This defect may close later over many months, but may also persist, and there has been no reported increased risk of guttural pouch infection in horses with this permanent fistula.

Guttural pouch empyema

Cases of guttural pouch empyema can be managed in many ways, depending on the extent of the disease. Most cases can be managed with a combination of medical treatment (isolation to prevent the spread of strangles and feeding from ground level) and transendoscopic removal of any chondroids through the normal pouch opening. Infrequently a surgical approach, such as the modified Whitehouse, is required (see Chapter 28). Another alternative is to create a large enough opening into the guttural pouch from the nasopharynx to remove inspissated material (Tate et al 1995, Hawkins et al 2001). This salpingo-pharyngeal fistula can be created in the standing sedated horse as described above. Not only will this fistula allow removal of chondroids with the endoscope but also any small amounts of material remaining after surgery will naturally empty over several weeks (Fig. 39.5).

Progressive ethmoid hematoma

Intralesional treatment of progressive ethmoidal hematoma (PEH) with 10% formalin is probably the most common current treatment of those hematomas that are readily

Fig. 39.4. Septal fenestration within the guttural pouch as a treatment for unilateral guttural pouch tympany.

Fig. 39.5. A fistula created by transendoscopic laser surgery between the pharynx and the right guttural pouch to assist in the treatment of guttural pouch empyema.

visible endoscopically. This treatment can be employed fairly successfully on both large and small PEHs, but recurrence (and formation of new lesions) is common with this disorder. An alternative to intralesional injection is transendoscopic laser ablation (Tate 2002). A diode laser cannot be used because high power is needed for non-contact ablation, and an Nd:YAG is recommended.

The power should be set at 100 W in continuous mode, and the laser should be directed at the center of the lesion initially, to cause cavitation. Once cavitation is created, the laser fiber can be used to slowly enlarge the cavitation in concentric circles. Since there is significant scatter irradiation that will cause marked lateral thermal damage, the perimeter of the PEH should not be targeted. This technique will minimize the risk of adverse effects on adjacent normal mucosa while still causing necrosis of the perimeter of the lesion. Once a significant amount of char develops during the laser treatment, surgery should be stopped. The char will prevent absorption of energy to the underlying targeted tissue. The laser treatment should be repeated every other day to allow removal of char and necrotic tissue before further treatments. A success rate approximating 70% has been reported using this technique on both primary lesions and lesions recurring after surgery.

Rothaug and Tulleners (1999) also reported using the Nd:YAG laser for treatment of PEHs but their technique

was to use the laser in contact fashion intraoperatively during a flap sinusotomy. They used a power setting of 18–20 W and they reported a success rate of close to 70% for unilateral lesions and 50% for bilateral lesions

Soft palate displacement

Inducing soft palate alterations with the laser to reduce snoring in people and displacement in horses has been investigated recently (Hogan et al 2002, Wang et al 2002). The theory behind such surgery is to cause an alteration of collagen within the tissue that results in shortening and stiffening of the soft palate. Hogan et al (2002) reported on 52 racehorses that were suspected to be experiencing intermittent dorsal displacement of the soft palate during exercise. They used a diode laser transendoscopically set at 15 W and applied it for 1–2 seconds at 2- to 4-mm intervals along the free margin of the palate. These horses also underwent a sternothyroid tenectomy and were given anti-inflammatory medications for several weeks, but were returning to exercise within days. Based on owner/trainer assessment as well as racing times, they reported a 90% improvement in performance with this combined therapy.

Complications of Laser Surgery

The most likely complication of laser surgery is collateral thermal damage that may result in irreparable damage to adjacent tissues. The absorption coefficient with laser light in the spectrum of the diode and Nd:YAG is not as great as with the CO_2 laser; thus there will be greater scatter and heat transfer to adjacent tissues with use of the diode and Nd:YAG lasers. While the adjacent tissues may look normal at the end of the procedure, there can be a delayed necrosis, because of the thermal energy transferred to these tissues. This is in part why contact surgery is preferred in many situations, to concentrate the energy in a smaller focal spot. Yet, even with contact surgery, excessive energy can be applied to the tissues. This problem can often be avoided with experience and proper technique. Other complications are extremely rare and more specifically associated with the particular procedure that is being performed.

Conclusion

The transendoscopic use of lasers has revolutionized our approaches to upper respiratory surgery. It is a minimally invasive approach that yields excellent results with a shorter convalescence period and that can often be performed on an outpatient basis. As new technology develops with different lasers and improved video-endoscopic equipment, even more procedures will be performed transendoscopically.

REFERENCES

Anastassiou C, Dellemann G, Weisberg O et al 2004 Fibers deliver CO_2 laser beams for medical applications. Photonics Spectra 12: 162–164

Bristol DG, Palmer SE, Tate LP 1994 Complications of Nd:YAG laser ventriculectomy in the horse. Veterinary Surgery 23: 397

Driessen B, Nann LE, Klein LV 2002 Use of a helium/oxygen carrier gas mixture for inhalation anesthesia during laser surgery in the airway of the horse. In: Steffey EP (editor) Recent Advances in Anesthetic Management of Large Domestic Animals. International Veterinary Information Service, Ithaca (www.ivis.org)

Ducharme NG, Goodrich L, Woodie B 2002 Vocal cordectomy as an aid in the management of horses with laryngeal hemiparesis/hemiplegia. Clinical Techniques in Equine Practice 1: 17–21

Hawkins JF, Andrews-Jones L 2001 Neodymium:yttrium aluminum garnet laser ventriculocordectomy in standing horses. American Journal of Veterinary Research 62: 531–537

Hawkins JF, Frank N, Sojka JE et al 2001 Fistulation of the auditory tube diverticulum (guttural pouch) with a neodymium:yttrium-aluminum-garnet laser for treatment of chronic empyema in two horses. Journal of the American Veterinary Medical Association 218: 405–407

Hay WP, Tulleners EP 1993 Excision of intralaryngeal granulation tissue in 25 horses using a neodymium: YAG laser (1986–1991). Veterinary Surgery 22: 129–134

Hogan PM, Palmer SE, Congelosi M 2002 Transendoscopic laser cauterization of the soft palate as an adjunctive treatment for dorsal displacement in the racehorse. In: Proceedings of the 48th Annual Convention of the American Association of Equine Practitioners, Orlando, FL, pp.228–230

King DS, Tulleners EP, Martin BB Jr et al 2001 Clinical experiences with axial deviation of the aryepiglottic folds in 52 racehorses. Veterinary Surgery 30: 151–160

Parente EJ 2002 Transendoscopic axial division of epiglottic entrapment. Clinical Techniques in Equine Practice 1: 9–12

Rothaug PG, Tulleners EP 1999 Neodymium:yttrium-aluminum-garnet laser-assisted excision of progressive ethmoid hematomas in horses: 20 cases (1986–1996). Journal of the American Veterinary Medical Association 214: 1037–1041

Sullins KE 2002 Noninvasive removal of equine uroliths: laser lithotripsy. Clinical Techniques in Equine Practice 1: 36–38

Tate LP 1991 Application of lasers in equine upper respiratory surgery. Veterinary Clinics of North America; Equine Practice 7: 165–195

Tate LP 2002 Noncontact free fiber ablation of equine progressive ethmoid hematoma. Clinical Techniques in Equine Practice 1: 22–27

Tate LP, Blikslager AT, Little EDE 1995 Transendoscopic laser treatment of guttural pouch tympanities in 8 foals. Veterinary Surgery 24: 367–372

Tetens J, Tulleners EP, Ross MW et al 1994 Transendoscopic contact neodymium:yttrium aluminum garnet laser treatment of tympany of the auditory tube diverticulum in two foals. Journal of the American Veterinary Medical Association 204: 1927–1929

Tulleners EP 1990 Transendoscopic contact neodymium: yttrium aluminum garnet laser correction of epiglottic entrapment in standing horses. Journal of the American Veterinary Medical Association 196: 1971–1980

Tulleners EP 1991 Evaluation of peroral transendoscopic contact neodymium:yttrium aluminum garnet laser and snare excision of subepiglottic cysts in horses. Journal of the American Veterinary Medical Association 198: 1631–1635

Wang Z, Rebeiz EE, Shapshay SM 2002 Laser soft palate "stiffening": an alternative to uvulopalatopharyngoplasty. Lasers in Surgery and Medicine 30: 40–43

40 Tracheal Disorders

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and Niamh Collins

Introduction

Primary tracheal disorders are uncommon in horses; however, the tracheal mucosa is sometimes grossly inflamed in horses with pulmonary disorders, and with virtually all types of equine pulmonary disease the trachea will invariably contain excessive volumes of abnormal respiratory secretions (Dixon et al 1995, McGorum et al 2000). Consequently, endoscopic examination of the trachea for the above findings should be part of a full equine pulmonary examination

Anatomy of Trachea

The equine trachea is a rigid but flexible tube that is circa 75–80 cm long in adult horses of the larger breeds and extends from the larynx to the carina where it divides into the two main-stem bronchi at the level of the fifth to sixth intercostal spaces. The trachea has a median position in the neck and also in the rostral thorax, where it lies in a fold of mediastinum, but at its termination, the trachea is slightly displaced to the right by the adjacent aortic arch. The equine trachea is composed of 48 to 60 individual tracheal rings of hyaline cartilage. Each individual tracheal ring is circa 10–12 mm wide, and is 2–3 mm thick dorsally and 5–7 mm thick ventrally. The space enclosed by each tracheal ring is circa 6 cm wide and 5 cm deep (dorso-ventrally) in adult thoroughbred horses but the shape and size of the trachea can vary slightly, depending on the degree of trachealis muscle contraction. The rostral two to four tracheal rings are often slightly laterally flattened, and if this flattening is extreme, it can be termed "scabbard trachea". These laterally flattened tracheal rings can be recognized by their palpable, protruding ventral ridge, but there is rarely clinical airflow obstruction at this site. The next few cervical tracheal rings may be circular in outline, but the more distal cervical tracheal rings usually have a more oval shape, with a slight dorsoventral flattening. The thoracic portion of the trachea is often more cylindrical in shape and may return to a slightly laterally flattened appearance where it contacts the aortic arch.

The longus capitis muscles lie on the dorsal aspect of both the cervical and thoracic trachea. At its origin, the esophagus is situated dorsally to the trachea, and it moves to the left of the trachea at the level of the fourth cervical vertebra. In some horses, the esophagus may lie more ventral to the trachea at around the sixth cervical vertebra, sometimes even lying below the level of the trachea at this site. The esophagus then moves back to the left side of the trachea in the lower cervical area, remaining so until the level of the fourth thoracic vertebra, when it moves dorsal to the trachea, and then to the right of the medial plane, before passing through the diaphragm (Hare 1975).

The paired thyroid glands, which are often mobile and of variable size in horses, lie on the lateral aspects of the first few tracheal rings. The strap muscles (sternothyrohyoideus muscles) lie ventrally to the cervical trachea throughout most of its length and are fused midline, at an indistinct junction beneath the rostral aspect of the trachea. At this site they are overlain by the variably thick panniculus (cutaneous colli) muscle. Anatomical knowledge of these structures is important to enable an emergency tracheostomy to be performed efficiently. More distally down the neck, the sternothyrohyoideus becomes the separate sternohyoideus and sternothyroideus muscles. The paired omohyoideus muscles are fleshy, ribbon-like muscles that are positioned ventrolateral to the trachea more rostrally, where they blend with the sternothyrohyoideus and cover the first few tracheal rings and the larynx (Figs 40.1 and 40.2). Distal to the third or fourth tracheal rings, these muscles diverge laterally towards the scapula. Lower down the neck the omohyoideus muscles lie between the carotid artery and the jugular vein and they also separate the jugular vein from the trachea for much of their length. Whilst the omohyoideus muscles have to be divided midline to perform a laryngotomy, they have diverged lateral to the trachea at the usual tracheostomy site (third to fifth tracheal rings). In general the trachea is relatively superficial rostrally and becomes progressively deeper under the thicker cervical muscles in the distal neck.

Running bilaterally along the dorsolateral aspect of the trachea are many vital structures including the carotid artery, sympathetic trunk, vagus and recurrent laryngeal nerves, and tracheal lymph ducts and cervical lymph nodes. The arterial supply to the trachea is segmental, from branches of the common carotid and the bronchoesophageal arteries. Its venous drainage is provided by branches of the jugular and bronchoesophageal veins. Sensory innervation of the trachea is via the recurrent laryngeal nerves and
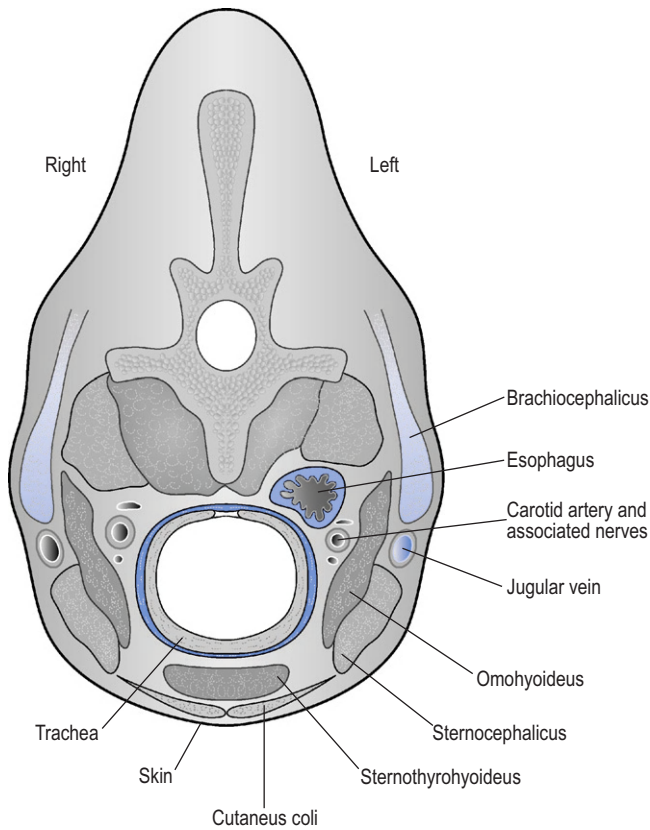
Fig. 40.1. Transverse section of the equine neck at the level of the fourth cervical vertebra showing the anatomical relationships of the trachea to adjacent structures. (From a drawing by M. Camburn.)

Right

Left

Brachiocephalicus

Esophagus

Carotid artery and associated nerves

Jugular vein

Omohyoideus

Sternocephalicus

Trachea

Skin

Sternothyrohyoideus

Cutaneus coli
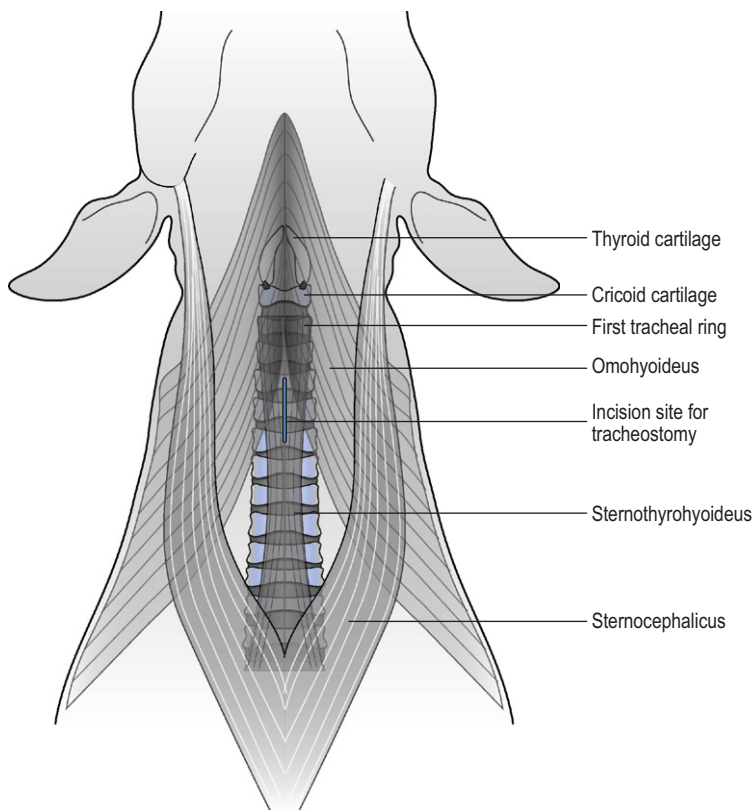
Fig. 40.2. Diagram of the ventral aspect of the cervical trachea and its relationships to the surrounding superficial muscles. The incision site for a tracheostomy is indicated. (From a drawing by M. Camburn.)

Thyroid cartilage

Cricoid cartilage

First tracheal ring

Omohyoideus

Incision site for tracheostomy

Sternothyrohyoideus

Sternocephalicus

other branches of the vagus; parasympathetic innervation is by branches of the vagus; and sympathetic innervation is by branches of the adjacent cervical sympathetic trunk. Lymphatic drainage is via the adjacent tracheal lymphatic trunk (Sisson & Grossman 1953).

Equine tracheal rings are incomplete dorsally, with the free ends of these rings sometimes overlapping in the cervical trachea but seldom meeting in the thoracic trachea. The free ends of the tracheal rings are connected by the dorsal tracheal ligament, which is composed of the transversely oriented trachealis smooth muscle that is attached to the inner (concave) surface of the tracheal rings, along with adjacent loose, areolar-containing connective tissue and the tracheal adventitia more dorsally (Bradley 1923). This elastic structure of the dorsal aspect of the trachea allows it to expand slightly during deep breathing, whilst the normally rigid nature of the tracheal rings prevents inspiratory collapse of the cervical trachea and expiratory collapse of the intrathoracic trachea.

The tracheal adventitia is the outer layer of the fascia that covers the cartilaginous rings and the intervening muscular and ligamentous structures of the larynx. It is attached to the deep tracheal fascia, which extends the length of the trachea to join the endothoracic fascia at the thoracic inlet (Hare 1975, Schummer et al 1979). Each tracheal ring is covered by and connected to adjacent rings by an intimately attached fibroelastic ligament, which is termed the annular ligament when it lies in the spaces between the tracheal rings. This arrangement of semi-rigid cartilaginous rings and elastic annular ligaments, along with the loose attachment of the tracheal adventitia to other tissues, gives the trachea great flexibility during head and neck movement. This movement is exemplified by the human trachea sliding in and out of the thorax during neck flexion. The cricotracheal ligament which attaches the first tracheal ring to the cricoid cartilage has a similar structure to the annular ligaments but is wider and under less tension. Definitive differentiation of the cricotracheal ligament from the annular ligament between the first and second tracheal rings is essential during laryngotomy.

The trachea is lined by a pale mucosa, containing numerous mucous and serous glands; it may have fine longitudinal folds. Ciliated epithelial cells are the predominant cell type in the equine large bronchi and trachea (Pirie et al 1990) with each cell containing about 200 cilia that beat 15–20 times/second. These cilia transport the mucus from the lungs proximally to the nasopharynx where it is normally swallowed. In the equine trachea, mucus is transported at a velocity of 13–20 mm/min (as reviewed by Dixon 1992), which is similar to tracheal mucus velocities in other mammals. In pulmonary disease, as a result of the increased production of more viscous respiratory secretions combined with decreased mucociliary clearance (primarily because of the loss of cilia), the respiratory secretions will accumulate in the trachea,

especially in the "sump-like" depression in the rostral thoracic trachea (Dixon et al 1995). Lowering of the head may play an important part in mucociliary clearance, especially in the presence of impaired mucociliary clearance and abnormal respiratory secretions, and preventing a horse from doing so by tying its head up for prolonged periods, such as occurs during prolonged air or road travel, may seriously compromise normal mucociliary clearance and thus predispose to pulmonary infections.

Endoscopy of the Trachea (Tracheoscopy)

With some pulmonary diseases, in particular with viral respiratory infections of the lower respiratory tract, and especially with aspiration tracheitis, e.g. choke or post laryngoplasty, the normally pale pink tracheal and bronchial mucosa may be swollen, reddened and have more prominent blood vessels. With most cases of chronic pulmonary disease, in particular recurrent airway obstruction, excessive volumes of mucopurulent respiratory secretions will be seen endoscopically in the tracheal lumen, but the tracheal mucosa will be of normal appearance, unless significant coughing is present, in which case the mucosa may be inflamed.

Tracheal cartilaginous nodules

Small, white nodular cartilaginous protrusions are endoscopically detected on the luminal aspects of tracheal cartilages, often throughout the length of the trachea, particularly in ponies (Chapter 1, Fig. 1.9B). No clinical significance should be attached to this developmental anomaly.

Post general anesthesia mucosal inflammation

Following endotracheal intubation, inflammation will always be present in the equine trachea (Holland et al 1986). If very excessive cuff pressure is used, there is a risk of severe ischemia of the tracheal mucosa (Touzot-Jourde et al 2005), and even of ischemic necrosis of the tracheal mucosa and deeper tissue with subsequent development of circumferential granulation tissue and later stricture, as is well documented in humans (Grillo et al 1995).

Extramural tracheal and bronchial compression

The cervical trachea can be compressed by grossly enlarged or abscessed adjacent lymph nodes (discussed later). Mediastinal tumors or grossly abscessed mediastinal lymph nodes may also cause compression of the intrathoracic trachea, or of one or both main-stem bronchi, and cause stridor and dyspnea.

Temporary tracheal collapse

In horses with severe expiratory dyspnea as the result of pulmonary disease, the raised intrathoracic pressure during expiration can cause a temporary collapse of the intrathoracic trachea, particularly of its dorsal ligament. Such temporary and often near total tracheal collapse can also be seen in all horses if they cough during bronchoscopy.

Left-sided intrathoracic tracheal deviation

A variable bulging of the caudal left side of the intra-thoracic trachea, circa 10 cm in front of the carina, is visible endoscopically in some horses. This is caused by the adjacent aorta pressing on the trachea and is of no clinical significance. On close observations the tracheal wall at this site may be seen to pulsate in time with the heartbeat.

Tracheal venous congestion

On endoscopy, the vasculature of the equine tracheal mucosa is not normally very prominent, but small, paired veins can usually be seen running up the lateral borders. Smaller venules or capillaries are visible on close inspection of the mucosa, especially around the carina. In horses with obstructed venous return to this area, such as from an intrathoracic mass or because of congestive heart failure, the lateral veins will become very prominent and possibly tortuous, and more prominent vessels will be apparent in the mucosa. Dyspnea, associated with the presence of frothy respiratory secretions and free fluid in the bronchi, may also occur in congestive heart disease.

Endoscopic appearance of the carina

The normally sharp appearance of the carina and of the bronchial bifurcations may become rounded and blunt as a result of mucosal swelling. The lumina of the main bronchi may narrow because of mucosal inflammation and/or bronchospasm, and this finding can be subjectively assessed endoscopically. In cases of pulmonary abscessation, it may be possible to identify exudate consistently flowing from an affected larger bronchus.

Cricotracheal Membrane (Ligament) Prolapse

The cricotracheal membrane is normally wider and under less tension than the tracheal annular ligaments. There are a limited number of clinical reports suggesting that excessive length and slackness of the cricotracheal liga-ment may allow it to prolapse deeply into the airway lumen as a result of the subatmospheric inspiratory airway pressures that occur during fast exercise. This prolapse is

said to significantly obstruct tracheal airflow and thus cause turbulence with resultant "noises" and reduced exercise performance (Pouret 1966, Goulden 1977). Goulden (1977) noted that affected horses had cricotracheal membranes up to 5 cm wide. This disorder is reputed to occur in thoroughbreds, which race with their head and neck in extension, thus tensing their cricotracheal ligament. Consequently, it is difficult to imagine how this prolapse occurs during fast exercise, unless there is gross slackness of this ligament or abnormally negative inspira-tory pressures within the rostral trachea, such as are caused by an intercurrent upper respiratory tract obstruc-tion. Surprisingly, this disorder does not appear to be reported in standardbreds, which often race with their necks flexed.

The reported treatment of cricotracheal membrane prolapse is to perform a transverse skin incision over the cricotracheal ligament followed by resection (imbrication) of much of the ventral aspect of this membrane and closure using mattress sutures. The aim of surgery is to leave a residual cricotracheal membrane circa 1.5 cm wide (Goulden 1977). There is little factual evidence on the significance of cricotracheal membrane collapse in exer-cising horses, nor of the response of affected horses to the above treatment.

Lateral Tracheal Flattening

Lateral tracheal flattening (scabbard trachea) occurs to some degree in the first few tracheal rings of many thoroughbreds and thoroughbred crosses but rarely, if ever, will this deformity cause clinical airflow obstruction. This condition can be recognized by palpation of the ventral aspect of the trachea in this region through the overlying muscles; distinctive ridges can be palpated on the ventral midline aspect of the deformed rings. Endoscopy will confirm this slight lateral flattening of the upper tracheal lumen, and will invariably show an adequate airway. No treatment is required for this deformity.

Dorsoventral Tracheal Collapse

Dorsoventral tracheal collapse is commonly recorded in many breeds of toy dogs (Dallman et al 1988, White 1995, Rudorf et al 1997) and can also occur in goats (Jackson et al 1986), and sheep and cattle (P.M. Dixon, personal observations). In Equids, this developmental dis-order mainly occurs in small ponies, especially Shetlands (Delahanty & Georgi 1954, Dixon 1988, Simmons et al 1988, Mair & Lane 1990) and miniature horses (Simmons et al 1988, Couteil et al 2004) but also occurs in larger breeds of ponies (Carrig et al 1973) and donkeys (Mair & Lane 1990, P.M. Dixon, personal observations). In many small ponies, the disorder remains undiagnosed because of both the lesser degrees of tracheal collapse and the usual
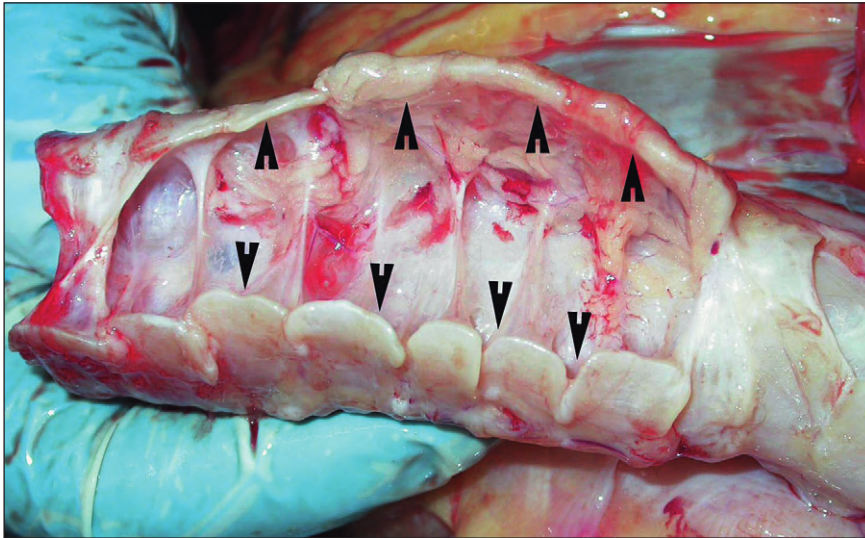
Fig. 40.3. Dorsal view of the intrathoracic trachea from a donkey which shows an abnormal vertical alignment of the (normally overlapping) free ends of the tracheal rings on both sides over seven tracheal rings (arrowheads). In this area the dorsal ligament is inserted circa 3–4 cm ventrally from the free ends of the tracheal rings.

low workload of these animals and thus it is an incidental post-mortem finding in many small ponies. This disorder appears to have a high prevalence in donkeys where it is usually asymptomatic, unless severe tracheal deformity or intercurrent pulmonary disorder, such as advanced pulmonary fibrosis, is also present (P.M. Dixon, personal observations).

Dorsoventral tracheal collapse is usually caused by a cartilage ring deformity, such that the normal rounded or oval tracheal ring is replaced classically by a flatter tracheal ring with a greatly reduced dorsoventral distance (Figs 40.3 and 40.4) that is further reduced by excessive movement of the slack and enlarged dorsal tracheal ligament (Delahanty & Georgi 1954, Carrig et al 1973, Dixon 1988). Many ponies and donkeys with tracheal collapse will have not only some dorsoventrally flattened tracheal rings (especially in their lower cervical trachea) but also other types of ring deformities at different sites (Fig. 40.4) that can also restrict the tracheal lumen size (Mair & Lane 1990, P.M. Dixon, personal observation). The free ends of some affected tracheal rings may protrude dorsally with the dorsal ligament attached 2–4 cm from the free ends of the tracheal rings and thus greatly decreasing the lumen of the trachea (Figs 40.3 and 40.4). In other affected cases with tracheal rings of relatively normal shape, there appears to be separation of the dorsal ligament from the dorsal aspect of the tracheal rings. This defect normally involves the mid and distal cervical, as well as the intrathoracic trachea, and may occasionally involve the main-stem bronchi (Fig. 40.4). Some affected ponies may also have some degree of tracheal rotation, with the dorsal aspect of trachea facing dorsolaterally (P.M. Dixon, personal observation).

The equivalent disorder in dogs has been associated with hypocellularity and decreased chondroitin sulfate content of the tracheal cartilage (Dallman et al 1988), suggesting that an inherited defect in chondrogenesis is present. There do not appear to be any studies of tracheal cartilage composition in Equids. However, most ponies develop clinical signs of tracheal collapse in middle age (Freeman 1991), thus indicating that some degenerative process of the tracheal cartilage or connective tissue (including separation of the dorsal ligament from the cartilage) may play a role in the pathogenesis of this disorder, in addition to the apparently developmental cartilage deformity. Some deformed rings in ponies may be excessively ossified (Mair & Lane 1990). The presence of chronic airway disease, which may cause tracheal mucosal thickening and also increase intrapleural pressure swings, and neuromuscular problems causing loss of tone of the trachealis muscle have also been implicated in the pathogenesis of this disorder in dogs (Hobson 1976, White 1995). Carrig et al (1973) proposed the latter as a potential etiology for tracheal collapse in ponies. A grading system has been used to structurally classify the severity of the disorder in dogs but because of a greater variation in tracheal ring shape in ponies with this disorder such a grading system is unlikely to be of clinical value.

The typical clinical signs in ponies are dyspnea and stridor, which commonly occur in hot or humid weather and may regress in autumn. This stridor may be very loud, with Delahanty & Georgi (1954) reporting a pony that made sounds like a truck horn that could be heard over 800 m away! The stridor is loudest when auscultated directly over areas of tracheal obstruction. Severe dyspnea may occur even at rest, because hyperventilation will exacerbate the partial tracheal obstruction in a self-perpetuating cycle. Exercise-induced pulmonary hemorrhage may occur in severe cases, presumably because of increasingly subatmospheric intrathoracic pressures. Coughing may also occur and it is unclear if this is the result of tracheal inflammation caused by abnormal
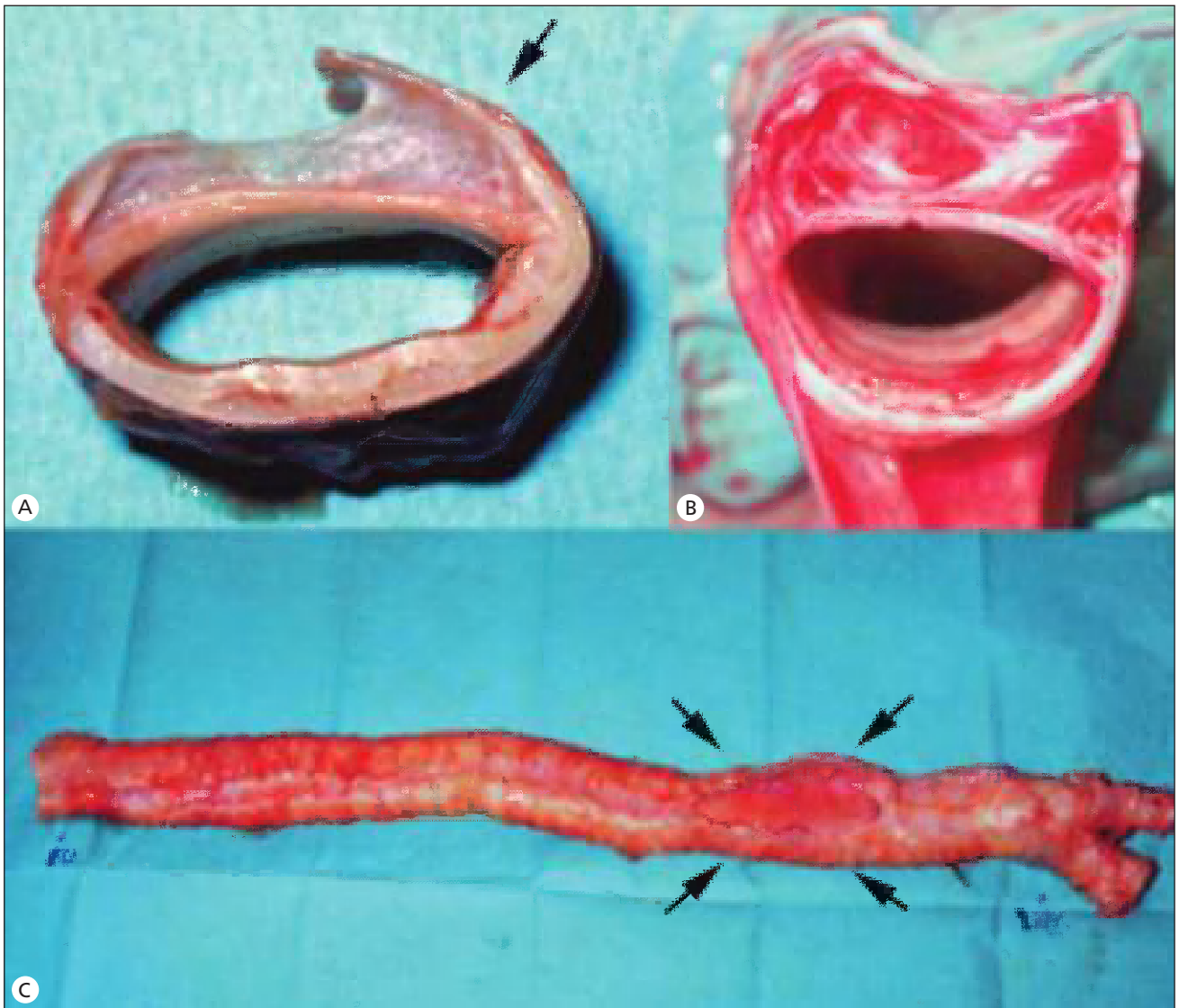
Fig. 40.4. (A) Cross-section of trachea showing separation of the dorsal ligament from the tracheal rings that are aligned normally on the left-hand side but slightly vertically misaligned on the right-hand side (arrow). The ventrally positioned dorsal ligament greatly reduces the cross-sectional area of the tracheal lumen. (B) Cross-section of the intrathoracic trachea of a donkey, showing the dorsal ligament attached at an abnormal, ventral site on the vertically positioned free aspect of the tracheal rings. (C) Dorsal view of the trachea of a pony showing a relatively normal configuration of the tracheal cartilages in the cervical and rostral thoracic area. There is a more vertical alignment and separation of the free ends of the tracheal rings in the mid thoracic region leading to a flattening of the trachea (arrows). There is also dorsoventral flattening of the left main bronchus (lower bronchus in figure).

contact of dorsal and ventral tracheal mucosa, or of underlying pulmonary disease that may have precipitated the clinical problem. In contrast, small dogs primarily present with coughing rather than stridor, with one report noting stridor in only 20–30% of dogs (White 1995).

Palpation of a collapsed cervical trachea may reveal a distinct, even sharp, edge on the lateral aspect of a trachea at the site where the rounded, lateral aspect of a normal trachea should be palpated. Additionally, the deformed trachea will usually not roll away with digital pressure. Palpation of the lateral edges of the flattened trachea can

be difficult, as the deeper, distal cervical trachea is most commonly affected. Additionally, some small ponies such as Shetlands have very thick skin and much subcutaneous fat, which make palpation difficult. As noted above, some affected ponies do not have this classical dorsoventral flattening, but have relatively normally shaped tracheal rings (possibly vertically aligned free ends of the tracheal rings), with separation of the dorsal ligament, while others have tracheal rotation and thus palpable abnormalities.

Endoscopy of affected cases will show the normal circular/oval appearance of the tracheal lumen to be

replaced by a wider, flatter and restricted lumen. Additionally, excessive dynamic movement of the dorsal tracheal wall and even airway occlusion may be observed during deep breathing, with inspiratory narrowing of the cervical tracheal lumen and expiratory narrowing of the intrathoracic tracheal lumen. The mucosa may be red and swollen so further compromising the tracheal lumen.

Lateral radiographs of the distal cervical and thoracic trachea may be diagnostic, demonstrating the flattened appearance of the trachea (Dixon 1988), but this may give a misleading normal appearance if a collapsed intrathoracic trachea is radiographed during inspiration, or a collapsed cervical trachea is radiographed during expiration. Radiography can also be misleading in cases where tracheal rotation is present along with collapse, where a widened (but collapsed) trachea will be found on lateral radiographs. The use of an image intensifier or of serial radiographs, taken at determined times of the respiratory cycle, could overcome the above limitations. Ultrasonography has been successfully used in the diagnosis of tracheal collapse in dogs (Rudorf et al 1997).

Treatment of such cases should initially be conservative, by restricting exercise and keeping affected cases indoors in cool boxes in hot weather and treating any intercurrent respiratory disease. Acutely affected horses will benefit from intranasal oxygen therapy that will reduce hyperventilation and thus decrease the degree of tracheal collapse. Severely affected cases may be hyperthermic, which increases the metabolic rate and thus respiratory rate, causing a vicious circle. Cooling down such ponies with a cold-water hose until their temperature is normal is beneficial. Parenteral corticosteroid therapy is also indicated in severely affected cases, to reduce the secondary tracheal mucosal inflammation that is likely to be present.

Surgical correction of affected cartilaginous cervical tracheal rings (as is commonly performed in miniature dogs) such as placing external stents (made from appropriately sized plastic tubing strips) over deformed tracheal rings (Simmons et al 1988) or inserting stainless steel sutures into every third tracheal ring along with plication (tucking in) of the slack dorsal membrane (Delahanty & Georgi 1954) has been performed in ponies with limited success. However, in many ponies, altering the shape of the tracheal rings may not be successful, because separation of the dorsal ligaments from the cartilage rings is the primary problem. Additionally, careful evaluation will usually reveal involvement of the intrathoracic trachea and, because only three or four intrathoracic rings can be exteriorized rostrally to the distal cervical area for surgical correction, this may be of limited benefit to the horse. Whilst intrathoracic surgery to correct more caudal tracheal deformities is technically feasible, it is not practical. Thus the sole correction of affected cervical tracheal rings is unlikely to be of major clinical benefit if widespread intrathoracic tracheal collapse is concurrently present.

An endoscopically introduced, expanding intraluminal stent has been recently used in a 7-month-old miniature horse foal to correct a life-threatening 7-cm-long segment of collapsed distal cervical and rostral thoracic trachea (Couteil et al 2004). The folded, self-expanding, metallic stent was placed in position under general anesthesia and expanded *in situ*. The stent later migrated distally but was repositioned endoscopically. Lower airway infection developed some months later, possibly caused by disruption of tracheal mucociliary clearance caused by multiple nodules of granulation tissue protruding through the mesh. Following antibiotic therapy and later, removal of the large nodules by electrocautery, resolution of the lower airway infection occurred and thus the repair was considered successful.

Other Congenital Defects of the Trachea

Congenital defects of the trachea, other than tracheal collapse, are rare in horses with only a few other types of deformities recorded, including a few reports of accessory or ectopic pulmonary tissue. For example, Davis et al (1991) recorded an accessory cervical tracheal bronchus in a 3-day-old foal. The foal presented with a large, air-filled sac in its cervical region caused by a rudimentary accessory lung that contained cartilaginous tissue and ciliated epithelium. Peek et al (1995) reported a combined tracheal and esophageal duplication cyst in a 4-month-old Arabian foal with a slowly developing cervical swelling that was successfully resected.

External Tracheal Trauma

Tracheal trauma is usually caused by blunt trauma, such as kicks to the rostral neck region that can cause severe tracheal damage, particularly from compression of the trachea between a hoof and the cervical vertebral body. Tracheal trauma can cause tearing of the mucosa and of the overlying annular ligaments, and fracture of tracheal rings. Penetrating tracheal injuries occur less commonly, such as by horses running into sharp objects including fence posts. In dogs and humans, sudden and severe chest trauma against a closed glottis can cause a massive increase in intratracheal pressure leading to tracheal rupture (Feat et al 2002) that often occurs at the junction of the distal trachea and main-stem bronchi but this injury does not appear to have been reported in horses.

A common presenting sign in horses with tracheal rupture is extensive subcutaneous emphysema and swelling over the rostral aspect of the neck (Scott 1978, Caron & Townsend 1984, Fubini et al 1985). External skin wounds are not always present. The emphysema may later spread to involve the head, trunk, and even limbs. Horses may later become stiff, and possibly febrile from secondary infection,

and epistaxis and coughing may also occur (Caron & Townsend 1984). In addition to possibly having a ruptured trachea, horses with emphysematous cervical swellings should always be suspected of suffering from a ruptured esophagus from identical trauma (Risnes & Mair 2003, P.M. Dixon, personal observation). Because the esophagus may lie ventrolateral, or even ventral, to the trachea at around the level of the sixth cervical vertebra in some horses, it is prone to trauma at this site. Penetrating skin wounds of the axilla can also lead to marked emphysema of the cervical area, but clinical examination will readily differentiate this type of injury from cervical tracheal or esophageal injury.

Cervical radiography of horses with tracheal or esophageal rupture will show extensive gas-infiltration subcutaneously, and along the fascial planes of the neck, in addition to variable soft tissue or fluid-filled swellings of the rostral and ventral neck regions. Thoracic radiography will also reveal pneumomediastinum and possibly pneumothorax. Endoscopy (tracheoscopy) will confirm if tracheal trauma has occurred, with evidence of mucosal tears, mainly on the dorsal and ventral aspects of the trachea (especially overlying the annular ligaments); possible prolapse of submucosal tissue into the tracheal lumen; distortion or partial occlusion of the lumen as a result of cartilage ring fractures; and/or local inflammation (Caron & Townsend 1984, Fubini et al 1985, authors' personal observations). Esophageal endoscopy to examine the integrity of the esophagus should *always* be performed on horses with neck swelling and emphysema that have had suspected neck injury. If the esophagus is ruptured, the prognosis is poor. The accumulated saliva, food and exudates should be drained ventrally and the esophageal wound left to heal by secondary intention, possibly with an indwelling nasogastric tube in the distal esophagus for 2–3 weeks, along with appropriate supportive therapy. If the cervical swelling and emphysema are solely the result of tracheal rupture, the prognosis is much better. In many instances of tracheal trauma with emphysema, the small tears of the tracheal mucosa will heal spontaneously. In addition to tetanus prophylaxis, non-steroidal anti-inflammatory agents should be administered to such cases to decrease the traumatic inflammation, along with broad-spectrum antibiotics to reduce the potential spread of pathogenic bacteria with entrapped air along the fascial planes. The emphysema present in such cases will usually resolve in 1–2 weeks, but may remain for up to a month in some horses (Caron & Townsend 1984).

Large rents in the tracheal mucosa with extensive herniation of soft tissue into the tracheal lumen can be sutured as described by Fubini et al (1985), who dissected the trachea free at the injury site and rotated it to allow suture repair of a progressively enlarging intratracheal hernia of the dorsal aspect of the trachea that contained trachealis muscle and subcutaneous tissue.

Fractures of the tracheal rings with gross distortion of the trachea may also occur and it may be difficult to evaluate the degree of cartilage ring damage in the presence of major local inflammation and emphysema. If available, computed tomography or magnetic resonance imaging of such lesions is the optimal method of assessing cartilage damage. If life-threatening tracheal obstruction occurs, a temporary tracheostomy can be performed more distally, depending on the site of the tracheal damage. If the site of a traumatic tracheal obstruction is so distal that a temporary tracheostomy cannot be performed, a more proximal tracheostomy can be performed and an endotracheal tube can be inserted through the tracheostomy and then manipulated through the damaged lumen into the thoracic trachea. Such tubes should be made of soft rubber without sharp edges to help prevent exacerbation of any mucosal ulceration and the subsequent development of a circumferential stricture. Additionally, such tubes should not have an inflated cuff because the lumen of such tubes will soon accumulate dry exudates (both from tracheal wound exudate and normal lower airway secretions) that will then cause an intraluminal obstruction that could prove fatal if the cuff on the tube is inflated. Finally, these tubes should not be advanced to the level of the carina, in case they enter a main-stem bronchus and bypass airflow to the other main-stem bronchus, causing a unilateral lung collapse with serious consequences.

Granulation tissue will usually develop at sites of mucosal damage but if focal it will contract when it subsequently becomes covered in mucosa. However, if the trauma has damaged the cervical tracheal mucosa circumferentially, or over most of its circumference, there is a risk that the granulation tissue that develops over these wounds may develop into a ring, or into a broad band of fibrous tissue over the following months, as occurs with laryngeal mucosal trauma (Chapter 38). A circumferential fibrotic ring is more serious than a partial circumference transtracheal band, because fibrotic rings may progressively contract into a smaller stricture that may eventually reduce the tracheal lumen to 1–2 cm in diameter (Fig. 40.5), leading to respiratory obstruction with stridor at rest, and possibly death by asphyxiation. After allowing the traumatic tracheal inflammation to subside, it may become obvious in other cases that resection of the damaged tracheal rings is required, as described later. If such damage involves only two or three tracheal rings, it may be possible to fully resect the damaged segment, and then appose the ends of the trachea. If more extensive damage occurs (i.e. if more than four tracheal rings are involved), it will be difficult to appose the normal tracheal segments without having excessive tension on the suture line. Such wounds will be prone to dehiscence and the later development of granulation tissue, leading to further fibrosis at the anastomosis sites. The prognosis for horses
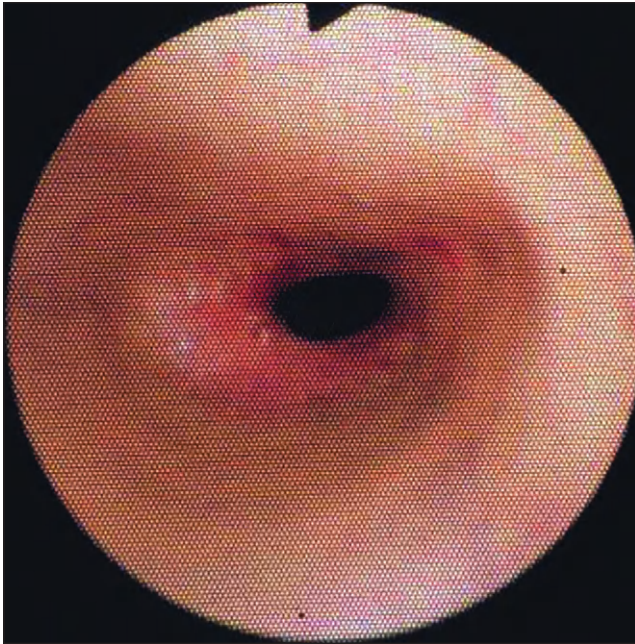
Fig. 40.5. Upper cervical trachea of a horse that sustained a kick injury to this region some months previously. At the time of injury, extensive emphysema and inflammation were present over the neck. The horse made an apparent clinical recovery, but subsequently developed stridor and exercise intolerance. The traumatic circumferential mucosal injury has healed with a fibrous stricture that has a diameter of circa 2 cm at the site of the stenosis.

with such wide areas of traumatically damaged cartilage rings is very poor, unless the damage is in the rostral cervical trachea and a permanent tracheostomy can be placed more distally.

Solid, chondroma-type swellings occasionally develop several months after traumatic damage to tracheal rings

and also following tracheostomy. These may enlarge to such an extent (up to 7–10 cm in diameter), that they cause almost total tracheal lumen obstruction (Fig. 40.6). Such solid swellings may also extensively distort adjacent tracheal rings. Following excision of these cartilaginous swellings, including the involved tracheal ring(s), mucosal stricture or tracheal collapse (as a result of the absence of tracheal ring structural support) may then develop. Consequently, such cases should have the affected rings fully removed and an end-to-end tracheal anastomosis performed, if feasible.

Focal Intraluminal Tracheal Lesions

Apart from granuloma formation post tracheostomy, focal intraluminal tracheal lesions are rare in horses. Charlton & Tulleners (1991) reported infected/necrotic midcervical tracheal proliferative lesions in two adult horses following intratracheal antibiotic administration in one, and following a transtracheal aspiration in the other. The masses were resected using a transendoscopic laser in one horse and combination of transendoscopic laser and conventional surgery in the other.

Tracheal Resection and Reconstruction Techniques

The trachea is flexible and mobile in its fascial attachments and so, in humans, excellent mobilization of the trachea can be achieved using tension-relieving sutures, allowing over half of the trachea to be resected with primary anastomosis achieved. The adjacent vital structures, in particular the recurrent laryngeal nerves, must be identified and protected during tracheal resection. Tate et al

Fig. 40.6. The ventral sections of two tracheal rings from either side of a temporary tracheostomy in a horse with *Streptococcus equi* var. *equi* (strangles) retropharyngeal abscesses. These firm, non-suppurative cartilaginous swellings enlarged over several months following the tracheostomy and caused gross tracheal obstruction (1 cm markers).

(1981) successfully resected three tracheal rings in each of three normal horses using the following technique, but removal of five rings in a further horse was unsuccessful because of excessive tension on the suture line. A 40-cm ventral midline skin and cutaneous colli incision is made, the sternothyrohyoideus muscles are separated, and careful blunt dissection is continued to fully isolate the trachea. Where possible, the affected tracheal cartilages are resected submucosally, without entering the tracheal lumen. Once the affected tracheal area is resected, the endotracheal tube is immediately advanced down to the distal segment. The mucosa is folded back over the two adjacent normal tracheal rings and sutured to their margins. The thick adventitia of the equine trachea will hold sutures whilst mucosal and cartilage sutures are being placed. The tracheal ends are temporarily maintained in place with towel clips while steel wire or monofilament nylon partial thickness, cartilaginous sutures are inserted. Tate et al (1981) also performed this technique on two clinical cases. One clinical case with post-tracheostomy cicatrix had five tracheal rings removed, with the use of a martingale to restrict neck movement postoperatively and a return to work at 8 weeks post surgery. A second clinical case died within 2 days of surgery as a result of myopathy. Necropsy on this case showed the tracheal anastomosis to be airtight. No iatrogenic laryngeal paralysis was observed in the four experimental and two clinical cases operated on by Tate et al (1981).

More extensive tracheal resection was performed by Carrig et al (1973) who resected 45 cm of cervical trachea in a horse with tracheal collapse and replaced it with a reinforced polyvinylchloride corrugated hose that was inserted 3 cm into both ends of the resected trachea. However, 3 months after surgery, granulation tissue formed at the proximal end of the prosthesis and was surgically removed. Subsequently (6½ months later), the pony was euthanased because of the reformation of intraluminal granulation tissue at the proximal end of the prosthesis.

Robertson & Spurlock (1986) operated on a 6-month-old standardbred foal that had had four midcervical tracheal rings fully sectioned midline for a tracheostomy at 2 months of age because of a *Streptococcus equi* cervical abscess. The cut rings had later inverted into the lumen with fibrous tissue formation that connected the cut ends obstructing the lumen. A fifth adjacent ring had a chondromatous thickening ventrally. The four inverted cartilages were partially sectioned on each side of the deformity and monofilament nylon sutures were inserted in the rings ventral to the cartilage incisions. External support made from 2- to 5-cm wide sections of a 60 ml polypropylene syringe barrel that was cut in half longitudinally were sutured over the ventral half of the deformed trachea, through appropriately placed holes. The wound was fully closed and a suction drain was inserted

for 24 h. The horse raced successfully as a 2-year-old. Yovich & Stashak (1984) used a similar repair to support tracheal rings that were deformed and eroded by a lipoma in an aged gelding.

Extramural Tracheal Compression

Occasionally, enlarged lymph nodes compress the trachea causing stridor. These lymph node abscesses are usually caused by *S. equi* infection (Randall & Myers 1973) and may involve the upper cervical chain, compressing the proximal cervical trachea. They can also involve the distal cervical trachea at the chest entrance (Randall & Myers 1973, Rigg et al 1985, Tessier et al 1996, P.M. Dixon, personal observation). Diagnosis is by a history, and possibly by intercurrent clinical signs, of strangles and confirmation can be by needle aspiration and/or ultrasonography of cervical swellings. Following local anesthesia of the overlying skin and subcutaneous tissues (except in emergencies), the abscessed lymph nodes should be drained. The cavity of the abscess should then be digitally explored and, if loculated, it should be opened into a common chamber. The abscess cavity should then be lavaged with dilute povidone iodine solution, having ensured that the drainage wound is large enough to prevent premature closure (Rigg et al 1985). As viable *S. equi* bacteria are commonly present in these abscesses, the clinical examination of such cases, and in particular endoscopy and surgical drainage, must be carried out with strict attention to hygiene and all protective clothing, equipment and premises must be thoroughly cleaned and sterilized.

Mediastinal tumors, mainly lymphosarcomas, or other cranial thoracic tumors including primary lung tumors, or grossly abscessed mediastinal lymph nodes may also cause compression of the intrathoracic trachea, or of one or both main-stem bronchi and thus cause wheezing and dyspnea. Such an endoscopic finding should lead to a careful thoracic examination, including radiography and ultrasonography. Thoracic tumors are usually untreatable in the horse.

Tracheobronchial Foreign Bodies

Inhaled foreign bodies are common in humans and dogs, but less common in horses because of their height. O'Connor (1950) described a range of laryngeal and tracheal foreign bodies in horses and stated that a fatal bronchopneumonia could develop if the foreign body was not coughed up. As noted (Chapter 38), long thin twigs or other long plant material will very occasionally be inhaled and, if they manage to bypass the larynx, they will become entrapped at the carina or in the main-stem bronchi (Lane 1981, Urquhart et al 1981, Brown & Collier 1983, Ferrucci et al 2003), predominantly the

right main-stem bronchus (Freeman 1991, P.M. Dixon, personal observation). If the branches, short leaves or thorns of these plants are directed rostrally, they will become progressively embedded in the airway wall like fishhooks, and are unlikely to be coughed out. Indeed, it is even difficult to retrieve such foreign bodies transendoscopically.

A history of sudden onset of continuous coughing is a very prominent finding in these cases. Malodorous breath will often be present. Other signs of pulmonary disease, such as increased respiratory rate or the presence of abnormal lung sounds, are usually absent, at least initially, unless secondary lung infection occurs. If significant pulmonary infection develops, abnormal lung sounds and possibly purulent, or blood-tinged, nasal discharge may occur. No response or only a temporary response (decreased nasal discharge) may occur following antibiotic therapy. Radiography may show a localized interstitial pattern caudal to the ventral cava (Brown & Collier 1983).

Endoscopically, local airway inflammation and purulent respiratory secretions may obscure a foreign body, especially shorter pieces of vegetation that are fully embedded within a main-stem bronchus. These types of object may significantly obstruct airflow and also obstruct the normal drainage of respiratory secretions from the affected bronchus and so induce severe, focal pulmonary infection. If the foreign body is not visible, culture of purulent exudate from the affected area will often reveal high numbers of a wide range of organisms that are unresponsive to antibiotic therapy. Such bacteriological findings in the presence of focal, purulent pulmonary disease should suggest that some predisposition to the local pulmonary infection is present. In such cases, the affected area should be transendoscopically lavaged to help determine if a bronchial foreign body or tumor is present.

When identified, bronchial foreign bodies may be removed using transendoscopic grasping or basket forceps. As noted, cranially facing branches and thorns will make the recovery of such vegetation difficult transendoscopically, and the midline stem may become inadvertently progressively broken off when grasped with the forceps. With persistence, most bronchial foreign bodies will eventually become loose and retrievable (Fig. 40.7). If a solid area of the plant stem cannot be grasped, basket forceps (as used to retrieve guttural pouch chondroid) can be pushed down the bronchus beside the plant and then opened to ensnare part or all of the plant. If still unsuccessful, a distal cervical tracheostomy can be performed under general anesthesia and a long, rigid grasping instrument (that will have to be bent significantly to permit its entry into the thoracic trachea and main-stem bronchi) can be used under flexible endoscopy guidance, to grasp and retrieve the foreign body (Urquhart et al 1981, Brown & Collier 1983).
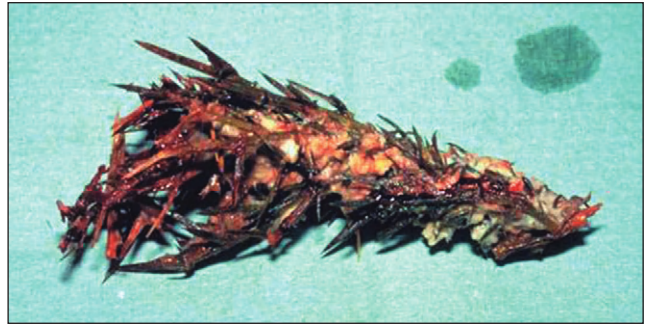
Fig. 40.7. This 6–7 cm long plant stem with caudally facing thorns was deeply embedded in the right main bronchus of a horse. For almost a year the horse coughed, had malodorous breath and intermittent purulent nasal discharge that temporarily responded to antibiotics. All signs resolved following the removal of the foreign body using transendoscopic biopsy forceps.

Tracheostomy in the Horse

A "tracheostomy" is a surgical opening into the trachea, with or without the use of a tracheostomy tube to keep this opening patent. The term "tracheotomy" was originally introduced by Heister in 1718 for this procedure (Wright 1914, Stevenson & Guthrie 1949) and this term is now used by some to describe the surgical procedure of creating a tracheostomy, although many authors including Woolridge (1928) use these words interchangeably. Tracheostomies have long been used in humans, with descriptions of this procedure in a Hindu text of 2000 BC, although Hippocrates later condemned this procedure because of the fear of carotid artery damage (Spector 1991). In horses, the benefits of tracheostomy have been recognized for over 150 years with Youatt (1846) stating:

> The respiratory canal is occasionally obstructed, to an annoying and dangerous degree. It has been anxiously inquired whether there might not be established an artificial opening for the passage of the air, when the natural one could no longer be used. It has now been ascertained that it is both a simple and safe operation.

There are three basic types of equine tracheostomy:

- *Temporary tracheostomy* – this is usually an emergency procedure where a smaller diameter tracheostomy tube is inserted, usually for a matter of days, to bypass an acute upper airflow obstruction.
- *Permanent tracheostomy* – this is an elective procedure in which a larger metal tracheostomy tube is used to bypass a permanent upper airway obstruction. The term "permanent tracheostomy" is not absolute because, for ease of management, many "permanent" tracheostomy tubes are removed at the end of each working season and then replaced at the beginning of the following season.

- *Permanent tracheal fistula (stoma)* – this has also been used to create a permanent bypass of the upper airways in both clinical and experimental cases (Shappell et al 1988).

The horse has a large relatively superficial trachea more rostrally, which readily facilitates tracheostomy. Additionally, because of the low sensitivity of the equine upper trachea, as compared to some other species, horses will tolerate the presence of a tracheostomy tube *in situ* very well, with little coughing, even immediately after its placement.

Indications for temporary tracheostomy

Severe upper airway obstructions can be caused by acute upper respiratory infections, most commonly strangles infection with accompanying large retropharyngeal abscesses. Other causes include nasopharyngeal cellulitis (e.g. following foreign body damage), or less commonly following viral or other infections in younger horses and foals that have relatively smaller upper airways, and also usually have pre-existing lymphoid hyperplasia of their nasopharynx. Upper respiratory infections can occasionally cause an epiglottitis, which can cause significant upper airflow obstruction as described in Chapter 38. Acute upper airway obstructions can also occur following anaphylactic reactions to insect stings or snake bites to the head or upper neck, in adverse injection reactions in the neck region that can cause laryngeal obstructions, or to smoke inhalation. Trauma to the head or throat regions may also cause serious perilaryngeal inflammation, e.g. horse throwing a rider and later causing self trauma to head after getting its bridle caught in a fence.

Such severe upper respiratory obstruction causes anxiety, greatly increased respiratory effort, tachypnea, tachycardia, hyperthermia, stridor, nostril flaring, and extension of the head and neck. Arterial blood gas analysis reveals marked hypoxemia ($P_AO_2 < 60$ mmHg) and hypercapnia ($P_aCO_2 > 50$ mmHg). With more severe airway obstruction, worsening hypoxemia ($P_AO_2 < 40$ mmHg) and hypercapnia, cyanosis and depression will develop. The accessory muscles of respiration (including the sternothyrohyoideus and orbicularis oris) may begin to contract during respiration and then death will shortly ensue.

A prophylactic temporary tracheostomy is indicated following major upper respiratory tract surgery, such as bilateral arytenoidectomy or correction of tracheal stenosis. A temporary tracheostomy is unnecessary after vocal cordectomy or ventriculectomy unless postoperative inflammation occurs to such a degree as to markedly obstruct the laryngeal lumen. Under these rare circumstances a temporary tracheostomy tube can be inserted through the laryngotomy wound. Horses occasionally develop temporary bilateral laryngeal paralysis during recovery from general anesthesia following any type of surgery (Dixon et al 1993; see Chapter 33). Equipment for an emergency tracheostomy should be readily available for this eventuality. A temporary tracheostomy may also be used for horses with acute hepatic encephalopathy-induced bilateral temporary laryngeal paralysis and in chronic grass sickness cases, which have severe rhinitis sicca (Milne et al 1994). A temporary tracheostomy can be used to permit inhalation anesthesia during major head surgery, e.g. arytenoidectomy. A distal temporary tracheostomy allows retrieval of bronchial foreign bodies and removal (or biopsy) of polyps and tumors of the lower trachea and bronchi.

Indications for permanent tracheostomy

Horses with inoperable nasal and sinus neoplasia can be maintained with a "permanent" tracheostomy for weeks to months, for example to enable a valuable stallion to complete a breeding season or allow a pregnant mare to foal. Some miniature horses have a temporary, but severe, bilateral nasal obstruction as the result of encroachment of maxillary cheek teeth eruption cysts into the nasal cavity, and may need a permanent tracheostomy tube for 12 months or so until these structures reduce in size (J. Easley, personal communication 2002). However, the ethics of this procedure are questionable. Major deformations of the nasal cavity and nasopharyngeal cicatrix formation can also be bypassed with a tracheostomy, as can severe acquired tracheal obstructions of the upper cervical trachea. Dorsoventral tracheal collapse in ponies is not amenable to this treatment because the distal cervical and intrathoracic trachea are usually worst affected.

In the UK, Ireland, and a limited number of other countries, a permanent tracheostomy can be used in performance horses (e.g. racehorses, hunters) to bypass performance-limiting upper respiratory obstruction, such as after failure of surgery to treat laryngeal paralysis or dorsal displacement of the soft palate. A permanent tracheostomy tube may be fitted in a horse with upper respiratory airflow problems, to assess the animal's athletic ability with the obstruction bypassed, and consequently to determine if conventional surgery is likely to be worthwhile. Some owners and trainers find a permanent tracheostomy aesthetically objectionable and do not permit their use. Some racing authorities, including those of most American states, do not allow racehorses to run with a tracheostomy and in general this treatment is currently uncommonly performed. Additionally, it is unclear if the unfiltered, unhumidified and unheated air entering the trachea at right angles via a tracheostomy has any adverse effects on pulmonary function.
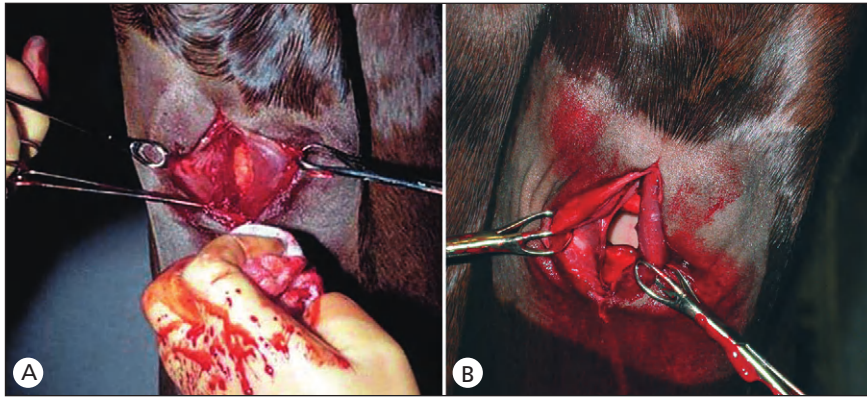
Fig. 40.8. Performing a tracheostomy. (A) For insertion of a temporary tracheostomy tube, the skin and the cutaneous colli muscle are incised vertically and the sternothyrohyoideus muscles are separated at the midline and retracted to expose the underlying trachea. The annular ligament between two tracheal rings is then incised to permit insertion of the tube. (B) For insertion of a permanent tracheostomy tube, the strap muscles have been further retracted with Lane's forceps. An elliptical segment of the dorsal aspect of the tracheal ring below the tracheal ligament incision has been resected to allow the insertion of a (wide) permanent tracheostomy tube. The pale dorsal wall of the trachea is visible in the center of the wound.

Surgical procedure

A temporary tracheostomy is usually an emergency procedure that is performed on the standing horse under local anesthesia. Thankfully, the anatomy of this area is relatively simple (Figs 40.1 and 40.2), with no vital structures overlying the standard tracheostomy site. Unless the airflow obstruction is in the mid-cervical trachea, the usual tracheostomy site is the upper third of the cervical trachea, commonly at the third to the fifth tracheal rings (Woolridge 1928), where the trachea is most superficial. Additionally, if tracheostomy-induced granulation/chondroma-type tissue causes subsequent tracheal obstruction, a second tracheostomy can be created more distally. A tracheostomy tube is also less likely to be rubbed on or trapped in doors or fences at this site, as compared to one located in the mid-neck region.

A midline area, circa 15 cm long and 7 cm wide, overlying the chosen tracheal site is shaved and the skin is prepared for surgery; 20–30 ml local anesthetic is infiltrated midline subcutaneously into the underlying muscles and directly over the trachea. The animal's head is then elevated to make the trachea more prominent, provided that this manipulation does not worsen the dyspnea in animals with gross laryngeal obstructions. It is also essential to have the head absolutely straight to ensure a midline incision. A 10-cm midline skin and subcutaneous incision will expose the underlying cutaneous colli (panniculus) muscle. There is no distinct midline division of this muscle over the trachea and so it must be incised midline beneath the skin incision. If present (and if time allows), hemorrhage from small arteries in the skin and panniculus muscle should be controlled with

hemostats; there should be minimal hemorrhage from dissection of the deeper layers.

It is helpful if an assistant laterally retracts the cutaneous colli muscle and skin with tissue forceps, to reveal the underlying sternothyrohyoideus muscles (Fig. 40.8). Provided that the skin and panniculus muscle incisions are midline (they may be off-center in emergency tracheostomies), the junction (sometimes ill-defined) between the paired bodies of these muscles can usually be identified as a pale fibrous line which can usually be avascularly divided with scissors (Share-Jones 1908). Separation of these muscles over a 7- to 8-cm length then reveals the trachea (Fig. 40.8), to which they are loosely attached by sparse connective tissue, and also exposes the paired omohyoideus muscles on the lateral aspects of the trachea. If the omohyoideus overlie the tracheostomy site, these muscles can be bluntly dissected off the trachea to more fully expose its anterior surface. In emergency situations, there may be insufficient time to follow the above procedures and one may have to make a deep midline incision through the skin and both muscle layers, down to the tracheal surface, without skin preparation or anesthesia. If a more rostral tracheostomy must be performed, this will involve sectioning between the omohyoideus muscles, where the line of demarcation between these paired, thin muscles is very indistinct.

Temporary tracheostomy procedure

As the horse's head is normally elevated during this surgery, it is important to ensure that the external skin wound and the tracheal incision site overlie each other. Before making the annular ligament incision, the horse's
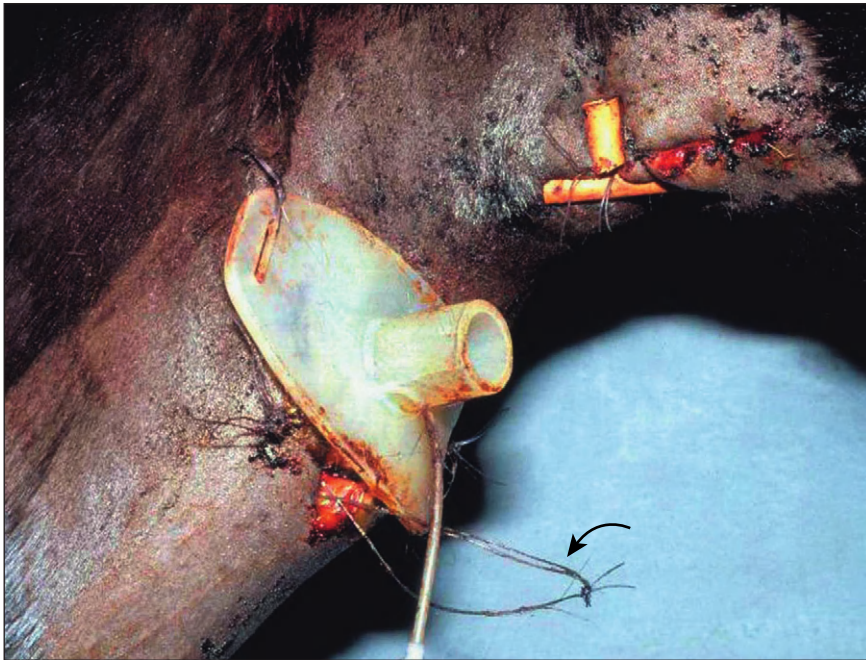
Fig. 40.9. This horse has a temporary tracheostomy tube inserted into its rostral trachea following extensive laryngeal surgery that might have caused severe laryngeal obstruction. In this case the temporary tube has been sutured to the skin because it is likely to be in this position for only 24 h. Note the nylon loop (arrow) which was sutured around the tracheal ring immediately distal to the tracheostomy site (with a shorter loop encircling the more proximal tracheal ring), to facilitate easy reinsertion of the tracheal tube if required.

head should be lowered to its normal resting position to select the optimum annular ligament incision site, which is usually the most distal annular ligament exposed. A transverse stab incision is made into the annular ligament and, in horses with severe dyspnea, this will induce a loud inspiratory noise, as air rushes into the trachea. Using straight scissors, this incision is extended on both sides, to create an opening large enough for the temporary tracheostomy tube. This incision should not be continued more than 180° through the circumference of the annular ligament in case the carotid artery or associated nerves are transected, and also to prevent tracheal stenosis following removal of the temporary tracheostomy tube. The temporary tracheostomy tube should be of soft silicone or plastic because the older style metal temporary tracheostomy tubes can cause severe mucosal damage, especially where their distal end contacts the ventral trachea. The largest tube that can be comfortably fitted through the tracheostomy incision should be manipulated through this opening and down the trachea. If a temporary tracheostomy tube is unavailable, one can readily be fashioned from the sawn barrel of a 30- or 50-ml syringe, a section of nasogastric tube or a plastic pipe, with holes drilled or burned into their wider parts to allow them to be taped or sutured to the neck.

It is helpful to insert two heavy monofilament nylon suture loops circa 15 cm long, encircling the tracheal rings immediately above and below the tracheostomy (Fig. 40.9). With moderate traction on these loops, the tracheal incision can be both exteriorized and dilated to facilitate tracheostomy tube reinsertion following removal for

cleaning and so minimizing the risk of its accidental insertion subcutaneously, or between the muscles and the trachea by inexperienced personnel. Gaping skin wounds can be loosely sutured, but marked inflammation always occurs and tightly suturing the tissues around the tracheostomy tube will restrict exudate drainage and promote local abscess formation or cellulitis and also promote the development of subcutaneous emphysema. A temporary tracheostomy tube should be left in place no longer than necessary and, if possible, should be gradually reduced in size to allow air to flow around it before its final removal.

Permanent tracheostomy procedure

To fit a permanent tracheostomy tube, the trachea is exposed as described above. Because the annular spaces in the horse are not large enough to accommodate a wide metal permanent tracheostomy tube, parts of two adjacent tracheal rings (Fig. 40.10) have to be removed to insert these tubes through the tracheal wall. A variety of instruments for accurately removing portions of tracheal rings (including Spooner's or McKenny's tracheotomes) were previously used when these procedures were more commonly performed, but they are now largely unavailable. Following incision of the chosen annular ligament, the midline of one of the adjacent tracheal rings is firmly grasped with large curved artery forceps. An elliptical incision is then made with a scalpel or less readily with heavy, sharp curved scissors. A firm grip should be maintained on the tracheal ring both to allow proper
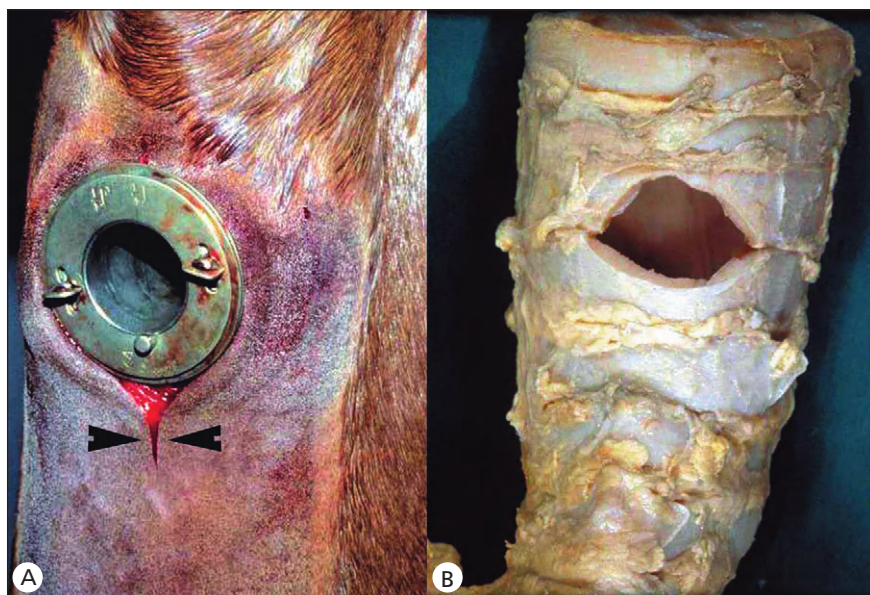
Fig. 40.10. (A) A permanent tracheostomy tube had been fitted to this horse. The ventral aspect of the wound (arrowheads) was not sutured to help prevent the development of post-tracheosotomy emphysema. (B) Tracheal specimen demonstrating the removal of an elliptical part of two adjacent tracheal rings to allow placement of a permanent tracheostomy tube.

control of the curved cartilage incision (the incision will tend to run straight and so can fully cut through the tracheal ring) and also to prevent inhalation of the excised piece of cartilage.

One should attempt to remove 75% of the width of tracheal ring (Share-Jones 1908) and avoid cutting fully through a tracheal ring, because following removal of the tracheostomy tube, the transected ends may invert into and partially occlude the tracheal lumen, particularly if two or more rings are transected. This problem can be particularly serious in foals, for example where a temporary tracheostomy has been performed using a temporary tracheostomy tube which has been found to be too large to fit through an annular space, with subsequent transection of tracheal rings to enable its insertion.

Aftercare: temporary tracheostomy

Depending on its vaccination status, the animal should be given tetanus antitoxin. Broad-spectrum antibiotics should be administered for 2–3 days to reduce the inevitable postoperative local infection that develops and so lessen the volume of exudate that causes a major problem in the first few days postoperatively by blocking the tracheostomy tube. It is absolutely imperative that the tube's lumen be kept fully patent, otherwise the blocked tracheostomy tube will act as an additional airflow obstruction to the one being bypassed and further compromise airflow. Temporary tracheostomy tubes with cuffs should not have the cuffs inflated (except during anesthesia or in intensive-care cases that are being ventilated) because intraluminal blockage of the tube may suffocate the horse. On the first day following insertion, temporary tracheostomy tubes should be inspected and cleaned if necessary two to four times, depending on degree of exudate accumulation present within the tube; twice daily examination and cleaning should suffice thereafter.

With temporary tracheostomies it is very helpful if a spare temporary tube is available so that they can be immediately exchanged for cleaning. The soiled tracheostomy tube should be soaked in a biological detergent, cleaned inside with a flexible bottle-brush, followed by thorough rinsing in water to remove all chemicals. The skin beneath the tracheostomy should be cleaned of blood, dried, and then coated in petroleum jelly immediately after tube insertion. Thereafter, the area below the tracheostomy should be cleaned, dried and again coated in petroleum jelly twice a day to facilitate skin cleaning and prevent excoriation. Horses with a temporary tracheostomy should be kept indoors in a box where they cannot rub and dislodge the tube. After the primary airway obstruction has resolved, the temporary tracheostomy tube and the two nylon loops are removed. The tracheostomy wound, which is inevitably contaminated, should always be allowed to heal by secondary intention, which usually takes 2–4 weeks.

All horses will develop localized emphysema following a tracheostomy and, consequently, subcutaneous crepitus will always be palpable around these wounds. However, the emphysema may extend up the neck to the larynx and head (Fig. 40.11), or caudally down to the ventral neck, especially when the tracheostomy skin wounds are sutured excessively tight. Such horses are usually otherwise asymptomatic and little effective treatment is needed or indeed can be given for this emphysema. Removing skin sutures above or below the tracheostomy that have become

Fig. 40.11. This horse had a permanent tracheostomy fitted 24 h previously, and has since developed extensive emphysema of the head and neck. Note the finger of the examiner deeply depressing the emphysematous subcutaneous tissues.

excessively tight because of postoperative inflammation may be of value in some cases. Performing minimal dissection during the placement of tracheostomy tubes will decrease dead space and help reduce the spread of emphysema.

Pneumothorax is a well-recognized complication of tracheostomy in humans, partly as a result of the short human neck and consequently of the risk of tracheal tubes being inadvertently misplaced rostral to the trachea and into the mediastinal space. Boy and Sweeney (2000) reported pneumothorax following temporary tracheostomy in three horses, and Kelly et al (2003) recorded pneumothorax in an 18-month-old filly following an emergency tracheostomy performed 2 h after sinus cyst excision. Kelly et al (2003) proposed that the subcutaneous emphysema dissected along the facial planes and migrated into the mediastinal space to cause pneumomediastinum and then extended through the pleura to cause pneumothorax. This mechanism is well described in humans, especially children (Swift & Rogers 1987, Waldron et al 1990).

As noted previously, all tracheostomy wounds are contaminated with microorganisms and all will have inflammation and exudation of the exposed muscles, subcutaneous and cutaneous tissues. Secretions will also be present in the tracheal lumen, especially in horses with pre-existing pulmonary disease. As a tracheostomy will interfere with conditioning of inhaled air, this may predispose to temporary lower airway inflammation that may be responsible for increased production of tracheal respiratory secretions, in the short term at least. A small percentage of cases will develop foul-smelling, anaerobic or mixed infections of the tracheostomy wound that lead to copious, malodorous, purulent exudate. In these cases, one should ensure maximal drainage of the wound by removing sutures in the ventral aspect of the wound. The tracheostomy tubes should be temporarily removed two or three times daily (e.g. whilst cleaning tubes) and the wound surfaces should be debrided of necrotic tissue using swabs soaked in dilute povidone iodine. Metronidazole (local and systemic) and penicillin should be administered until the malodor and excessive discharge resolve.

Aftercare: permanent tracheostomy

In heavier type horses, particularly if a permanent tracheostomy has to be re-fitted lower down the neck, where there is greater muscle cover by the sternocephalicus and also by the larger (and at this site) separate bodies of the sternothyroideus and sternohyoideus muscles, postoperative inflammation can cause complications (Dixon 1988). Woolridge (1928) noted that when a permanent tracheostomy is fitted there is always inflammation of the tracheostomy site and therefore the neck on the tube used during this period should be 1–1.5 cm longer than the skin to tracheal lumen thickness. The inevitable postoperative swelling of the tissues overlying the trachea can cause such pressure on the permanent tracheostomy tube that it cannot be dismantled and removed for cleaning without considerable force. This inflammation may also cause the internal flange of the tube to press on the tracheal mucosa, and cause pressure necrosis of the mucosa and even cartilage. This is one reason for the development of tracheal granulation, scar tissue, chondroma-type swellings, and septic chondritis (Woolridge 1928) which can cause local tracheal obstruction. This inflammation can also cause the external flange of the tube to press on the skin and can even cause the flange to sink below the skin surface and eventually become totally embedded in scar tissue. An appropriate diameter, longer-necked permanent tracheostomy tube which can accommodate the tissue
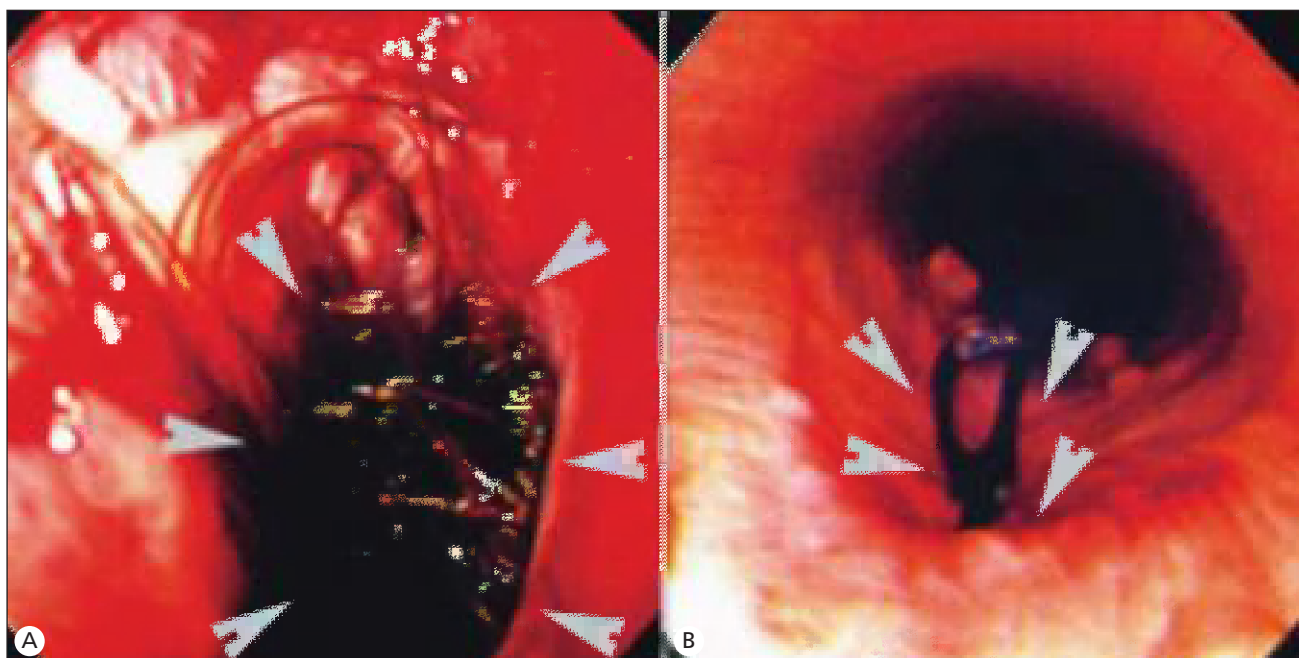
Fig. 40.12. (A) Intratracheal endoscopy image taken after removal of a permanent "tracheostomy tube". The tracheostomy wound did not heal because the external epithelium had grown into the trachea. Epithelium, with hair on it, is present on the margins of the wound forming a fistula (arrowheads). (B) Intratracheal view of a tracheal fistula showing an open curette being used to remove the conjoining epithelial surfaces at the fistula (arrowheads) and so allow the wound to close by granulation.

swelling without causing undue pressure on the tracheal mucosa or skin surface should therefore be used in this situation. In some animals it may be necessary to resort to removal of sections of these overlying muscles before fitting the tube, especially if it must be fitted lower down the neck.

The removal for cleaning of permanent tracheostomy tubes by owners who have little regard for the gentle and hygienic handling of tissues can be another factor in the exacerbation of postoperative inflammation and the development of intratracheal granulomas/chondromas. After permanent tracheostomy the surgeon should, if possible, remove and fully clean the tube daily for the first few days and then instruct the owner how to clean it *in situ* daily with a blunt-ended blade, such as a round-ended table knife. Animals that have been shown to have tracheal granuloma formation should not have their tubes removed for cleaning at any stage, with all cleaning being done *in situ*. In all cases where a permanent tube is removed for cleaning, it should be replaced as quickly as possible because local inflammation can make replacement impossible even within a few hours.

If a permanent tube is removed at the end of a season's work or on retirement to stud, the wound will normally heal over fully within weeks. If full healing does not occur, this usually indicates the development of continuity between the tracheal mucosa and skin, i.e. a tracheal fistula. This can generally be treated by freshening up the edges of the fistula (Fig. 40.12).

Post-tracheostomy airway obstruction

Following removal of a temporary or permanent tracheostomy tube, excessive granulation tissue (Fig. 40.13), which can possibly develop into a web (Fig. 40.14) and/or cartilaginous proliferation (Fig. 40.6), may continue to develop at the tracheostomy site. If excessive, this may lead to significant airflow obstruction. This is more common following a permanent tracheostomy. Sectioning the annular ligament over more than 180° of the tracheal circumference during a temporary tracheostomy procedure risks immediate damage to the carotid artery, vagosympathetic trunk and recurrent laryngeal nerve, and in the long term risks tracheal obstruction by causing a band of healed mucosa to protrude into the rostral half of the tracheal lumen (Freeman 1991). Following a permanent tracheostomy, a permanent tracheal obstruction may occur if tracheal rings are fully cut to accommodate the tracheostomy tube. Following removal of the permanent tube, the cut ends and attached mucosa may fold back into the tracheal lumen and cause a permanent obstruction.

Permanent Tracheal Fistulation

A permanent tracheal fistula (stoma) has long been used to create a permanent bypass of the upper airways in both clinical (McClure et al 1995) and experimental (Shappell et al 1988) horses, as is commonly performed in ruminants
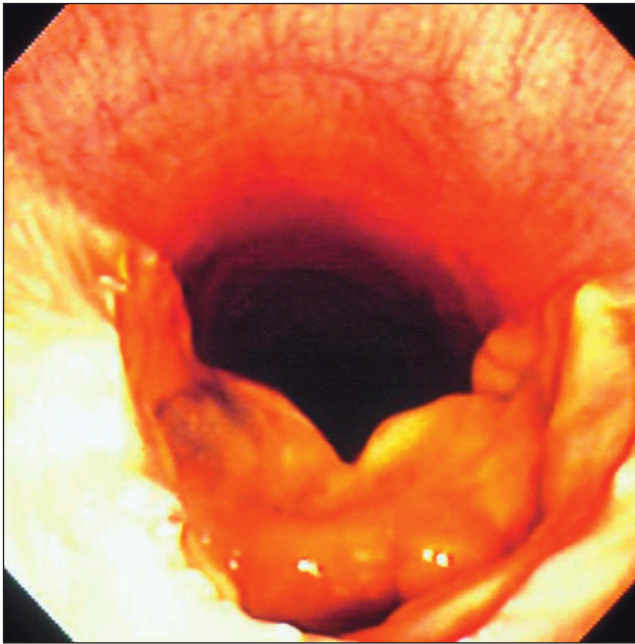
Fig. 40.13. Endoscopic image of the rostral trachea of a horse that had a temporary tracheostomy tube fitted for 1 week, some months previously. Damage to the tracheal mucosa around the tracheostomy site led to granulation and scar tissue formation, causing clinically insignificant tracheal obstruction at this time. Some tracheal cartilage swelling may also be present that can progress to form tumor-like tracheal obstructions (see Fig. 40.6).

Fig. 40.14. A ventral tracheal stenosis (web) (arrowheads) that developed following placement of a temporary tracheostomy in the upper trachea for 1 week.

with chronic laryngeal infections (Goulding et al 2003). Smaller tracheal fistulas will contract in size and may become blocked with dried tracheal secretions so larger fistulas are desirable if they are required for the long term. For this procedure, a vertical midline incision as described above can be made (Shappell et al 1988) with later resection of some of the sternothyrohyoideus muscles if excessive tension is present on the trachea to skin sutures. An alternative approach, as used in ruminants, is to fully resect all tissues above the tracheostomy site (McClure et al 1995). For this technique, a 3 × 6 cm rectangular section of skin and subcutaneous tissue and cutaneous colli (often very thin this far rostrally) is removed from the ventral midline neck, beginning 3 cm behind the larynx. Local anesthesia of the tracheal mucosa is reported to reduce coughing and make patients more comfortable during this procedure.

The overlying sternothyrohyoideus muscles are clamped and resected at both ends of the skin resection, exposing the rostral aspect of the second to fifth tracheal rings. Parallel incisions are made through the cartilages only, on the lateral aspects of the exposed tracheal rings, 1.5–2.0 cm (depending on size of horse) to each side of the midline; then, the rostral aspects (circa 30% of circumference) of the incised tracheal rings are dissected free and resected. The vertical incision in the center of the trachea

(less its cartilages) is extended into a (double) "Y"-shaped incision at each end of the wound. The flaps of the trachea are everted and sutured directly to the skin. If excessive tension is present (often at the caudal aspect of the wound), the medial aspects of the sternothyrohyoideus muscle can be resected. Such fistulas have been maintained for 32 months in experimental ponies (Shappell et al 1988). Unlike permanent tracheostomies, these large fistulas will predispose horses to the development of pulmonary disease (Shappell et al 1988) and, when used in clinical cases, they sometimes have to be reversed to treat such pulmonary infections.

Tracheal Tumors and Growths

Malignant tracheal tumors are rare in all species, with for example the incidence of human tracheal tumors being only 2.7 cases per million people per year, mainly squamous cell carcinomas and adenoid cystic carcinomas. Sweeney (1997) illustrated intratracheal squamous cell carcinomas in an 18-year-old Arab cross and a 35-year-old Morgan horse. Benign lesions including papillomas, fibromas, chondromas, and adenomas are more common (Spector 1991). Wenger & Caron (1988) reported tracheal mastocytosis in an Arabian cross that presented with a stridor of 3 days duration. The stridor was loudest during eating, which was thought to be the result of the esophageal food bolus compressing the dorsal tracheal
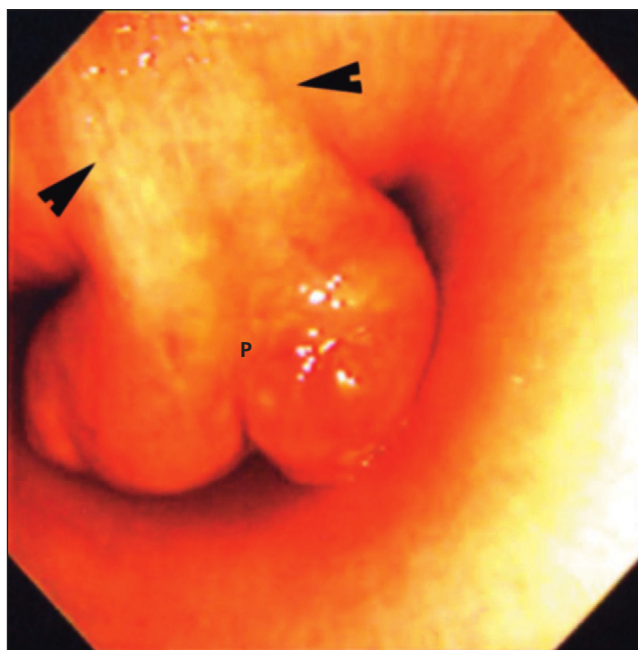
Fig. 40.15. Endoscopy of the distal cervical trachea of a horse with severe dyspnea showing a bi-lobed pedunculated mass (a polyp "P") attached by a pedicle (arrowheads) to the dorsal trachea. This mass is almost totally obstructing the tracheal lumen.

ligament, thereby further compromising the tracheal lumen. Endoscopy revealed a large, rounded mass attached to the left dorsolateral aspect of the trachea, 5 cm distal to the larynx. The mass was removed via a laryngotomy and its identity was confirmed histologically. Collins et al (2005) reported an eosinophilic polyp of the distal cervical trachea in a 12-year-old Irish draft cross that presented with coughing and stridor (Fig. 40.15). The lesion was successfully resected using a distal cervical tracheostomy.

REFERENCES

Boy MG, Sweeney CR 2000 Pneumothorax in horses: 40 cases (1980–1997). Journal of the American Veterinary Medical Association 216: 1955–1959

Bradley CO 1923 The Head and Neck. Topographical Anatomy of the Head and Neck of the Horse. W Green, Edinburgh. p.7

Brown CM, Collier MA 1983 Tracheobronchial foreign body in a horse. Journal of the American Veterinary Medical Association 182: 280–281

Caron JP, Townsend HGG 1984 Tracheal perforation and widespread subcutaneous emphysema in a horse. Canadian Veterinary Journal 25: 339–341

Carrig CB, Groenendyk S, Seawright AA 1973 Dorsoventral flattening of the trachea in a horse and its attempted treatment. Journal of the American Radiographic Society 14: 32–36

Charlton C, Tulleners E 1991 Transendoscopic contact neodymium: yttrium aluminum garnet laser excision of tracheal lesions in two horses Journal of the American Veterinary Medical Association 199: 241–243

Collins NM, Barakzai S, Dixon PM 2005 Tracheal obstruction by an eosinophilic polyp in a horse. Equine Veterinary Education 17: 128–131

Couteil LL, Gallatin LL, Blevins W et al 2004 Treatment of tracheal collapse with an intraluminal stent in a miniature horse. Journal of the American Veterinary Medical Association 225: 1727–1732

Dallman MJ, McClure RC, Brown EM 1988 Histochemical study of normal and collapsed tracheas in dogs. American Journal of Veterinary Research 49: 2117–2125

Davis DM, Honnas CM, Hedlund CS et al 1991 Resection of a cervical tracheal bronchus in a foal. Journal of the American Veterinary Medical Association 198: 2097–2099

Delahanty DD, Georgi JR 1954 A tracheal deformity in a pony. Journal of the American Veterinary Medical Association 125: 42–44

Dixon PM 1988 Tracheostomy in the horse. In Practice 10: 249–253

Dixon PM 1992 Respiratory mucociliary clearance in the horse in health and disease, and its pharmaceutical modification. Veterinary Record 131: 229–235

Dixon PM, Railton DI, McGorum BC 1993 Temporary bilateral laryngeal paralysis in a horse associated with general anaesthesia and post anaesthetic myositis. Veterinary Record 132: 29–32

Dixon PM, Railton DI, McGorum BC 1995 Equine pulmonary disease: a case control study of 300 referred cases. Part 3: Ancillary diagnostic findings. Equine Veterinary Journal 27: 428–435

Feat S, Le Clech G, Riffaud L et al 2002 Complete cervical tracheal rupture in children after closed trauma. Journal of Pediatric Surgery 37: 1–3

Ferrucci F, Croci C, Zucca E et al 2003 Use of a trans-endoscopic technique to remove a bronchial foreign body in a Standardbred colt. Equine Veterinary Education 15: 228–232

Freeman DE 1991 Trachea. In: Beech J (editor) Equine Respiratory Disorders. Lea & Febiger, Philadelphia, pp.389–402

Fubini SL, Todhunter RJ, Vivrett SL et al 1985 Tracheal rupture in two horses. Journal of the American Veterinary Medical Association 187: 69–70

Goulden BE 1977 Some unusual cases of abnormal respiratory noises in horses. New Zealand Veterinary Journal 25: 398–390

Goulding R, Schumacher J, Barrett DC et al 2003 Use of a permanent tracheostomy to treat laryngeal chondritis and stenosis in a heifer. Veterinary Record 152: 809–811

Grillo HC, Donahue DM, Mathisen DI et al 1995 Postintubation tracheal stenosis. Treatment and results. Journal of Thoracic and Cardiovascular Surgery 109: 486–493

Hare WC 1975 Equine respiratory system. In: Getty R (editor) Sisson and Grossman's The Anatomy of the Domestic Animals, 5th edn. WB Saunders, Philadelphia, pp.498–523

Hobson HP 1976 Total ring prosthesis for the surgical correction of collapsed trachea. Journal of the American Animal Hospital Association 12: 822–828

Holland M, Snyder JR, Steffey EP et al 1986 Laryngotracheal injury associated with nasotracheal intubation in the horse. Journal of the American Veterinary Medical Association 189: 1447–1450

Jackson PG, White RA, Dennis R et al 1986 Tracheal collapse in a goat. Veterinary Record 119: 160

Kelly G, Prendergast M, Skelly C et al 2003 Pneumothorax as a complication of tracheostomy in a horse. Irish Veterinary Journal 56: 153–156

Lane JG 1981 Fibreoptic endoscopy. In Practice 3: 24–30

Mair TS, Lane JG 1990 Tracheal obstructions in two horses and a donkey. Veterinary Record 126: 303–304

McClure SR, Taylor TS, Honnas CM et al 1995 Permanent tracheostomy in standing horses: techniques and results. Veterinary Surgery 24: 231–234

McGorum BC, Dixon PM, Radostitis OM et al 2000 Clinical examination of respiratory system. In: Radostitis OM, Mayhew IG, Houston DM (editors) Veterinary Clinical Examination and Diagnosis. WB Saunders, London, pp.299–348

Milne EM, Woodman MP, Doxey DL 1994 Use of clinical measurements to predict the outcome in chronic cases of grass sickness (equine dysautonomia). Veterinary Record 134: 438–440

O'Connor JJ 1950 Operations on the head and neck. In: Dollar's Veterinary Surgery, 4th edn. Bailliere, Tindall, Cox, London, pp.308–314

Peek SF, DeLahunta A, Hackett RP 1995 Combined oesophageal and tracheal duplication cyst in an Arabian filly. Equine Veterinary Journal 27: 475–478

Pirie M, Pirie HM, Cranston S et al 1990 An ultrastructural study of the equine lower respiratory tract. Equine Veterinary Journal 22: 338–342

Pouret E 1966 Laryngeal ventriculectomy with suturing of the laryngeal saccules. In: Proceedings of the 12th Annual meeting of the American Association of Equine Practitioners, p.207

Randall RW, Myers VS 1973 Partial tracheal stenosis in a horse. Veterinary Medicine: Small Animal Clinician 68: 264–266

Rigg DL, Ramey DW, Reinerston EL 1985 Tracheal compression secondary to abscessation of cranial mediastinal lymph nodes in a horse. Journal of the American Veterinary Medical Association 186: 283–284

Risnes I, Mair TS 2003 Traumatic oesophageal rupture in horse complicated by subsequent rupture of the common carotid. Equine Veterinary Education 35: 120–125

Robertson JT, Spurlock GH 1986 Tracheal reconstruction in a foal. Journal of the American Veterinary Medical Association 189: 313–314

Rudorf H, Herrtage ME, White RA 1997 Use of ultrasonography in the diagnosis of tracheal collapse. Journal of Small Animal Practice 38: 513–518

Scott EA 1978 Ruptured trachea in the horse: a method of surgical reconstruction. Veterinary Medicine: Small Animal Clinician 73: 485

Schummer A, Nickel R, Sack WO 1979 The Viscera of the Domestic Animals. Springer-Verlag, New York, pp.238, 276

Shappell KK, Stick JA, Derksen FJ et al 1988 Permanent tracheostomy in Equidae: 47 cases (1981–1986). Journal of the American Veterinary Medical Association 192: 939–942

Share-Jones JT 1908 The Surgical Anatomy of the Horse, Part I. Bailliere, Tindall, Cox, London, pp.73–77

Simmons TR, Peterson M, Parker J et al 1988 Tracheal collapse due to chondrodysplasia in a miniature horse foal. Equine Practice 10: 39–42

Sisson S, Grossman JD 1953 The Anatomy of the Domestic Animals. WB Saunders, Philadelphia, p.533

Spector GJ 1991 Respiratory insufficiency, tracheostenosis and airway control. In: Ballenger JJ (editor) Diseases of the Nose, Throat, Ear, Head and Neck. Lea & Febiger, Philadelphia, pp.530–569

Stevenson RS, Guthrie D 1949 History of Otolaryngology. E & S Livingstone, Edinburgh, pp.17, 31

Sweeney CR 1997 Trachea and bronchi. In: Traub-Dargatz JL, Brown CM (editors) Equine Endoscopy, 2nd edn. Mosby, St Louis pp.97–105

Swift AC, Rogers JH 1987 The outcome of tracheostomy in children. Journal of Laryngology and Otology 101: 936–939

Tate LP, Koch DB, Sembrat RF et al 1981 Tracheal reconstruction by resection and end-to-end anastomosis in the horse. Journal of the American Veterinary Medical Association 178: 253–258

Tessier GJ, Neuwirth LA, Merritt AM 1996 Peritracheal abscess as the cause of tracheal compression and severe respiratory distress in a horse. Equine Veterinary Education 8: 127–130

Touzot-Jourde G, Stedman NL, Trim CM 2005 The effects of two endotracheal tube cuff inflation pressures on liquid aspiration and tracheal wall damage in horses. Veterinary Anaesthesia and Analgesia 32: 23–29

Urquhart KA, Gerring EL, Shepherd ML 1981 Tracheobronchial foreign body in a pony. Equine Veterinary Journal 13: 262–264

Waldron J, Padgham ND, Hurley SE 1990 Complications of emergency and elective tracheostomy – a retrospective study of 150 consecutive cases. Annals of the Royal College of Surgeons of England 72: 218–220

Wenger IE, Caron JP 1988 Tracheal mastocytosis in a horse. Canadian Veterinary Journal 29: 563–565

White R 1995 Unilateral arytenoid lateralisation and extra-luminal polypropylene ring prostheses for correction of tracheal collapse in the dog. Journal of Small Animal Practice 36: 151–158

Woolridge GH 1928 Encyclopaedia of Veterinary Medicine, Surgery and Obstetrics. Volume II. Frowde H, Hodder, Stoughton, London, pp.949–954

Wright J 1914 A History of Laryngology and Otology. Lea & Febiger, Philadelphia, p.65

Youatt W 1846 The Anatomy and Diseases of the Neck and Neighbouring Parts: Tracheotomy. The Horse, new edition. C Knight, London, pp.219–220

Yovich JV, Stashak TS 1984 Surgical repair of a collapsed trachea caused by a lipoma in a horse. Veterinary Surgery 13: 217–221

Recurrent Airway Obstruction (Heaves) and Summer-pasture-associated Obstructive Pulmonary Disease

Jean-Pierre Lavoie

Introduction and Case Definition

It has been known since antiquity that stabled horses have an increased risk of developing a chronic, recurrent, and debilitating respiratory syndrome. The terms that have been used to describe this syndrome are numerous and relate to the clinical presentation (heaves, broken-wind, chronic obstructive pulmonary disease, recurrent airway obstruction), the suggested etiologies (allergic airway diseases, hay sickness), or the lung pathology (emphysema, chronic bronchiolitis, small airway disease). The variable terminology and inconsistent case definitions have led to conflicting reports and considerable confusion concerning the etiopathogenesis, diagnosis, and therapy of equine chronic respiratory diseases. A recent consensus statement, from clinicians and researchers interested in the field of equine respiratory diseases, recommended the use of heaves or recurrent airway obstruction (RAO) to describe a syndrome of mature horses with chronic airway obstruction (periods during which labored breathing is present), reversible by dust control in the environment or the use of bronchodilators (Robinson 2001). In this chapter, both terms will be used interchangeably. As major differences exist between equine RAO and human chronic obstructive pulmonary disease (poorly reversible airway obstruction occurring primarily in smokers), this latter term is no longer appropriate to describe this condition of horses (Robinson 2001). The term inflammatory airway disease (IAD) is used to describe a condition of horses with lower airway inflammation which does not result in episodes of labored breathing. Presently, the relationship between IAD and RAO is unknown.

While RAO is observed in horses that are stabled and fed hay, a similar clinical presentation is observed in some horses when pastured; the condition is reversible by housing affected horses in a dust-free stable. This syndrome has been called summer-pasture-associated obstructive pulmonary disease (SPAOPD) and shares many clinical and pathological similarities with RAO. SPAOPD and RAO do not appear to be mutually exclusive and horses with heaves may also develop airway obstruction when pastured in the absence of hay or straw exposure (Mair 1996b).

Etiology and Pathogenesis

The strong association between environment and both RAO and SPAOPD is well established. While it is now recognized that inflammation of the distal airways is central to these syndromes, the mechanisms by which the environment leads to chronic airway inflammation and to the clinical signs of respiratory diseases remain poorly defined. The finding that only a subset of horses will develop RAO or SPAOPD was the basis for the "allergy" hypothesis, in which predisposed individuals mount an antigen-specific inflammatory response (hypersensitivity reaction) to components of environmental dust. However, it has recently been suggested that RAO may result from a non-specific inflammatory response to inhaled pro-inflammatory agents including molds, endotoxins, particulates and noxious gases which are present in the breathing zone of stabled horses (Table 41.1). The etiology

Table 41.1. Pro-inflammatory agents present in the breathing zone of stabled horses that could contribute to development of airway inflammation	
Bacteria	Endotoxin Lipoteichoic acid Peptidoglycan Bacterial DNA Short formylated peptides Proteases Toxins
Molds	Allergens Glucans Proteases Mycotoxins
Forage mites	Allergens Proteases
Plant debris	
Inorganic dust components	
Noxious gases	Ammonia Hydrogen sulfide Methane

of SPAOPD is unknown. While inhaled pollens or outdoor molds are a probable cause, tracheal secretions from affected horses do not have increased concentrations of mold-specific or pollen-specific immunoglobulin G (IgG) and IgE antibodies (Seahorn et al 1997). The possibility that SPAOPD results from ingestion of a pasture-derived pneumotoxin has not been eliminated.

Hypersensitivity reactions

Type I hypersensitivity reaction (allergy)

Because exacerbation of clinical signs can be provoked by the inhalation of dusty hay, researchers have postulated that heaves is an allergic reaction to inhaled molds and fungi. A number of anecdotal findings support this hypothesis, including the observation that some RAO-affected horses in midwestern USA may become completely asymptomatic when moved to central USA, even when exposed to locally produced moldy hay (N.E. Robinson, personal communication). An emphasis has been directed toward the possible roles of *Faenia rectivirgula* (formerly *Micropolyspora faeni*), *Aspergillus fumigatus* and *Thermoactinomyces vulgaris*, as they are abundant in poor quality hay (Woods et al 1993). Consistent with this possibility, inhalation of *F. rectivirgula* or *A. fumigatus* caused airway obstruction in animals with RAO, but not in controls (McPherson et al 1979b, Derksen et al 1988). Inhalation challenge with timothy hay extracts did not elicit clinical exacerbation in affected horses, suggesting that the condition does not result from an allergic response to the hay *per se* (Lowell 1964).

Studies of atopic human asthmatics and various animal models of allergic pulmonary disease have highlighted a typical biphasic response when sensitized animals are exposed to inhaled allergens. The "early-phase response" occurs within minutes after susceptible subjects have inhaled an allergen. It is initiated by the activation of cells bearing allergen-specific IgE, primarily mast cells, through activation of FcεRI receptors. Mast cell activation leads to liberation of numerous pro-inflammatory mediators, which induce mucus secretion, vasodilation, microvascular leakage, and airway smooth muscle contraction. Together, these changes result in a narrowing of the airway lumen and airflow obstruction that typically last less than 45 min to 1 h. Involvement of IgE-mediated mast cell degranulation in RAO is supported by the positive passive cutaneous anaphylaxis test observed using sera from horses with heaves (Eyre 1972), the 30-min response after intradermal injection of molds and fungi (Halliwell et al 1979), and the elevated levels of IgE in bronchoalveolar lavage fluid (BALF) (Halliwell et al 1993, Schmallenbach et al 1998) and serum (Eder et al 2000) of affected horses. However, an early-phase response is not a clinical feature of RAO, as hours or days of stabling are usually required before airway obstruction ensues. This suggests that

an IgE-mediated mast cell response is not central to the pathogenesis of heaves. Furthermore, the increase in allergen-specific IgE in horses with RAO may be the result of environmental or genetic factors which are unrelated to disease pathogenesis (Eder et al 2001). An early-phase response is also not a feature of SPAOPD. Antigen-specific IgE for various fungi, thermophilic actinomycetes, and a forage mite in tracheal fluids of horses with SPAOPD were not elevated, suggesting that if an allergic response is implicated, it may be the result of unrelated environmental allergens (Seahorn et al 1997). The "late-phase response" occurs 6–9 h after allergen provocation and is reminiscent of the time-course of recruitment of neutrophils and development of airway obstruction in RAO (Fairbairn et al 1993b). While it was previously believed that the late-phase response resulted primarily from the release of mediators by sensitized mast cells, it is now recognized that T cells, particularly CD4$^+$ T cells, play a key role in coordinating the asthmatic late-phase response. The underlying mechanisms by which T cells initiate and propagate the inflammatory response include the elaboration of T helper type 2 (Th2)-type cytokines and chemokines and the interaction with pulmonary leukocytes. Recent reports suggest involvement of Th2-type cytokines in RAO and SPAOPD (see under Lymphocytes below).

In summary, the absence of an early response after inhalation challenges or following natural exposure to moldy hay indicates that a classical type I hypersensitivity reaction to molds is not central to RAO pathogenesis. Whether molds or other pro-inflammatory components of moldy hay may elicit a late-phase response independently from mast cell degranulation remains to be elucidated.

Type III hypersensitivity (Arthus) reaction

Type III hypersensitivity reactions to inhaled allergens result from local pulmonary deposition of immune complexes and the resultant activation of the complement system. This reaction requires presensitization, which is demonstrable by the presence of precipitating antibodies against the offending antigen in the serum of affected individuals. Farmer's lung disease in humans, a condition associated with a type III hypersensitivity reaction to *F. rectivirgula* and other thermophilic actinomycetes, shares many epidemiological similarities with RAO. As in RAO, it affects middle-aged farmers and the disease peaks at the time of year when farmers feed stored hay to farm animals. Similar to RAO, there is a neutrophilic inflammation in the airway secretions of affected patients. Initial reports indicating that RAO-affected horses have high serum precipitating antibodies to *F. rectivirgula* and that they develop wheals 4 h after intradermal injection of mold extracts suggested that RAO involved type III hypersensitivity (Halliwell et al 1979). However, the lung pathology in farmer's lung disease is strikingly different to that in RAO, with the former being a bronchiolitis and alveolitis with

granuloma formation and extensive fibrosis leading to a restrictive respiratory pattern. Also, fever, which is a characteristic finding in farmer's lung disease, is not a feature of heaves. Furthermore, RAO-affected horses that responded to inhalation challenge with *F. rectivirgula* often lack serum-precipitating antibodies, suggesting that the latter are indicative of high exposure to the antigen as a result of poor air hygiene, rather than of an impaired immune response (Lawson et al 1979). For these reasons, a type III hypersensitivity reaction is not currently considered to be central to RAO pathogenesis.

Non-antigen-specific inflammatory responses

Typical stabling conditions expose horses to a mixture of irritant gases and airborne dusts which have been shown to cause airway inflammation in humans. Among these, the contribution of endotoxins, molds, and particulates to RAO has been recently investigated.

Endotoxins

Inhalation of endotoxin by humans and animals induces inflammatory lung disease that has many similarities with RAO. Experimental endotoxin inhalation causes a dose-dependent airway neutrophilia, both in RAO-affected and control horses, but airway dysfunction occurs only in horses with RAO (Pirie et al 2001). However, the concentration of endotoxins required to cause airway obstruction is much greater than the level of exposure occurring during natural hay and straw challenges. Furthermore, the potency of nebulized hay dust suspension to induce airway neutrophilia and airway obstruction is not related to its endotoxin content (Pirie et al 2002). These findings suggest that although endotoxins may contribute to airway inflammation in horses, other components of stabled dust, such as molds, are more important for the etiopathogenesis of RAO (Pirie et al 2001). An additional argument against an important role of inhaled endotoxin in RAO is the recent observation that concentrations of airborne endotoxin in horse stables in Scotland, where RAO is prevalent, are similar to those in stables in Australia, where RAO is comparatively rare (Malikides 2004). The greater response to inhaled endotoxin in horses with RAO compared with control horses is nonetheless reminiscent of the finding that human asthmatics are more sensitive to inhaled endotoxin than healthy control subjects. It has been proposed that atopy may enhance the sensitivity to inhaled endotoxins in asthmatic subjects through the activation of airway macrophages and granulocytes.

Molds

The contribution of mold components, including allergens, glucans, proteases, and mycotoxins, to allergic airway inflammation is well known in animals and humans. It has been demonstrated that β-D-glucan, a component of the cell wall of molds, yeasts, and certain bacteria and plants, can induce inflammation of the airways by non-immune-mediated mechanisms. In horses, hay dust suspensions with a higher content of β-glucan are more likely to induce airway inflammation, supporting the concept that molds are involved in the pathogenesis of RAO (Pirie et al 2002). However, whether immune mechanisms are involved or not in this response remains to be determined.

Noxious gases

Stabled animals are exposed to various noxious gases that can induce airway inflammation, including ammonia, hydrogen sulfide and methane. However, while the threshold concentrations of these gases that are required to induce inflammation in equine airways is unknown, their levels in stables are generally lower than those in environments used for food-producing animals (Clarke et al 1987). Nevertheless, because horses with RAO have non-specific airway hyperresponsiveness, these gases may exacerbate airway obstruction in affected horses.

Bacterial and viral infection

There is currently no evidence indicating that RAO is an infectious process. The bacteria that are often present in the tracheal secretions of RAO-affected horses likely reflect colonization of the airways as a result of impaired mucociliary clearance because histological and cytological findings are not indicative of infection. It had been suggested, based on circumstantial evidence, that influenza and other respiratory infections may predispose horses to develop RAO.

Pathophysiology

Airway obstruction, inflammation, mucus accumulation, and tissue remodeling have been shown to contribute to the pathophysiology of RAO.

Airway obstruction

Airway smooth muscle

Bronchospasm is a key feature in RAO, as indicated by the marked improvement in clinical signs and pulmonary function within minutes of administration of bronchodilators. Airway smooth muscle contraction is controlled by the autonomic nervous system, centrally, via local axonal reflexes, and through activation of receptors for a number of circulating excitatory and inhibitory molecules that are present on the smooth muscle cell membrane. Anticholinergic agents and β₂-adrenergic agents have similar bronchodilator potency, suggesting that bronchospasm is mediated predominantly via muscarinic receptors (Robinson 2001). Bronchorelaxation also appears to be defective in RAO, due to a reduced inhibitory non-adrenergic

non-cholinergic (NANC) response (Yu et al 1994). Despite these findings, RAO is not considered to be the result of a primary defect of the airways or their neuromuscular control mechanisms (reviewed by Robinson 2001).

Numerous inflammatory mediators, including serotonin, endothelin-1, histamine, and leukotriene D_4 (LTD_4), increase tension in equine smooth muscle cells and cause bronchoconstriction. However, while they may contribute to increased cholinergic airway tone, the latter two mediators are not believed to be major contributors to bronchospasm as neither antihistamines nor leukotriene antagonists are effective for the treatment of heaves (Marr et al 1998a, Olszewski et al 1999, Lavoie et al 2002b).

Airway inflammation

Pulmonary inflammation is central to the pathogenesis of chronic lung diseases and to the development of airway obstruction. The contribution of the various leukocytes to this inflammatory response is emerging.

Neutrophils

Airway neutrophilia is a characteristic feature of RAO and SPAOPD. Neutrophils accumulate in the airways of RAO-affected horses within 6 h after exposure to moldy hay (Fairbairn et al 1993b, Pirie et al 2001). The airway neutrophilia persists thereafter if RAO-affected horses are continually exposed to stable dust (Jean 1996). Blood and airway neutrophils are activated in RAO, further supporting their contribution to lung inflammation (Tremblay et al 1993, Marr et al 1997, Pellegrini et al 1998). However, the mechanisms responsible for their activation and recruitment into the airways, as well as their contribution, if any, to bronchospasm, mucus accumulation, and airway remodeling, remain poorly understood.

Interleukin-8 (IL-8) is a neutrophil chemokine that may be important in the recruitment of neutrophils to the airways in RAO. IL-8 is synthesized by many cells in the lung, including neutrophils, macrophages, epithelial cells, endothelial cells, and smooth muscle cells, and its expression is increased in RAO (Franchini et al 2000). In human asthmatics, the levels of IL-8 correlate both with neutrophil numbers and the degree of lung dysfunction. Other cytokines and chemokines that promote tissue neutrophilia, such as macrophage inflammatory protein (MIP)-2, IL-1β and tumor necrosis factor-α (TNF-α), are also up-regulated in RAO (Franchini et al 2000, Giguere et al 2000). Inhalation of LTB_4 induces airway neutrophilia, suggesting that it may also contribute to neutrophil recruitment in equine airway diseases (Marr et al 1998b). Bronchoalveolar lavage (BAL) granulocytes of horses with heaves have delayed apoptosis, which may contribute to the airway neutrophilia (Turlej et al 2001).

When activated, neutrophils may cause lung injury by releasing as many as 50 histotoxins, including reactive

oxygen species (superoxide, H_2O_2, OH^-), proteases (elastase, collagenase, metalloproteinase-9), lipid mediators (LTB_4, platelet-activating factor, thromboxane A_2, LTA_4), microbicidal products (lactoferrin, myeloperoxidase, lysozyme), and nitric oxide. The concentrations of many of these mediators are increased in the airways of RAO-affected horses. Neutrophils also produce potent pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and the chemokines IL-8 and MIP-2 (Joubert et al 2001). These are likely to play an active role in the pathogenesis of RAO. However, neutrophils are unlikely to be direct contributors to bronchospasm as their secretory products do not increase equine airway smooth muscle tone *in vitro* (Olszewski et al 1999).

It has been shown that stabling may also lead to airway neutrophil infiltration in horses without RAO, and that this may occur in the absence of clinical signs of airway disease (Tremblay et al 1993, Holcombe et al 2001). However, neutrophils from these horses appear to be in a reduced state of activation compared to those of RAO animals (Tremblay et al 1993).

Lymphocytes (Th1/Th2 paradigm)

Recent studies, using molecular tools and murine models, have highlighted the complex interactions involved in the modulation of inflammatory lung diseases. Most cells present within the lung, including epithelial cells, myocytes, and neurons, in addition to traditional inflammatory cells, contribute to this modulation. Among these, T cells ($CD4^+$ cells, more specifically Th2 cells) play a central role in allergic airway inflammation by secreting cytokines including IL-4, IL-5, IL-9, and IL-13. Th1 cells are important for cell-mediated immunity because they produce interferon (IFN)-γ, IL-2, and other cytokines. The cytokines produced by each subset of cells act in a paracrine fashion to promote the growth of their own phenotype. Thus, once a particular pathway has been entered, the response will be skewed toward that cell line. However, complete polarization into either type of response appears to be very uncommon during natural animal diseases. The current belief is that the balance between the Th1- and Th2-type responses, rather than the complete polarization into either phenotype, is important for the modulation of airway inflammation.

The polarization of uncommitted T cells (Th0) into either a Th1 or a Th2 response is the result of complex interactions between immune cells, including interactions between the T-cell receptor/CD3 complex and the antigen/major histocompatibility complex on antigen-presenting cells and the presence of additional signals produced by costimulatory or accessory molecules. Although the capacity of equine T helper cells to differentiate into Th1 and Th2 subsets has not been thoroughly investigated, *in vitro* culture of equine $CD4^+$ T cells following chronic allergenic stimulation has induced a Th2 cytokine profile

characterized by an increased expression of IL-4 mRNA and a decreased expression of IL-2 mRNA (Aggarwal & Holmes 1999). Furthermore, consistent with the capacity of horses to develop a Th2-type cell response, ovalbumin inhalation by sensitized ponies increased the BAL cell mRNA expression of the Th2-type cytokines IL-4 and IL-13, and decreased the expression of IFN-γ, a Th1 cytokine (Bowles et al 2002).

Lower airway inflammation, reversible airway obstruction, and bronchial hyperresponsiveness are characteristic of both equine heaves and human asthma. Asthma is associated with a predominant Th2-type cytokine expression in BAL cells and it had been postulated that a similar process occurs in RAO and SPAOPD. Consistent with this, BAL cells from horses with RAO which had been stabled for several months had an increased expression of the mRNA for Th2-type cytokines IL-4 and IL-5, when compared to control horses, while the expression of IFN-γ mRNA was decreased (Lavoie et al 2001). These changes were not detected when the horses were at pasture, but started within 24 h of exposure to moldy hay (Cordeau et al 2004). Furthermore competitive reverse transcription–polymerase chain reaction (RT-PCR) revealed increased expression of IL-4 mRNA in RAO-affected horses after 3 weeks of exposure to moldy hay (Giguere et al 2002). Real-time RT-PCR analysis of BAL and peripheral blood cells from horses affected with SPAOPD also demonstrated that clinical disease was associated with elevated levels of mRNA for IL-4 (Beadle et al 2002). These findings were consistent with the concept that RAO and SPAOPD are associated with a predominant Th2-type cytokine response. IL-4 promotes development and growth of the Th2 cell phenotype and is essential for the induction of B-cell isotype switching to IgE production (Lasky & Brody 1997). The increase in IL-4 mRNA expression in heaves is coherent with the elevated levels of antigen-specific IgE in serum and BAL of horses with heaves (Halliwell et al 1993, Schmallenbach et al 1998, Eder et al 2000). The significance of the increased IL-5 mRNA expression that was noted in these studies is less clear, since increased expression of IL-5 mRNA is usually associated with tissue eosinophilia, which is an inconsistent finding in heaves. The recent finding that horses with RAO have high levels of the transcription factor nuclear factor-κB (NF-κB), consisting mainly of truncated p65 homodimers rather than classic p65–p50 heterodimers, may contribute to the lack of BAL eosinophilia in heaves (Bureau et al 2000a), as loss of the p50 locus in knockout mice ablates the eosinophilic airway response (Yang et al 1998).

While a Th2-type response is typically associated with tissue eosinophilia, pooling of neutrophils in the lower airways of affected horses is characteristic of RAO. Interestingly, allergen inhalation by sensitized ponies resulted in a predominant Th2-type response and BAL neutrophilia, suggesting that neutrophils rather than

eosinophils predominate in equine allergic pulmonary diseases (Bowles et al 2002). Furthermore, mRNA expression of IL-17, a cytokine secreted by activated T cells that indirectly promotes maturation, chemotaxis, and activation of neutrophils, was increased in horses with heaves following exposure to moldy hay (Debrue et al 2004). There is also evidence that Th2-type cytokines are implicated in the modulation of the neutrophilic inflammatory response. Injection of IL-4 into humans causes a neutrophilia (Gilleece et al 1992) and accelerates the maturation of myelocytes into neutrophils (Bober et al 1995). Functional IL-4 receptors are present on human neutrophils and their activation leads to cytoskeletal rearrangements, *de novo* protein synthesis, and inhibition of neutrophil apoptosis (Girard et al 1997). Also, horses with RAO have significantly increased numbers of neutrophils that express IL-5 and IL-9 receptors compared to control horses (Al-Dewachi et al 2002). These results provide a possible mechanism by which Th2-type cytokines could play a role in heaves by stimulating neutrophils to release various pro-inflammatory mediators, thereby amplifying the inflammatory response.

However, while the above findings support the contribution of Th2-type cytokines to RAO, other studies have reported contradictory results. The concurrent increases in the mRNA expression of both IL-4 and IFN-γ noted in some studies suggested a mixed Th1 and Th2 response (Beadle et al 2002, Giguere et al 2002, Ainsworth et al 2003). In another study, increased IFN-γ without changes in Th2-type cytokines suggested a predominant Th1-type response (Ainsworth et al 2003). The apparently contradictory conclusions of these studies may be explained by differences in the methods used for tissue collection and cytokine measurements, duration of exposure to moldy hay before the sampling (chronicity of inflammatory response) and differences in factors such as deworming, vaccination, and the quantity and composition of airborne dust in the environment. These findings also highlight the complexity of the factors that determine the heaves phenotype and suggest that multiple pathways may lead to this syndrome. Clearly, more work will be required before a definite conclusion can be reached concerning the contribution of Th1/Th2-type cytokines to heaves.

Mast cells

Liberation of pro-inflammatory mediators by mast cells following cross-linkage of their membrane-bound IgE receptors is central to acute allergic responses. Within minutes after exposure to an allergen, sensitized mast cells release vasoactive molecules, proteases, cyclooxygenase metabolites, cytokines, and chemokines. Among those, histamine contributes to the initial bronchospasm, vascular leakage, and increased mucus secretion and serves as a surrogate marker of mast cell activation. In RAO-affected horses, allergen challenge increased the concentration of

histamine in the pulmonary epithelial lining and decreased BAL mast cell numbers, presumably attributable to their degranulation (Derksen et al 1988, McGorum et al 1993b). Furthermore, pulmonary mast cells from RAO-affected horses degranulate more than mast cells from control horses in response to *in vitro* fungal antigen challenge, suggesting presensitization of mast cells from RAO-affected horses (Hare et al 1999).

Eosinophils

Bronchoalveolar eosinophilia is uncommon in RAO and peribronchial eosinophilic infiltrates are inconsistent findings, indicating that eosinophils are not pivotal in RAO pathogenesis.

Macrophages

Macrophages are a family of antigen-presenting cells that contributes to tissue damage and repair in various inflammatory conditions through phagocytosis and the production of a wide range of molecules. Pulmonary macrophages have been divided into four categories based primarily on their anatomical locations, but only those macrophages that can be retrieved by BAL (presumably of the alveolar type) have been studied in RAO. BAL macrophages from RAO-affected horses have a higher density than those from control horses, suggesting an elevated state of activation (Tremblay et al 1993). Compared to the general population, high-density macrophages are generally more cytotoxic and release more pro-inflammatory mediators including superoxide anion, IL-1 and thromboxane B_2. Equine macrophages also have the capacity to produce pro-inflammatory cytokines such as TNF-α, IL-1β, IL-8 and MIP-2, which are potent chemoattractants for neutrophils (Franchini et al 1998, Joubert et al 2002).

Epithelial cells, smooth muscle, and other parenchymal and mesenchymal cells

While the contribution of leukocytes to inflammation has long been recognized, there is emerging information highlighting the critical role of mesenchymal and parenchymal cells in the modulation of airway inflammation and tissue remodeling. Epithelial cells are important in many lung diseases as a consequence of their role as a physical and functional barrier to inhaled gases and particles and because of the extensive array of receptors they possess and mediators they produce. While our understanding of the role of epithelial cells in RAO and SPAOPD is limited, emerging information indicates that they may be important modulators of the inflammatory response. For instance, bronchial epithelial cells of horses with heaves have an increased expression of transcription factor NF-κB, a regulator of inflammatory gene expression. The bronchial activity of NF-κB is correlated with airway function and BAL neutrophilia, and may therefore play a key role in both airway inflammation and obstruction in
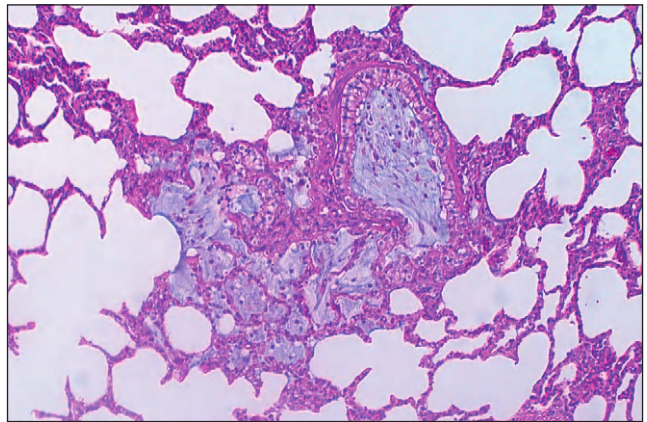
Fig. 41.1. Marked goblet cell hyperplasia and mucus accumulation in a bronchiole from an RAO-affected horse that failed to improve with turning out to pasture and corticosteroid administration. The mucus appears to plug the bronchioles and pools of mucus may be seen in adjacent alveoli.

heaves (Bureau et al 2000a,b). Also, bronchial epithelial cells of horses with SPAOPD have increased immunoreactivity to inducible nitric oxide synthase (iNOS) (Costa et al 2001). The iNOS-derived nitric oxide (NO) may contribute to the amplification of the airway inflammation, and may possibly down-regulate Th1 cells, favoring immune deviation towards a Th2-type response. Furthermore, goblet cell hyperplasia and increased mucin accumulation contribute to airway obstruction and clinical exacerbation.

Mucus accumulation

Airway mucus accumulation is a consistent finding in horses with RAO and is associated with neutrophilic airway inflammation and coughing (Robinson et al 2003). When RAO-affected horses have been pastured for several weeks and most components of lower airway disease, including bronchospasm and inflammation, have resolved, mucus accumulation persists at higher levels than in healthy horses (Jefcoat et al 2001). This persistent mucus accumulation may contribute to the residual lung function deficits noted in RAO horses when they are in remission. While poorly documented, mucus plugging of airways may also occur in some severely affected heaves horses that are relatively unresponsive to environmental change and to therapy (Fig. 41.1).

A 5-h duration hay and straw challenge resulted in increased tracheal secretions in RAO-affected horses but not in controls (Pirie et al 2001). Mucus accumulation in RAO exacerbation is accompanied by decreased mucus clearability resulting from a large increase in mucus viscoelasticity (Gerber et al 2000). Both in exacerbation and in remission of RAO, there are quantitative and qualitative changes in the carbohydrate side chains of
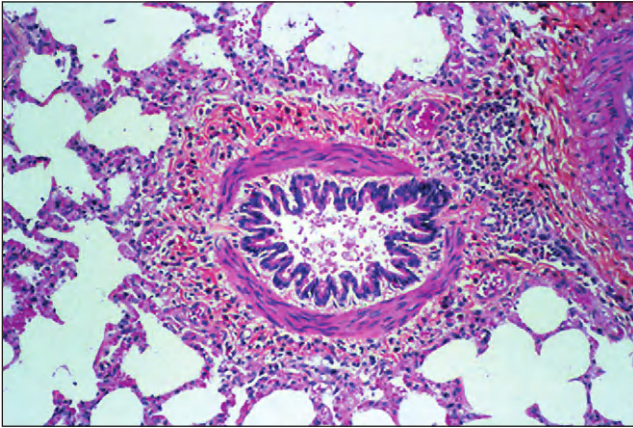
Fig. 41.2. Photomicrograph of the distal airways of a horse with RAO. Peribronchiolar infiltrates with primarily mononuclear cells and increased smooth muscle mass are present. There are mucus and inflammatory cells within the airway lumen.

airway mucins, and in particular of α-1,2-fucose (Jefcoat et al 2001). This may be linked both to mucus characteristics and to the expression of specific mucin genes. MUC5AC, a signature mucin in airway disease of humans and experimental models, but not MUC2, shows robust expression in large and small airways of RAO-affected and control horses (Gerber et al 2003).

Airway remodeling

Features of airway remodeling in RAO that decrease the caliber of the airway lumen include increased smooth muscle mass, peribronchial fibrosis and epithelial cell hyperplasia (Fig. 41.2). These features may contribute to progressive impairment in airway function in affected horses. The increased smooth muscle mass, in particular, may accentuate airway obstruction due to passive narrowing of the airway lumen and increased force of muscle contraction. Furthermore, while the role of airway smooth muscle has been traditionally restricted to the control of regional ventilation, new evidence indicates that smooth muscle is capable of secreting various cytokines, chemokines, and growth factors that may be important in RAO.

Pathology

Gross pathology

Macroscopic findings in RAO and SPAOPD are variable and not homogeneously distributed throughout the lungs. The lungs may be grossly normal, may be slow to collapse or may appear hyperinflated, primarily in the cranial lobes and in the periphery of the caudal lobes. Their normally pink color may be abnormally pale as a result of hyper-

inflation (Beech 1991). Exudate is commonly found within the airways. Severely affected horses may have emphysema, both vesicular and interlobular. Right ventricular hypertrophy, a common sequel to chronic lung disease in other species, is not a characteristic finding in RAO, although the weight of the right ventricle wall may be increased compared to that of the left ventricle (Dixon et al 1982).

Histopathology

In RAO and SPAOPD, mucus, neutrophils, and cellular debris are commonly present within the airway lumina (Figs 41.1 and 41.2). The mucus may appear to plug the bronchioles and pools of mucus may be seen in adjacent alveoli (Thurlbeck & Lowell 1964, Kaup et al 1990). The distal airways are the primary site of the microscopic lesions. Common findings include inflammation of the distal airway wall, epithelial metaplasia and desquamation, increased smooth muscle mass, and peribronchial fibrosis. Inflammation of the airway wall is mainly the result of chronic bronchiolitis with predominantly lymphocytic and plasmacytic infiltration of the bronchiolar and peribronchiolar regions (Thurlbeck & Lowell 1964, Winder & von Fellenberg 1987, Nyman et al 1991). Goblet cell hyperplasia and metaplasia, and fibrosis of bronchiolar submucosa and alveoli are also common. Aggregates of lymphocytes forming small nodules and follicles around the vasculature of the small airways may also be seen (Winder & von Fellenberg 1987). Peribronchial fibrosis and accumulations of eosinophils and mast cells are inconsistent findings. The large airways may have loss of ciliated epithelial cells, and peribronchial inflammation of varying severity may be observed focally (Kaup et al 1990). All sizes of airways show increased airway smooth muscle mass but this is most marked in the distal airways (Herszberg et al 2004). Emphysema (enlarged air spaces resulting from destruction of alveolar walls) has been reported in RAO, although alveolar hyperinflation as the result of air trapping is more common (Thurlbeck & Lowell 1964, Gerber 1973). Furthermore, the subpleural emphysema occasionally seen in horses with heaves must be interpreted with caution as it may also be observed in older horses without respiratory disease (Thurlbeck & Lowell 1964). Nevertheless, areas of alveolar emphysema are associated with markedly increased numbers of Kohn's pores, allowing increased collateral ventilation (Kaup et al 1990). Fibrosis, epithelial cell metaplasia, and mononuclear cell infiltrates of the alveolar region may be present in areas with peribronchial inflammation (Winder & von Fellenberg 1987, Kaup et al 1990). The changes observed in the alveolar regions are believed to be the result of bronchiolar obstruction and extension of the bronchiolar inflammation to the adjacent alveoli. When bronchiectasis is present, the cartilaginous plates of the dilated bronchus may be widely separated from each other and the chondrocytes may appear disorganized.

The number of elastic fibers in the lamina propria may be greatly reduced (Lavoie et al 2004).

SPAOPD is characterized by an accumulation of mucus, degenerated and sloughed epithelial and inflammatory cells (predominantly neutrophils) within the airways, and variable peribronchial smooth muscle hypertrophy and inflammation (Costa et al 2000). Fibrosis and emphysema are not prominent features of SPAOPD.

Clinicopathological correlations

In RAO, a good structural–functional relationship has been observed between bronchiolar alterations in necropsy specimens and clinical disease severity (Viel 1983, Kaup et al 1990). As the histological changes are focal and heterogeneously distributed, transthoracic lung biopsy results may not necessarily correlate with the necropsy findings. However, in one study, clinical scores were correlated with the severity of peribronchial mast cell and bronchiolar neutrophilic infiltrations in lung biopsies (Naylor et al 1992). In SPAOPD, histological findings in transthoracic lung biopsies were highly correlated with those found in post-mortem tissue samples (Costa et al 2000).

Epidemiology

Prevalence

The true prevalence of RAO and SPAOPD, as currently defined, is unknown. However, chronic pulmonary disorders are the most common cause of premature loss through disease in the Swiss horse population (Gerber 1973).

Breed, sex, gender, and age

Horses with RAO and SPAOPD are usually 7 years of age or older and all breeds and genders are affected. Results from a large retrospective epidemiological study conducted in a North American referral center suggested that females may be at increased risk of developing RAO, a finding anecdotally reported 150 years ago (Dun 1859, Couetil & Ward 2003). However, in most reported studies, both sexes were equally affected. There is also conflicting evidence concerning breed predilection, with thoroughbreds being either the most likely or the least likely breed to be affected with RAO (McPherson et al 1979a, Couetil & Ward 2003).

Genetic factors

It has long been suggested that RAO is a heretable condition, but only recently has a genetic predisposition to chronic respiratory diseases been documented in German warmbloods and Lippizaner horses (Dun 1859, Marti et al 1991). In a study of German warmbloods on a large stud farm, 17%, 48%, and 67% of the progeny had chronic pulmonary disease when, respectively, none, one or both parents had the disease (Marti et al 1991). Similarly, on a Lippizaner stud farm, 6%, 35% or 44% of the progeny had respiratory diseases when none, one or two parents had a history of chronic respiratory diseases. The study also showed that the offspring from an affected stallion had an increased risk of developing chronic respiratory disease when stabled in a poorly ventilated barn or when fed poor quality hay, suggesting that the phenotype is determined by environmental and genetic risk factors.

Clinical Manifestations

Natural history

RAO and SPAOPD are both seasonal disorders, but they occur at different times of the year. As RAO is associated with exposure to hay and straw, it is more common when horses are stabled during the winter. In contrast, clinical exacerbation of SPAOPD occurs during the warm summer months, mainly from June to September, when horses are grazing (Mair 1996b, Seahorn et al 1996). Horses with SPAOPD usually recover in autumn and are commonly unaffected during winter. However, those horses that have both RAO and SPAOPD may have respiratory disease throughout the year, and present a significant diagnostic and managemental challenge. Both conditions usually recur from year to year if horses are exposed to the appropriate environments.

Respiratory manifestations

When in disease remission, horses with RAO and SPAOPD appear clinically healthy at rest and their respiratory rate and breathing patterns are usually indistinguishable from those of unaffected horses. However, because of lung remodeling and persistent low-grade airway inflammation and mucus accumulation, affected horses may remain exercise intolerant or present with an occasional cough at the onset of exercise or when eating. Auscultation during normal tidal breathing is often unremarkable although during forced rebreathing some horses may have wheezes throughout the lung fields and expiratory crackles primarily at the periphery of the lungs. Interestingly, a period of clinical remission of between 1 and 3 months' duration did not improve morphological changes in lung biopsies from RAO horses, suggesting that altered airway morphology may persist even when clinical signs have resolved (Kvart et al 1986).

During periods of disease exacerbation, the clinical signs of horses with RAO and SPAOPD are non-specific and include serous, seromucous or mucopurulent nasal discharge, coughing, exercise intolerance, and labored

breathing. The frequency and severity of the coughing episodes increases as the disease progresses; thus severely affected horses have paroxysmal bouts of deep non-productive coughing. In severe cases, an increased respiratory rate, extended neck and head, flared nostrils (Fig. 41.3) and double expiratory effort are evident. A "heave line" caused by hypertrophy of the external abdominal oblique muscles may develop. The anus may "pump", as a result of forced expiration, and flatulence may occur, especially when the horse coughs. The appearance and severity of the clinical signs tend to wax and wane in RAO. The duration of the exacerbations varies from days to weeks and some horses may be asymptomatic at rest between exacerbations even if they are maintained in the same deleterious environment. As some horses may require exposure to moldy hay for 1 month before showing signs of heaves (Grünig et al 1989), short duration exposure to moldy hay may be ineffective for the detection of heaves-susceptible horses in a prepurchase situation.

During severe clinical exacerbations, crackles and wheezes may be auscultated without the use of a rebreathing bag and thoracic auscultation may detect areas with decreased audibility of breath sounds. Percussion of the thorax may occasionally reveal increased resonance of the ventral and caudal borders of the lung fields as a result of air trapping.

Other manifestations

Importantly, horses with mild to moderate RAO and SPAOPD have good appetite and remain alert and afebrile. Severe cases may develop weight loss, likely as a result of the increased energy expenditure resulting from the increased work of breathing (Mazan et al 2004). Horses with recurrent fever episodes and severe clinical airway obstruction may have secondary bacterial bronchopneumonia or bronchiectasis (Mair 1996a, Lavoie et al 2004). Heart rate and cardiac output usually remain within

Fig. 41.3. RAO-affected horse with respiratory distress. Note the characteristic extended position of the head and neck and the dilated nostrils.

normal ranges, although tachycardia and pulmonary hypertension are common during clinical exacerbation (Nuytten et al 1988, Seahorn et al 1996). This increase in pulmonary vascular resistance is likely the result of hypoxemia as it is closely correlated to P_{AO_2} in horses with RAO (Nuytten et al 1988).

Assessment of Lung Function

Clinical scores

Clinical scores have been developed to estimate the severity of airway obstruction in horses with both RAO and SPAOPD. These are more useful for the identification of group differences for research purposes than for quantifying the degree of airway obstruction in individual horses. They are based on grading the clinical signs commonly found during severe exacerbation of RAO such as cough, nasal flaring, nasal discharges, abdominal muscle excursion, and movement of the anus. A simple clinical scoring system which graded nasal flaring and abdominal effort was found to correlate well with, but be less sensitive than, standard lung mechanics measurements in RAO (Rush et al 1998b, Robinson et al 2000). In SPAOPD, a simple scoring system based on nasal flare and abdominal lift was strongly correlated with maximal change in pleural pressure (Costa et al 2000).

Respiratory mechanics

Lung function measurement facilitates objective assessment of the degree of airway obstruction. It is primarily used in research settings because it requires specialized equipment and expertise, and has a low sensitivity. Sequential respiratory mechanics measurements are especially useful to assess treatment efficacy.

Robinson et al (2000) reviewed the changes in lung function that occur in heaves:

> *the diffuse obstruction of the airways leads to increased pulmonary resistance due to airflow and tissue resistance and increased dynamic elastance (or decrease in dynamic compliance), which reflects peripheral airway obstruction, parenchymal tissue remodeling and an uneven distribution of ventilation. To maintain ventilation, a greater effort by the respiratory muscles is applied to the thoracic wall to expand and compress the lungs, resulting in an increase in the variation of pleural pressure during each respiration. Also, while the tidal volume remains generally unaffected or is only mildly decreased, increased ventilation is achieved by increasing the respiratory rate and changing breathing strategy by moving air at the end of inhalation and the beginning of expiration, when the airways are at their widest. This leads to increases in both peak inspiratory and expiratory flow. In severe cases, the rapid contraction of thoracic muscles at the beginning of expiration is followed by a more prolonged contraction of abdominal muscles at the end of expiration, which cause the typical doubled expiratory efforts observed of RAO.*

Using standard respiratory mechanics techniques (measurement of esophageal pressures and flow rates), lung function parameters of horses with heaves in clinical remission are not significantly different from those of normal horses. However, airflow limitation appears to be only partially reversible in some horses with RAO, especially when using more sensitive measuring techniques, such as determination of airway hyperresponsiveness, forced oscillation, forced expiration, and volumetric capnography.

Airway hyperresponsiveness describes an excessive response of the airways to intrinsic chemical mediators, such as histamine, or to synthetic analogs of acetylcholine, such as methacholine and carbachol. The cause of airway hyperresponsiveness in RAO is unknown but may include reduced airway caliber as a result of airway remodeling (increased airway smooth muscle mass, goblet and epithelial cell hyperplasia, and peribronchial fibrosis), impaired inhibitory mechanisms that normally limit smooth muscle contraction, and sensitization of cholinergic nerves and smooth muscle by inflammatory mediators to facilitate smooth muscle contraction (Robinson 2001). This characteristic feature of RAO is of clinical relevance because affected horses have increased susceptibility to develop airway obstruction in response to a wide range of specific (such as mold or pollen allergens) and non-specific (such as cold air, dry air, exercise, irritant dusts) triggers (Obel & Schmiterlöw 1948). While RAO-affected horses have increased airway hyperresponsiveness even when stabled in a low-dust environment (Votion et al 1999, van Erck et al 2003), when they are at pasture their airway responsiveness is indistinguishable from that of controls (Derksen et al 1985, Fairbairn et al 1993a, Votion et al 1999).

Forced oscillation techniques are more sensitive and less invasive than standard respiratory mechanics measurements for the diagnosis of heaves (Mazan et al 1999, van Erck et al 2003). These techniques may gain popularity for the evaluation of equine lung function following the commercial availability of a system measuring impulse oscillometry, a forced oscillation method that generates pulse-shape signals in a wide range of frequencies (IOS MasterScreen, Jaeger GmbH, Wurzburg, Germany). Similarly, the *forced expiration technique* and *volumetric capnography* are more sensitive that standard respiratory mechanics for the detection of airway obstruction (Couetil et al 2000). *Whole body plethysmography* has also been used to study airway function of horses with SPAOPD (Beadle 1985); however, the technical difficulties

associated with this method have limited its use in both clinical and research settings. *Respiratory inductance plethysmography* is more practical and may prove helpful for the assessment of airway function in field studies (Hoffman et al 2001).

Gas exchange

Arterial hypoxemia is a characteristic finding during clinical exacerbation of RAO that reflects uneven distribution of ventilation as the result of diffuse but variable airway obstruction. Arterial blood gas analysis allows assessment of the degree of respiratory dysfunction in severely affected RAO horses and assessment of the response to therapy. However, the degree of hypoxemia is poorly correlated with pulmonary mechanics (Nuytten et al 1988). In severe cases, $P_{A}O_2$ values less than 50 mmHg may be observed and may be accompanied by hypercapnia, although $P_{a}CO_2$ usually remains within normal limits (Willoughby & McDonnel 1979, Kvart et al 1986). Arterial acidemia is rare because of the compensatory metabolic alkalosis (Picandet et al 2004).

The prolonged nitrogen washout in horses with RAO is consistent with the presence of small airway disease and correlates with the severity of histological lung changes (Willoughby & McDonnel 1979, Viel 1983). Increased dead space ventilation and ventilation of high $\dot{V}_A/\dot{Q}$ regions were not correlated with clinical signs or lung biopsy (Nyman et al 1991). However, there was a strong correlation between the extent of bronchiolar epithelial hyperplasia and ventilation of high $\dot{V}_A/\dot{Q}$ regions and dead space (Nyman et al 1991). Alveolar clearance, measured using scintigraphy, is a sensitive technique to quantify the ventilation–perfusion mismatch in RAO (Votion et al 1999).

Laboratory Findings

Pulmonary cytology and bacteriology

Cytological examination of respiratory tract secretions is commonly used for the diagnosis of equine lower airways diseases. Since RAO is associated with diffuse lung pathology, the cytological findings of BAL samples from different lung segments show good correlation (McGorum et al 1993a, Jean 1996).

Macrophages and lymphocytes are the predominant cell populations in BAL from normal horses and the proportions of other cell types are negligible (Fig. 41.4). RAO and SPAOPD are characterized by a BAL neutrophilia (RAO neutrophils > 25%, controls ≤ 5%), the degree of which is poorly correlated with the severity of clinical signs (Grünig et al 1989). In a study where BAL was performed at monthly intervals for 3 months, on no occasion did any of the horses with RAO have < 25% neutrophils in BAL, suggesting that there is insignificant intra-horse variability in neutrophil counts given continuous dust challenge (Jean 1996). This finding is in agreement with the recent proposal that > 25% neutrophils in BAL is necessary in a research setting for a horse to qualify as being affected with RAO (Robinson 2001). However, in advanced cases, labored breathing is occasionally observed in horses with only mild (10–20%) airway neutrophilia. As stabling of both young (Holcombe et al 2001) and older horses which are free of clinical respiratory disease (Tremblay et al 1993) may induce an airway neutrophilia, the results of BAL cytology should be interpreted in conjunction with the history and other clinical findings. Importantly, the airway neutrophilia in RAO and SPAOPD should not be interpreted as evidence of bacterial infection, even if degenerate neutrophils with karyolysis and cytoplasmic vacuolation are also noted. In addition to the airway neutrophilia, large amounts of mucus and increased numbers of exfoliated epithelial cells may be seen within the airways. The presence of fungal elements in BAL cell preparations suggests impaired mucociliary clearance and/or exposure to high levels of airborne organic dust, rather than fungal infection of the airways. Curschmann's spirals, which are coils of inspissated mucus fibrin, may be observed (Fig. 41.4). RAO-affected horses rarely have increased numbers of metachromatic cells and eosinophils in their airway secretions (Winder et al 1990, Vrins et al 1991).

Tracheal aspirates comprise secretions from both the peripheral and central airways throughout the lungs. Neutrophil counts in tracheal aspirates are poorly correlated with BAL neutrophil counts and with lung histology in RAO (Larson & Busch 1985, Derksen et al 1989, Winder et al 1990, Traub-Dargatz et al 1992). Furthermore, the neutrophil percentages found in tracheal washes of normal horses vary widely, and the cytological features observed in RAO are non-specific and occur in a number of other inflammatory lung diseases. The presence of bacteria in tracheal aspirates from RAO- and SPAOPD-affected horses, in the absence of clinical signs of bacterial infection (fever, anorexia, depression), most likely represents secondary colonization of the large airways and reduced mucociliary clearance, rather than primary bacterial infection.

Hematology and serum biochemistry

The hemogram, leukogram, and fibrinogen concentration are usually within reference ranges in RAO and SPAOPD. Mild and inconsistent increases in packed cell volume, as the result of elevated mean erythrocyte corpuscular volume, have been reported in some horses with chronic respiratory diseases (Gerber 1973). These analyses may, however, be performed to aid detection of secondary bacterial infection in severely affected horses that are febrile or unresponsive to therapy.
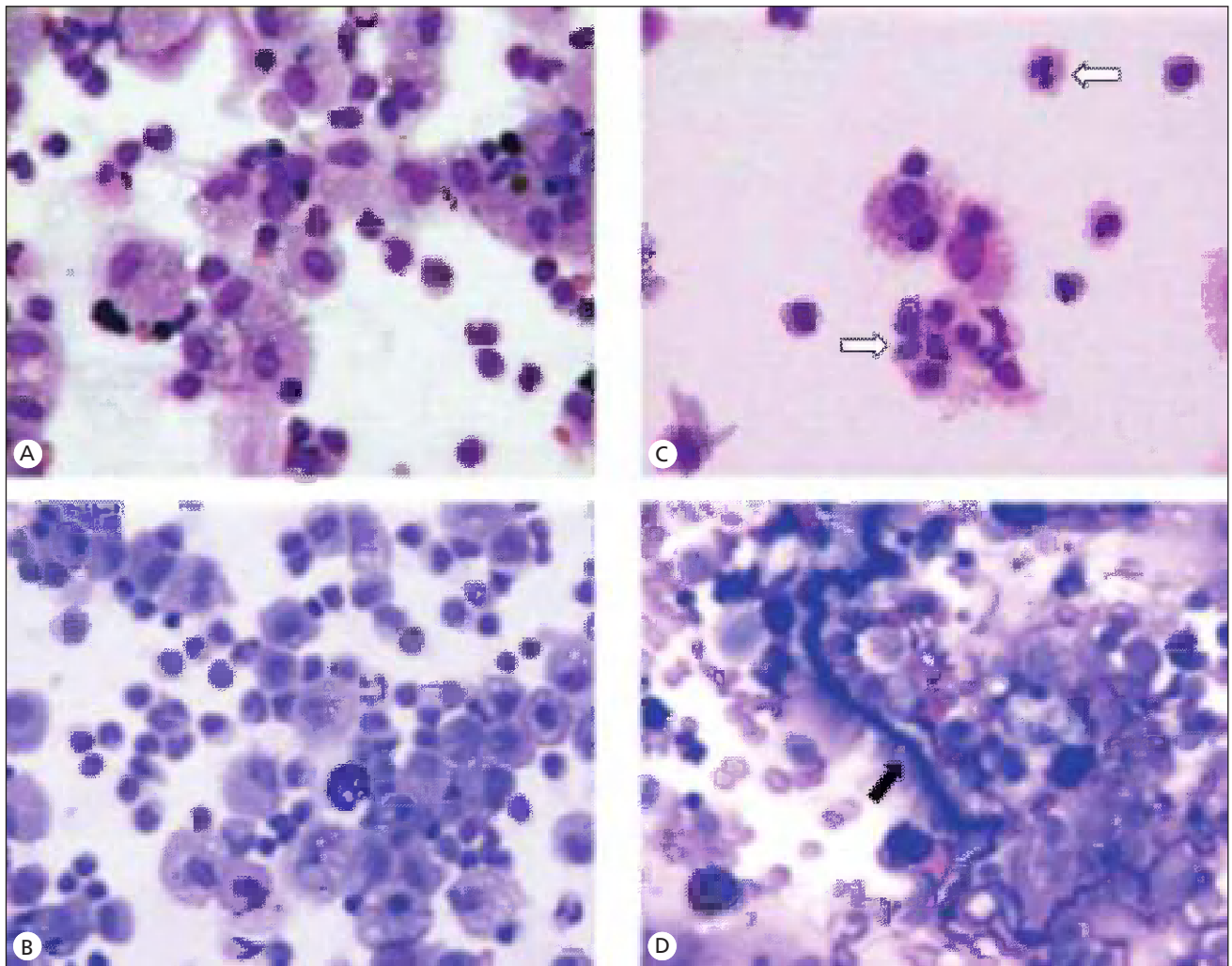
Fig. 41.4. BAL cytology from a control horse (A,B) and a horse with RAO (C,D). While lymphocytes and macrophages predominate in the control, there are numerous neutrophils (white arrows) in the RAO-affected horse. A Curschmann's spiral (black arrow) is also present (D).

Imaging techniques

Endoscopy

Endoscopy of the lower airways of horses with RAO and SPAOPD generally reveals variable amounts of mucopus within the trachea. The tracheal carina may be thickened and the tracheal and bronchial mucosa may be hyperemic (Mair 1996b). Dynamic collapse of the intrathoracic airways may occur during coughing. The presence of distended and irregular bronchial walls suggests bronchiectasis, a sequel of severe RAO (Lavoie et al 2004).

Radiography

Radiographic examination of the thorax is often unremarkable but may reveal an increased bronchointerstitial pattern. Flattening or concavity of the diaphragm is indicative of alveolar hyperinflation, and may be reversible when affected horses attain clinical remission (McPherson

et al 1978, Seahorn & Beadle 1993). Localized radiolucent areas, suggestive of emphysematous bullae, occur rarely in advanced cases. The presence of single or multiple distended bronchi is indicative of bronchiectasis (Fig. 41.5).

Other imaging techniques

Thoracic ultrasonography of RAO-affected horses is usually unrewarding, although the surface of the visceral pleura may have irregular echogenicity. Scintigraphic determination of the alveolar clearance rate is a sensitive method of detecting lung damage that facilitates detection of RAO-affected horses even when they are clinically asymptomatic (Votion et al 1999).

Immunological testing

Intradermal allergen testing is an accepted method for the detection of allergen hypersensitivity in atopic humans and
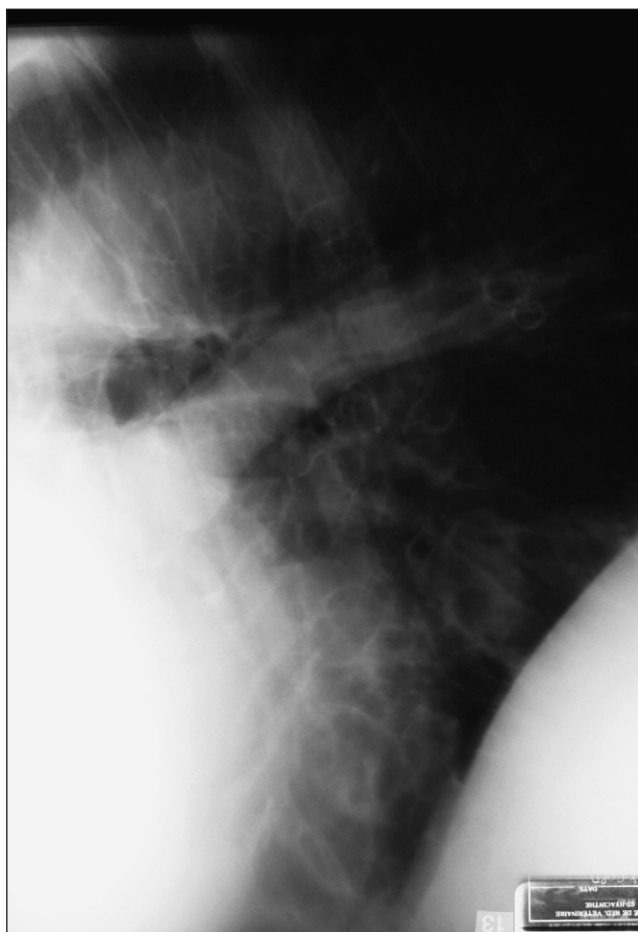
Fig. 41.5. Thoracic radiograph of a horse with cylindrical bronchiectasis. Multiple cavitary lesions are present throughout the lung field and the bronchial walls are thin and regular.

Table 41.2. Criteria commonly used for the clinical diagnosis of RAO and SPAOPD	
Signalment	Horses are usually 7 years of age or older No sex or breed predispositions
History	Respiratory problem of > 3 months' duration Period of labored breathing (nasal flaring, expiratory effort), reversible with broncho-dilator, corticosteroids, pasture (RAO) or stabling (SPAOPD)
Clinical findings	Horses are afebrile, bright, and alert unless in respiratory distress Labored breathing with doubled expiratory effort and heaves line may be present Crackles and wheezes are audible on thoracic auscultation
BAL cytology	>25% neutrophils during disease exacerbation Normal cytology when in disease remission
Thoracic radiography	Not performed routinely May be used to exclude other conditions such as pneumonia or neoplasia Usually normal chest, but bronchointerstitial patterns, hyperinflation, and emphysematous bullae may be present Bronchiectasis in severe cases

domestic animals. Horses with RAO have immediate (30–60 min), late-phase (4–6 h), and delayed (24–48 h) reactions to environmental allergens. However, intradermal testing is not discriminating of health status because most horses have positive skin reactions to common barn allergens, although horses with heaves tend to have a greater number of positive skin reactions at both 30 min and 4 h. A radioallergosorbent test and two enzyme-linked immunosorbent assays are commercially available in the USA, but they failed to reliably detect allergen hypersensitivity when compared to intradermal testing (Lorch et al 2001). For these reasons, intradermal mold antigen testing and the evaluation of serum antibody titers against common environmental antigens appear to be of little value in the diagnosis of equine RAO.

Analysis of exhaled markers of inflammation

Analysis of inflammatory mediators or related molecules in breath condensate and exhaled breath is being evaluated

as a non-invasive method for the diagnosis of airway inflammatory diseases. To date, this methodology is not used in clinical settings.

Diagnosis and Differential Diagnosis

Diagnosis

In clinical practice, a diagnosis of RAO and SPAOPD (Table 41.2) is generally based on the signalment (horses > 7 years of age), history (bright, alert, coughing episodes of > 3 months' duration, normal appetite, absence of pyrexia) and results of clinical examination (double expiratory effort, dilated nostrils, abnormal breath sounds on auscultation of the cervical trachea and both hemithoraces). The diagnosis can be confirmed by demonstrating airway neutrophilia and the reversibility of airway obstruction with environmental dust control or with bronchodilators (Table 41.3). Inhaled bronchodilators are well tolerated in horses and may improve the clinical signs of airway obstruction within minutes of administration.

Differential diagnosis

A thorough history and clinical examination usually suffice to differentiate RAO from other conditions that may cause clinical signs of lower airway disease.

Table 41.3. Medications recommended for the treatment of acute exacerbations of RAO and SPAOPD: suggested dosages are indicative only

Medication	Dosage
Corticosteroids	
Dexamethasone	0.04–0.1 mg/kg q24h IV 0.164 mg/kg q24h PO, preferably before feeding
Dexmethasone-21-isonicotinate	0.04 mg/kg q3 days IM
Isoflupredone acetate	0.03 mg/kg q24h IM
Triamcinolone acetonide	20–40 mg* IM
Beclomethasone dipropionate (CFC) Beclomethasone dipropionate (HFA)	3,500 μg/horse, q12h in MDI (Equine Aeromask®) 1,320 μg/horse, q12h in MDI (3M Equine Aerosol Delivery System®)
Fluticasone propionate (CFC)	2,000 μg/horse, q12h in MDI (Equine Aeromask®)
Bronchodilators	
Clenbuterol	0.8–3.2 μg/kg PO q12h 0.8 μg/kg IV
Aminophylline	5–10 mg/kg PO q12h
Fenoterol	1–2 mg/horse, q1h, in MDI (Equine Aeromask®)
Albuterol	0.8–2 μg/kg, q1h, in MDI (3M Equine Aerosol Delivery System, Equine Aeromask®)
Pirbuterol	1.3 μg/kg q7h, in MDI (3M Equine Aerosol Delivery System, Equine Aeromask®)
Salmeterol	0.5–1 μg/kg, q6h, in MDI (Equine Aeromask®)
Ipratropium bromide	180–450 μg/horse, q6h, using a mechanical nebulizer 0.4–1 μg/kg, q6h, in MDI (Equine Aeromask®) 2,400 μg/horse, q6h, using a DPI (EquiPoudre®)
Cromones	
Sodium cromoglycate	80 mg/horse, q24h for 4 days using a mechanical nebulizer 200 mg/horse, q12h, in MDI (Equine Aeromask®)

* The usual dose for a horse that weighs 450–500 kg.
CFC, chlorofluorocarbon; DPI, dry powder inhaler; HFA, hydrofluoralkane; MDI, metered-dose inhaler.

IAD

IAD and RAO share a number of clinical, cytological, and functional similarities. However, the lack of labored breathing in IAD permits differentiation from RAO. The differences between the two disorders are further highlighted in Table 42.1 in Chapter 42.

Bronchopneumonia

Manifestations of sepsis, such as fever, depression, decreased appetite, and weight loss, are usually present in bacterial or fungal bronchopneumonia and pleuro-pneumonia but are absent in RAO. However, in horses with severe exacerbation of heaves, or when bronchiectasis is present, febrile episodes, anorexia and weight loss may occur. In these horses complete blood count, plasma fibrinogen estimation, and thoracic radiography may be necessary to exclude the presence of bacterial infections.

Viral infection

The short duration of the respiratory symptoms resulting from viral infections allows their differentiation from RAO.

Chicken hypersensitivity pneumonitis

This uncommon condition develops when horses share stabling with chickens, especially when the chickens roost above the stables (Mansmann et al 1975). The clinical signs of this disorder resemble those of RAO, and include coughing, obvious airway obstruction, and rapid reversal of clinical signs when horses are removed from the causal allergens.

Neoplasia

Thoracic neoplasia may present with numerous clinical signs, some of which may resemble RAO. Thoracic radiography and ultrasonography, cytological analysis

Table 41.4. Practical management of RAO

1. **The clinical signs of RAO are reversible, but chronic cases may develop incompletely reversible lung dysfunction**

2. **Reduce airborne dust levels**
    a. Most effective means to control the clinical signs and prevent the progression of RAO
    b. May take up to 6 weeks before clinical signs subside
    c. Remove hay from diet
    d. Turning horse out to pasture without hay supplement is ideal
    e. Pelleted hay and cubes are practical but may be associated with development of stereotypic behavior
    f. Hay silage may be associated with botulism in certain countries
    g. Use low dust bedding such as wood shavings or shredded paper
    h. Ensure adequate stable ventilation
    i. Prevent exposure to dust particles (house horse away from hay stores, remove horse from stable while mucking out the stall)

3. **Corticosteroids**
    a. Most effective drugs
    b. Clinical signs will return if not combined with environmental dust control
    c. May be associated with severe side effects
    d. Inhaled corticosteroids are preferable for prolonged therapy, however:
         (i)   Expensive and time consuming
         (ii) Inadequate administration techniques may result in treatment failure
        (iii) Mask is poorly tolerated when labored breathing is present

4. **Bronchodilators**
    a. Offer symptomatic relief only
    b. Should be combined with environmental dust control and/or corticosteroid therapy
    c. Variable efficacy in horses with labored breathing
    d. May improve the delivery of inhaled corticosteroids to the small airways

of lower airway specimens and histological findings of transthoracic or bronchial biopsies may help confirm the diagnosis.

Lungworm

Horses with *Dictyocaulus arnfieldi* infection may have clinical signs similar to those of RAO, including paroxysmal coughing, crackles and wheezes on thoracic auscultation, and increased respiratory effort (George et al 1981). Clinical diagnosis of lungworm is based on a history of contact with mules or asses and, occasionally, the presence of eosinophils and immature *D. arnfieldi* in tracheal aspirates. The resolution of the clinical signs with appropriate parasiticidal drugs may further help to differentiate lungworm infection from RAO.

Treatment of RAO and SPAOPD

(Tables 41.3 and 41.4)

Environmental control

Reduction of exposure to environmental dust is essential for the successful long-term management of horses with RAO and SPAOPD. In RAO, remission of clinical signs and airway inflammation is best achieved by pasturing affected horses all year long. When this is not possible, exposure to airborne stable dust can be diminished by changing the feedstuff and bedding, decreasing the degree of agitation of the source material (which occurs primarily during eating,

and cleaning of the stable) and increasing ventilation to remove the airborne dust (Clarke et al 1987, Woods et al 1993). The replacement of hay and straw by less dusty feed and bedding material is central to the management of horses with RAO. Pelleted or cubed hay, hay silage and hydroponic hay are well tolerated and relatively free of dust, although mild degrees of inflammation and abnormal lung function may persist when horses are fed grass silage and bedded on wood shavings, as indicated by increased airway responsiveness (Vandenput et al 1998a, Votion et al 1999). Soaking hay in water for 2–4 h before feeding may control heaves in some horses, while it affords only partial or no improvement in others (Beech 1991). Wood shavings, shredded paper, peanut kernels, and peat moss are good substitutes for straw, although a recent study failed to find differences in airway function in heaves-susceptible horses fed silage that were bedded on good quality straw or shavings (Vandenput et al 1998b). This may be explained by the finding that good quality straw has low dust particle content (Vandenput et al 1997). Other common recommendations include use of a box stall away from the hay chute, removing the horse from the stable when cleaning the box stall, and watering the aisles before sweeping to decrease the airborne dust. Proper ventilation is also important, although identifying the optimal ventilation system to minimize dust levels is problematic.

The reversal of clinical signs of RAO with strict environmental changes may start within a few days but may take

up to 3–4 weeks (Thomson & McPherson 1984). The remission time correlates with age and the duration and severity of illness (Thomson & McPherson 1984). In a study where clinical cases of heaves were re-evaluated after 6 weeks of environmental dust control, only seven of 73 horses had BALF neutrophil ratios exceeding 8% (Dixon et al 1995). Horses kept permanently outdoors and fed grass or hay substitutes usually remain free of clinical signs, airway inflammation, bronchial reactivity, and abnormal alveolar clearance. However, supplementing horses with hay while at pasture may lead to airway neutrophilia, even in the absence of abnormal airway function at rest (Tremblay et al 1993). Horses do well when kept outdoors, even in very cold conditions, provided they have enough food, fresh water (heated water tub), and shelter.

Environmental control is also central to the successful management of SPAOPD. Affected horses should be removed from pasture and placed in a low-dust environment (Beadle 1983). As with RAO, it may take several days before an improvement in clinical signs is noted. However, in some cases, and in horses with labored breathing, stabling alone may not be sufficient and administration of bronchodilators and corticosteroids may also be required (Mair 1996b, Seahorn et al 1996).

Medications

Medications are required for the control of RAO when appropriate environmental dust control cannot be fully implemented or when more rapid relief of airway obstruction is required (see Chapter 7).

Anti-inflammatory agents

As inflammation is believed to play a central role in the pathophysiology of RAO, a number of anti-inflammatory agents have been evaluated for the treatment of RAO including corticosteroids, phosphodiesterase inhibitors, anti-leukotriene therapies, and other non-steroidal anti-inflammatory drugs.

Corticosteroids

Corticosteroids are the most potent anti-inflammatory drugs currently available for the treatment of RAO. They improve airway function by decreasing smooth muscle contraction by inhibiting the effects of inflammatory cells and their mediators, potentiating the bronchodilatory effects of catecholamines, and by reducing mucus production. Interestingly, horses that fail to improve with bronchodilators alone may have a marked improvement in airway function when treated with corticosteroids and environmental dust control. Corticosteroids may be administered orally, systemically or by inhalation. A number of corticosteroids have proven efficacy for the acute management of clinical exacerbations of RAO (Table 41.3). After the clinical signs have subsided, drugs are empirically administered at decremental doses and on alternate days until an effective maintenance dose is reached or treatment can be discontinued. Prolonged systemic administration of corticosteroids is to be avoided because of the widely feared, but poorly documented, side effects such as laminitis and enhanced susceptibility to infections. With recommended dosages of corticosteroids, pulmonary inflammation often persists and, unless a strict environmental regimen is concurrently implemented, the clinical signs of RAO are likely to recur rapidly following the cessation of drug administration.

Reported adverse effects of systemic corticosteroid administration to RAO-affected horses include adrenal suppression, altered bone metabolism, laminitis, bacterial pneumonia, neutrophilia, lymphopenia, and eosinopenia. To date, the only adverse effect attributed to inhaled corticosteroids is a decrease in serum cortisol (Rush et al 1997). While the clinical significance of the adrenal suppression remains to be determined, laminitis and bacterial pneumonia appear to be very uncommon when corticosteroids are used for the treatment of heaves.

Systemic corticosteroids

DEXAMETHASONE Dexamethasone is the most commonly used corticosteroid for the treatment of RAO in North America. While clinical improvements may be observed within a few hours of therapy (Cornelisse et al 2004), a delay of a week or longer can be expected before achieving the maximal improvement in lung function when concurrent dust control measures are not implemented. In horses with labored breathing, dexamethasone may be combined with a bronchodilator to provide more rapid symptomatic relief. Episodes of severe airway obstruction have been effectively treated with dexamethasone [initial dose 0.04–0.1 mg/kg, q24h intravenously (IV)] (Rush et al 1998b, Lavoie et al 2002a). Intramuscular administration of dexamethasone-21-isonicotinate (0.04 mg/kg) every 3 days was considered effective in eight of nine horses (Robinson et al 2002). A single oral administration of a dexamethasone solution formulated for intravenous use (0.164 mg/kg) improved the airway function of horses with RAO for up to 30 h (Cornelisse et al 2004). In the same study, fasting the horses before drug administration improved drug efficacy. However, oral administration of lower dosages of dexamethasone (0.02–0.05 mg/kg, q24h for 7 days) produced inconsistent improvement (Cesarini et al 2004). Administration of dexamethasone (0.05–0.1 mg/kg q24h IV) for 10–14 days attenuated airway obstruction but pulmonary neutrophilia persisted (Rush et al 1998a, Lavoie et al 2002b, Robinson et al 2002). Thus the control of airway neutrophilia

with dexamethasone appears to be dose related, and while compared in different studies, IV administration of dexamethasone at 0.1 mg/kg, but not at 0.05 mg/kg, resulted in a significant decrease in BALF neutrophilia (Lavoie et al 2002b, Robinson et al 2002).

ISOFLUPREDONE ACETATE Isoflupredone acetate administered by the intramuscular route (0.03 mg/kg q24h) is as effective as dexamethasone (0.04 mg/kg IV q24h) in improving the airway function of horses with RAO (Picandet et al 2003). Isoflupredone has a greater mineralocorticoid effect than dexamethasone but the hypokalemic myopathy reported in cattle and in people treated with this drug has not been reported in horses.

PREDNISOLONE Prednisolone is considered to be less potent and toxic than the aforementioned corticosteroids and has been used for the treatment of mildly affected horses or for maintenance therapy (Beech 1991). Prednisolone administration [0.4 mg/kg intramuscular (IM) q24h] for 3 days failed to improve the airway function of RAO horses, but improved airway hyperreactivity to inhaled histamine in seven of eight horses. The clinical significance of these findings remains to be ascertained. Oral prednisolone (1 mg/kg q48h) controlled clinical signs in a horse with RAO that was only partially responsive to strict environmental control and bronchodilators (Mair 1996a). However, after 4 months of prednisolone administration, the horse developed a severe bacterial pneumonia.

PREDNISONE Prednisone, orally administered, is poorly absorbed in horses and appears to be of little benefit, if any, in the treatment of RAO (Traub-Dargatz et al 1992, Peroni et al 2002, Robinson et al 2002).

TRIAMCINOLONE ACETONIDE Triamcinolone acetonide at a dosage of 20–40 mg/500 kg IM is a potent corticosteroid that improved the airway function of RAO-affected horses for up to 5 weeks, even in severely affected cases (Lapointe et al 1993). Because long-acting corticosteroids are more likely to induce adverse effects such as laminitis, triamcinolone is not recommended for the routine control of RAO.

Inhaled corticosteroids

Inhalation therapy is well suited for corticosteroid administration because of the large number of glucocorticoid receptors present on bronchial epithelial cells and vascular endothelial cells. Inhalation therapy provides the maximal concentration of drug at the effector sites and minimizes side effects. Inhaled corticosteroids are therefore preferable over systemic administration when prolonged therapy is required. Inhaled drugs are delivered to a horse using a mechanical nebulizer, a metered dose inhaler (MDI), and, less commonly, with dry powder inhalers (DPI). MDIs are advantageous because they are easy to use and the amount of drug delivered is constant. MDIs are administered to horses using a mask and a spacer, or other specially developed devices to maximize drug delivery to the lower airways. Administration of drugs by positioning the MDI directly into a nostril is not recommended because it is unlikely to result in delivery of therapeutic dosages of drug.

Efficacy varies according to drug potency, the inhalation device, and the propellant. Until recently, chlorofluorocarbons (CFCs) were the most commonly used propellant for MDIs. However, because of their detrimental effect on the ozone layer, they are being replaced with newer environmentally friendly gas propellants. As these alternative propellants are delivered in a manner that does not mimic CFCs, the potency of some drugs is greatly affected. As these propellants become available, marked variations in drug efficacies and potential toxicities might be expected if they are not evaluated in horses. For these reasons, the dosages recommended here are indicative only and are likely to change with various combinations of devices and propellants. Masks used in combination with an MDI or a DPI will increase airflow resistance and therefore may not be suitable for the initial treatment of horses in respiratory distress.

BECLOMETHASONE DIPROPIONATE Beclomethasone dipropionate administered via an MDI improves respiratory mechanics in RAO affected horses within 3–4 treatment days (Table 41.3). The maximal beneficial effects are usually observed during the first week of therapy. Beclomethasone also significantly decreased neutrophil percentages in BAL and prevented the increase of CD4$^+$ T cells associated with exposure to moldy hay (Rush et al 1998a). However, the magnitude of response to aerosolized beclomethasone was less than that to dexamethasone (0.1 mg/kg IV q24h) (Rush et al 1998b). There is a threefold range in the dosages of beclomethasone that have been reported to be effective for the treatment of RAO (Ammann et al 1998, Rush et al 1998a,b). The lowest dosage was effective when using a hydrofluoroalkane propellant attached to a device that has been shown to increase drug delivery to the distal airways of horses, but which is not currently commercially available (Rush et al 1998a,b).

FLUTICASONE PROPIONATE Fluticasone propionate (Table 41.3) is a newer, potent corticosteroid that relieves airway obstruction and decreases neutrophil percentages in BAL when administered by an MDI and mask to RAO affected horses (Giguere et al 2002). Fluticasone also decreases the expression of IL-4 mRNA in BAL and increases the IFN-γ : IL-4 ratio, suggesting that a modulation towards a Th1 response may contribute to the benefit of corticosteroids in RAO (Giguere et al 2002). Interestingly, there

was no reduction in IL-8 in BAL with this therapy, indicating that other mediators are also important contributors in the persistence of airway neutrophilia.

The information available to date in horses suggests that the short-term administration of inhaled corticosteroids is efficacious and well tolerated but unfortunately these drugs have short residual effects when therapy is discontinued. Since a delay in response is expected with inhaled corticosteroids, when used to treat horses in respiratory distress they should be combined with faster acting drugs, such as bronchodilators or systemic corticosteroids in this situation. Horses may become reluctant to inhale the medication after a while, although replacing the poorly tolerated drug with another of the same class will often overcome this problem.

Phosphodiesterase inhibitors
Methylxanthine derivatives

Aminophylline and **pentoxifylline** are methylxanthine derivatives with non-specific phosphodiesterase (PDE) inhibitory properties. The PDEs are a family of enzymes that catalyze the hydrolysis of cAMP and cGMP and thereby terminate their role as secondary messengers in mediating cellular responses to various hormones and neurotransmitters. Activation of cAMP by PDE may be a common mechanism to facilitate pro-inflammatory effects of cytokines and other proliferative agents. Aminophylline is used primarily as a bronchodilator in horses but it also enhances mucociliary clearance, respiratory drive, and contractility of the diaphragm and modulates immune function. Adverse effects, such as excitability, tachycardia, muscular tremors, and sweating, are commonly observed. Theophylline administered intravenously at 12 mg/kg over a 15-min period improved airway function in seven of 14 horses within 45 min of administration (Pearson & Riebold 1989); however, horses became excitable and hyperesthetic and some developed muscle tremor. Theophylline plasma concentrations of 10 μg/ml or more result in bronchodilation in ponies with RAO, but adverse effects are noted when the concentration exceeds 16 μg/ml (McKiernan & Koritz 1990). Because of their low therapeutic index, aminophylline and other salts of theophylline are not routinely used for the management of RAO. Recent findings in humans indicate that low-dose theophylline has anti-inflammatory and immunomodulatory effects and potentiates the effects of corticosteroids in patients with asthma (Barnes 2003). However, administration of theophylline [5 mg/kg q12h *per os* (PO)] for 7 days failed to improve airway obstruction of RAO-affected horses or to increase the efficacy of low-dose dexamethasone (Cesarini et al 2004). The potential synergetic effects of more prolonged administration of theophylline and corticosteroids in horses remain to be evaluated. Oral administration of **etamiphylline**, a theophylline derivative, also

failed to improve the pulmonary function of horses with RAO (Thomson & McPherson 1983).

Pentoxifylline, a non-specific PDE inhibitor, is currently approved in some countries for the treatment of navicular disease in horses. It is also a bronchodilator, inhibits neutrophil recruitment to inflammatory sites, and at high concentrations is a potent inhibitor of TNF-α production. High doses of pentoxifylline (16 g/horse, q12h) were as beneficial as atropine for the relief of airway obstruction in RAO horses, but failed to reduce BAL neutrophilia (Leguillette et al 2002). However, the oral absorption of pentoxifylline is limited and the efficacy of more practical lower dosages needs to be assessed.

Selective PDE inhibitors

Selective PDE inhibitors, particularly of the PDE4 subtype, have been studied for the treatment of lower inflammatory airway diseases in people owing to the expression of PDE4 in airway smooth muscle, pulmonary nerves, and almost all inflammatory and immune cells relevant to the pathogenesis of asthma. A selective PDE4 inhibitor effective at inhibiting the *ex vivo* production of inflammatory mediators by equine leukocytes was ineffective for the treatment of heaves-affected horses (Lavoie et al 2002a).

Anti-leukotrienes

Of the various mediators that are known to be involved in pulmonary inflammatory diseases, leukotrienes are considered to be amongst the most important. Leukotrienes are metabolites of the arachidonic acid produced via the 5-lipoxygenase enzyme and its essential cofactor, the 5-lipoxygenase-activating proteins (FLAP). Cysteinyl leukotrienes (LTC_4, LTD_4, LTE_4) are potent bronchoconstrictors that also increase airway vascular permeability and mucus production. Furthermore, the main receptor for cysteinyl leukotrienes, $CystLT_1$, is present on the bronchi of horses (Lavoie et al 2002b). However, administration of the LTD_4 receptor antagonist, a 5-lipoxygenase inhibitor and a FLAP antagonist was ineffective in treating RAO (Marr et al 1998a, Robinson et al 1998, Lavoie et al 2002b, Kolm et al 2003). These results are consistent with the finding that BALF from RAO horses contains only low concentrations of cysteinyl leukotrienes (Watson et al 1992).

Cromones

The mechanism of action of cromones in heaves is unknown but may include stabilization of inflammatory cells and a local effect on nerve endings. They have been used primarily prophylactically, to prevent clinical exacerbation, but data regarding their efficacy in RAO are conflicting. In one study the preventative effect of *sodium cromoglycate* (80 mg q24h for 4 days using a mechanical nebulizer) persisted up to 3 weeks (Thomson & McPherson 1981), while in another report that used 520 mg, q24h for

2 days, it failed to prevent clinical exacerbation, although it did attenuate pulmonary resistance in a dose-dependent manner (Soma et al 1987). Sodium cromoglycate can also be administered using an MDI (10–12 mg/horse, once a day). A similar mast cell blocker is *nedocromil sodium*, which can be administered at a dose of 10–20 puffs (1 mg/puff) three times per day.

Non-steroidal anti-inflammatory drugs

Although various metabolites of the cyclooxygenase pathway have been detected in airway secretions of horses with chronic airway diseases, non-steroidal anti-inflammatory drugs are not of therapeutic benefit in RAO. The administration of antihistamines is also of limited value for the treatment of RAO.

Bronchodilators

Bronchodilators are used to relieve obstruction caused by airway smooth muscle contraction and to aid clearance of airway secretions. Unlike corticosteroids, their action is rapid in onset but short-lived. Bronchodilator administration should be combined with strict environmental dust control and/or corticosteroid administration because inflammation of the lower airways may progress despite the transient improvement in clinical signs observed with these drugs. As a result of their rapid onset of action, bronchodilators are particularly helpful when immediate relief of clinical signs is required. Administration of bronchodilators in RAO may cause a transient worsening of hypoxemia, possibly as the result of an increase in physiological dead space (Gallivan & McDonell 1989). Although this rarely appears to lead to clinical problems, it may be advisable to combine inhaled bronchodilators with intranasal oxygen insufflation in horses with severe respiratory distress and profound hypoxemia. The agents most commonly used for bronchodilation in horses are β_2-adrenergic agonists, antimuscarinic agents, and methylxathine derivatives.

β_2-adrenergic agonists (Table 41.3)

Clenbuterol has bronchodilating effects and increases mucociliary transport. Side effects such as tachycardia and sweating are rarely seen with lower oral doses but are more frequent with intravenous administration and may persist for up to 3 h (Thomson & McPherson 1983). The clinical efficacy of clenbuterol, at the lower recommended dosage (0.8 μg/kg q12h), in horses with RAO is inconsistent if exposure to dusty hay and bedding is maintained (Thomson & McPherson 1983, Erichsen et al 1994). With higher dosages (up to 3.2 μg/kg) its efficacy improves, but so do the frequency and severity of the adverse effects (Erichsen et al 1994). This poor response may in part result from the rapid desensitization and down-regulation of β_2-adrenoreceptors following administration of therapeutic doses of clenbuterol (Abraham et al 2002). As this effect

can be prevented or reversed by dexamethasone administration, the therapeutic benefits of combined use of glucocorticoids and β_2-agonists should be investigated (Abraham et al 2002). In SPAOPD, a dosage of 0.8 mg/kg IV improved lung function from 30 min to 2 h post administration (Beadle 1985).

Terbutaline sulfate, another β_2-adrenergic agonist, is rapidly cleared from the blood following intravenous administration to horses, and has low bioavailability when administered orally (Torneke et al 2000). However, terbutaline (0.02 mg/kg in saline, total volume 4 ml) administered using a mechanical nebulizer resulted in bronchodilation that lasted up to 4 h (Murphy et al 1980).

Fenoterol, *albuterol*, *pirbuterol* and *salmeterol* are also β_2-agonists with potent bronchodilator effects that can be administered by inhalation (Table 41.3). With the exception of salmeterol and pirbuterol, bronchodilation induced by inhaled β_2-agonists occurs rapidly and adverse effects are minimal. However, the beneficial effects are short-lived (duration <1 h) thus requiring frequent drug administration. Salmeterol has an onset of action of 30–60 min but its effect lasts up to 6 h (Henrikson & Rush 2001). Pirbuterol administration improves lung function within 5 min of drug administration and its effect lasts up to 7 h (Derksen et al 1996). The use of sympathomimetic agents such as ephedrine which stimulate both α- and β-receptors has decreased because they are less efficacious than more specific β_2-adrenergic agonists, and are more likely to be associated with adverse effects.

Anticholinergics

As a result of their potentially severe adverse effects, anticholinergic drugs are generally not administered systemically for the treatment of heaves. *Atropine* (0.01–0.02 mg/kg IV) provides rapid bronchodilation of short duration (less than 2 h) but is associated with tachycardia, mydriasis, increased viscosity of respiratory secretions, ileus, and occasionally severe abdominal pain (Beadle 1983, Thomson & McPherson 1983). Adverse effects are also observed when atropine (0.02 mg/kg in saline, total volume 4 ml) is administered using a mechanical nebulizer (Murphy et al 1980). *Ipratropium bromide* has an onset of action of approximately 15–30 min and bronchodilation in RAO may last up to 4–6 h. It is well tolerated when administered by inhalation (Robinson et al 1993, Duvivier et al 1999).

Antioxidants

There is evidence that oxidative stress may contribute to airway inflammation in RAO (Deaton et al 2004). A placebo-controlled study demonstrated that administration of dietary antioxidant supplements for 4 weeks to horses with RAO that were in clinical remission improved exercise tolerance but did not improve BAL cytology or other pulmonary markers of oxidative stress (Kirschvink et al 2002).

Expectorant, mucolytic, and mucokinetic agents

Expectorants are drugs that increase pulmonary secretion while mucolytic agents loosen secretions. The term "mucokinetic agent" may be preferred as it indicates that the agent aids clearance of the respiratory tract secretions. Although the administration of mucokinetic agents may help loosen the secretions in the large airways, evidence of their efficacy in improving the clinical signs of RAO is sparse. *Clenbuterol*, because of its bronchodilator and mucokinetic properties, is currently the drug of choice for aiding clearance of mucus from the airways. Oral iodides and nebulized acetylcysteine may facilitate expectoration but their efficacies have not been determined (Genetzky & Loparco 1985). Iodide should be administered with caution as it is an irritant and can induce or exacerbate bronchospasm.

Overhydration by administration of extremely high volumes of isotonic saline solution (30 liters at 10 liters/h, q24h, for 1–3 days) combined with bronchodilators or mucokinetic agents has been used to treat airway obstruction of horses with heaves (Deegen 1981). The proposed beneficial effects of this treatment are improved mucus transport and removal of mucus plugs related to the liquefaction of excessively viscous mucus. Under controlled conditions, however, no improvement in lung function was observed in heaves-affected horses when isotonic saline (10 liters/h, for 3 h) was administered as sole therapy (Jean et al 2004). This treatment should be administered with caution as a number of side effects, including labored breathing and colic, were observed with its use. Antitussive agents are not indicated in the treatment of equine heaves, as coughing is a beneficial mechanism essential for the clearance of respiratory secretions.

Antibiotics

Since bacterial colonization of airways is not uncommon in RAO, antimicrobials have been used before the administration of corticosteroids, in an attempt to reduce the risk of secondary bacterial infection. However, this practice is probably not warranted unless there is clinical evidence of infection, such as fever, pyrexia, depression, and leukocytosis.

Alternative therapies

Currently, there is an increased interest from horse owners in the use of alternative therapies, such as acupuncture and herbal medicines, for the treatment of equine diseases. However, there is only very limited information concerning their efficacy and toxicity. Film-coated extracts of *Thymus vulgaris* and *Primula veris* administered orally for 1 month to RAO-affected horses failed to improve the clinical signs, P_AO_2 and BAL neutrophilia, but significantly improved lung mechanics (van den Hoven et al 2003).

Immunological intervention

The use of allergen hyposensitization or immunotherapy to treat RAO has increased because of the commercial availability of *in vitro* serum allergy tests. Although uncontrolled reports suggest efficacy, to the author's knowledge, there is no critical scientific evidence to support their use for the treatment of RAO at present.

Case Outcome

RAO and SPAOPD are considered to be reversible conditions because marked improvement in airway function is seen in almost all horses that are properly managed. Presently, however, there are no good prognostic indicators and published information on the long-term outcome is sparse (Ainsworth et al 1998, Nuytten et al 1988). Follow-up information from horses diagnosed with RAO and treated with environmental changes and oral steroid therapy indicate that even with therapy, 78% of horses periodically experience episodes of heaves and 41% of owners believe that their horses have compromised athletic capacity (Ainsworth et al 1998). This suggests that the RAO may not be fully reversible and that lung remodeling may even progress despite appropriate therapy.

While the condition will rapidly deteriorate in some horses that do not receive appropriate therapy, the clinical signs may wane and wax, alternating between periods of clinical exacerbations and remission, even when horses are maintained in the same environment. Anecdotal findings from our and other laboratories indicate that horses that have repeatedly developed severe airway obstruction when exposed to moldy hay may occasionally remain asymptomatic when exposed to moldy hay, even for prolonged periods of time.

Death rarely occurs because of RAO *per se*; however, severely affected horses may be euthanized because owners are unable or unwilling to change the environment of horses or, rarely, because the horse fails to respond to therapy. While poorly documented, some horses with bronchiectasis or severe mucus plugging of airways have failed to respond or have responded poorly to environmental control, and to corticosteroid and bronchodilator therapy (Lavoie et al 2004). Glucocorticoid resistance, defined as a failure to respond to systemic corticosteroids but continued response to bronchodilators, has been reported in one horse with RAO (Stamper et al 2002).

REFERENCES

Abraham G, Brodde OE, Ungemach FR 2002 Regulation of equine lymphocyte beta-adrenoceptors under the influence of clenbuterol and dexamethasone. Equine Veterinary Journal 34: 587–593

Aggarwal N, Holmes MA 1999 Characterisation of equine T helper cells: demonstration of Th1- and Th2-like

cells in long-term equine T-cell cultures. Research in Veterinary Science 66: 277–279

Ainsworth DM, Eicker SW, Bennett BJ 1998 Outcome of horses diagnosed with and treated for heaves. World Equine Airway Symposium, Guelph, Canada

Ainsworth DM, Grunig G, Matychak MB et al 2003 Recurrent airway obstruction (RAO) in horses is characterized by IFN-gamma and IL-8 production in bronchoalveolar lavage cells. Veterinary Immunology and Immunopathology 96: 83–89

Al-Dewachi O, Joubert P, Taha R et al 2002 Expression of IL-5 and IL-9 receptors on neutrophils in horses with heaves. 98th International Conference, American Thoracic Society, Atlanta, GA

Ammann VJ, Vrins AA, Lavoie JP 1998 Effects of inhaled beclomethasone dipropionate on respiratory function in horses with chronic obstructive pulmonary disease (COPD). Equine Veterinary Journal 30: 152–157

Barnes PJ 2003 Theophylline: new perspectives for an old drug. American Journal of Respiratory and Critical Care Medicine 167: 813–818

Beadle RE 1983 Summer pasture-associated obstructive pulmonary disease. In: Robinson NE (editor) Current Therapy in Equine Medicine. WB Saunders, Philadelphia, pp.512–516

Beadle RE 1985 Experiences with whole-body plethysmography in horses with obstructive pulmonary disease. Lung Function and Respiratory Diseases in the Horse, International Symposium in Hannover, Germany

Beadle RE, Horohov DW, Gaunt SD 2002 Interleukin-4 and interferon-gamma gene expression in summer pasture-associated obstructive pulmonary disease affected horses. Equine Veterinary Journal 34: 389–394

Beech J 1991 Chronic obstructive pulmonary disease. Veterinary Clinics of North America; Equine Practice 7: 79–91

Bober LA, Waters TA, Pugliese-Sivo CC et al 1995 IL-4 induces neutrophilic maturation of HL-60 cells and activation of human peripheral blood neutrophils. Clinical and Experimental Immunology 99: 129–136

Boey H, Rosenbaum R, Castracane J et al 1989 Interleukin-4 is a neutrophil activator. Journal of Allergy and Clinical Immunology 83: 978–984

Bowles KS, Beadle RE, Mouch S et al 2002 A novel model for equine recurrent airway obstruction. Veterinary Immunology and Immunopathology 87: 385–389

Bureau F, Bonizzi G, Kirschvink N et al 2000a Correlation between nuclear factor-kappaB activity in bronchial brushing samples and lung dysfunction in an animal model of asthma. American Journal of Respiratory and Critical Care Medicine 161: 1314–1321

Bureau F, Delhalle S, Bonizzi G et al 2000b Mechanisms of persistent NF-kappa B activity in the bronchi of an animal model of asthma. Journal of Immunology 165: 5822–5830

Cesarini C, Hamilton E, Picandet V et al 2004 Efficacy of theophylline associated with a low dose of dexamethasone in the treatment of horses with heaves. 22th Symposium of the Veterinary Comparative Respiratory Society, Montreal, Canada

Clarke AF, Madelin TM, Allpress RG 1987 The relationship of air hygiene in stables to lower airway disease and pharyngeal lymphoid hyperplasia in two groups of Thoroughbred horses. Equine Veterinary Journal 19: 524–530

Cordeau ME, Joubert P, Dewachi O et al 2004 IL-4, IL-5 and IFN-gamma mRNA expression in pulmonary lymphocytes in equine heaves. Veterinary Immunology and Immunopathology 97: 87–96

Cornelisse CJ, Robinson NE, Berney CE et al 2004 Efficacy of oral and intravenous dexamethasone in horses with recurrent airway obstruction. Equine Veterinary Journal 36: 426–430

Costa LR, Seahorn TL, Moore RM et al 2000 Correlation of clinical score, intrapleural pressure, cytological findings of bronchoalveolar fluid, and histopathological lesions of pulmonary tissue in horses with summer pasture-associated obstructive pulmonary disease. American Journal of Veterinary Research 61: 167–173

Costa LR, Seahorn TL, Moore RM et al 2001 Plasma and bronchoalveolar fluid concentrations of nitric oxide and localization of nitric oxide synthesis in the lungs of horses with summer pasture-associated obstructive pulmonary disease. American Journal of Veterinary Research 62: 1381–1386

Couetil LL, Ward MP 2003 Analysis of risk factors for recurrent airway obstruction in North American horses: 1,444 cases (1990–1999). Journal of the American Veterinary Medical Association 223: 1645–1650

Couetil LL, Rosenthal FS, Simpson CM 2000 Forced expiration: a test for airflow obstruction in horses. Journal of Applied Physiology 88: 1870–1879

Deaton CM, Marlin DJ, Smith NC et al 2004 Pulmonary epithelial lining fluid and plasma ascorbic acid concentrations in horses affected by recurrent airway obstruction. American Journal of Veterinary Research 65: 80–87

Debrue M, Lajoie-Kadoch S, Joubert P et al 2004 Stabling is associated with increased expression of IL-17 mRNA in bronchoalveolar cells of horses with heaves. 100th International Conference, American Thoracic Society, Orlando, FL

Deegen E 1981 Massive intravenous infusions: a novel secretolytic therapy for horses with chronic obstructive pulmonary disease (COPD). Proceedings of the American Association of Equine Practitioners 27: 27–33

Derksen FJ, Robinson NE, Armstrong PJ et al 1985 Airway reactivity in ponies with recurrent airway obstruction (heaves). Journal of Applied Physiology 58: 598–604

Derksen FJ, Robinson NE, Scott JS et al 1988 Aerosolized *Micropolyspora faeni* antigen as a cause of pulmonary dysfunction in ponies with recurrent airway obstruction (heaves). American Journal of Veterinary Research 49: 933–938

Derksen FJ, Brown CM, Sonea I et al 1989 Comparison of transtracheal aspirate and bronchoalveolar lavage cytology in 50 horses with chronic lung disease. Equine Veterinary Journal 21: 23–26

Derksen FJ, Olszewski M, Robinson NE et al 1996 Use of a hand-held, metered-dose aerosol delivery device to administer pirbuterol acetate to horses with "heaves". Equine Veterinary Journal 28: 306–310

Dixon PM, Nicholls JR, McPherson EA et al 1982 Chronic obstructive pulmonary disease anatomical cardiac studies. Equine Veterinary Journal 14: 80–82

Dixon PM, Railton DI, McGorum BC et al 1995 Equine pulmonary disease: a case control study of 300 referred cases. Part 4: Treatments and re-examination findings. Equine Veterinary Journal 27: 436–439

Dun F 1859 Veterinary Medicines: Their Actions and Uses. Sutherland and Knox, Edinburgh

Duvivier DH, Bayly WM, Votion D et al 1999 Effects of inhaled dry powder ipratropium bromide on recovery from exercise of horses with COPD. Equine Veterinary Journal 31: 20–24

Eder C, Crameri R, Mayer C et al 2000 Allergen-specific IgE levels against crude mould and storage mite extracts and recombinant mould allergens in sera from horses affected with chronic bronchitis. Veterinary Immunology and Immunopathology 73: 241–253

Eder C, Curik I, Brem G et al 2001 Influence of environmental and genetic factors on allergen-specific immunoglobulin-E levels in sera from Lipizzan horses. Equine Veterinary Journal 33: 714–720

Erichsen DF, Aviad AD, Schultz RH et al 1994 Clinical efficacy and safety of clenbuterol HCl when administered to effect in horses with chronic obstructive pulmonary disease (COPD). Equine Veterinary Journal 26: 331–336

Eyre P 1972 Equine pulmonary emphysema: a bronchopulmonary mould allergy. Veterinary Record 91: 134–140

Fairbairn SM, Lees P, Page CP et al 1993a Duration of antigen-induced hyperresponsiveness in horses with allergic respiratory disease and possible links with early airway obstruction. Journal of Veterinary Pharmacology and Therapeutics 16: 469–476

Fairbairn SM, Page CP, Lees P et al 1993b Early neutrophil but not eosinophil or platelet recruitment to the lungs of allergic horses following antigen exposure. Clinical and Experimental Allergy 23: 821–828

Franchini M, Gilli U, Akens MK et al 1998 The role of neutrophil chemotactic cytokines in the pathogenesis of equine chronic obstructive pulmonary disease (COPD). Veterinary Immunology and Immunopathology 66: 53–65

Franchini M, Gill U, von Fellenberg R et al 2000 Interleukin-8 concentration and neutrophil chemotactic activity in bronchoalveolar lavage fluid of horses with chronic obstructive pulmonary disease following exposure to hay. American Journal of Veterinary Research 61: 1369–1374

Gallivan GJ, McDonell WN 1989 An evaluation of the multi-breath nitrogen washout as pulmonary function test in dairy cattle. Canadian Journal of Veterinary Research 53: 133–142

Genetzky RM, Loparco FV 1985 Chronic obstructive pulmonary disease in horses: Part 1. Compendium on Continuing Education for the Practicing Veterinarian 7: S407–S414

George LW, Tanner ML, Roberson EL et al 1981 Chronic respiratory disease in a horse infected with *Dictyocaulus arnfieldi*. Journal of the American Veterinary Medical Association 179: 820–822

Gerber H 1973 Chronic pulmonary disease in the horse. Equine Veterinary Journal 5: 26–31

Gerber V, King M, Schneider DA et al 2000 Tracheobronchial mucus viscoelasticity during environmental challenge in horses with recurrent airway obstruction. Equine Veterinary Journal 32: 411–417

Gerber V, Robinson NE, Venta RJ et al 2003 Mucin genes in horse airways: MUC5AC, but not MUC2, may play a role in recurrent airway obstruction. Equine Veterinary Journal 35: 252–257

Giguere S, Viel L, Lee E 2000 Cytokine messenger RNA (mRNA) expression in bronchoalveolar cells from normal horses and horses with heaves, and effect of therapy with inhaled fluticasone propionate. 18th Annual Veterinary Medical Forum, ACVIM, Seattle, WA

Giguere S, Viel L, Lee E et al 2002 Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate. Veterinary Immunology and Immunopathology 85: 147–158

Gilleece MH, Scarffe JH, Ghosh A et al 1992 Recombinant human interleukin 4 (IL-4) given as daily subcutaneous injections – a phase I dose toxicity trial. British Journal of Cancer 66: 204–210

Girard D, Paquin R, Beaulieu AD 1997 Responsiveness of human neutrophils to interleukin-4: induction of cytoskeletal rearrangements, de novo protein synthesis and delay of apoptosis. Biochemical Journal 325: 147–153

Grünig G, Herman M, Howald B et al 1989 Partial divergence between airway inflammation and clinical signs in equine chronic pulmonary disease. Equine Veterinary Journal 21: 145–148

Halliwell RE, Fleischman JB, Mackay-Smith M et al 1979 The role of allergy in chronic pulmonary disease of horses. Journal of the American Veterinary Medical Association 174: 277–281

Halliwell REW, McGorum BC, Irving P et al 1993 Local and systemic antibody production in horses affected with chronic obstructive pulmonary disease. Veterinary Immunology and Immunopathology 38: 201–215

Hare JE, Viel L, Conlon PD et al 1999 In vitro allergen-induced degranulation of pulmonary mast cells from horses with recurrent airway obstruction (heaves). American Journal of Veterinary Research 60: 841–847

Henrikson SL, Rush BR 2001 Efficacy of salmeterol xinafoate in horses with recurrent airway obstruction. Journal of the American Veterinary Medical Association 218: 1961–1965

Herszberg B, Ramos-Barbon D, Martin JG et al 2004 Airway smooth muscle remodeling in an equine model of chronic asthma. 100th International Conference, American Thoracic Society, Orlando, FL

Hoffman A, Kuehn H, Riedelberger K et al 2001 Flowmetric comparison of respiratory inductance plethysmography and pneumotachography in horses. Journal of Applied Physiology 91: 2767–2775

Holcombe SJ, Jackson C, Gerber V et al 2001 Stabling is associated with airway inflammation in young Arabian horses. Equine Veterinary Journal 33: 244–249

Jean D 1996 Etude de la variabilité des valeurs de mécanique respiratoire et de la cytologie du lavage bronchoalveolaire chez les chevaux atteints de maladie pulmonaire obstructive chronique (MPOC) et de chevaux exempts de cette maladie. MSc thesis, Faculté des Études Supérieures, St-Hyacinthe, Université de Montréal

Jean D, Vrins A, Lavoie JP 2004 Respiratory and metabolic effects of massive administration of isotonic saline solution in heaves-affected and control horses. Equine Veterinary Journal 36: 628–633

Jefcoat AM, Hotchkiss JA, Gerber V et al 2001 Persistent mucin glycoprotein alterations in equine recurrent airway obstruction. American Journal of Physiology. Cell Physiology 281: C704–712

Joubert P, Silversides DW, Lavoie JP 2001 Equine neutrophils express mRNA for tumour necrosis factor-alpha, interleukin (IL)-1beta, IL-6, IL-8, macrophage-inflammatory-protein-2 but not for IL-4, IL-5 and interferon-gamma. Equine Veterinary Journal 33: 730–733

Joubert P, Cordeau ME, Boyer A et al 2002 Quantification of cytokine mRNA expression by pulmonary macrophages in horses affected by heaves. 20th Symposium of the Veterinary Comparative Respiratory Society, Boston, MA

Kaup F-J, Drommer W, Damsch S et al 1990 Ultrastructural findings in horses with chronic obstructive pulmonary disease (COPD). 2: Pathological changes of the terminal airways and the alveolar region. Equine Veterinary Journal 22: 349–355

Kirschvink N, Fievez L, Bougnet V et al 2002 Effect of nutritional antioxidant supplementation on systemic and pulmonary antioxidant status, airway inflammation and lung function in heaves-affected horses. Equine Veterinary Journal 34: 705–712

Kolm G, Zappe H, Schmid R et al 2003 Efficacy of montelukast in the treatment of chronic obstructive pulmonary disease in five horses. Veterinary Record 152: 804–806

Kvart C, Carlsen J, Dahl L et al 1986 Changes in arterial oxygen tension in COPD horses after reduction of exposure to dust in the feed and environment. Second International Conference on Equine Exercise Physiology, San Diego, LA. ICEEP Publications

Lapointe JM, Lavoie JP, Vrins AA 1993 Effects of triamcinolone acetonide on pulmonary function and bronchoalveolar lavage cytologic features in horses with chronic obstructive pulmonary disease. American Journal of Veterinary Research 54: 1310–1316

Larson VL, Busch RH 1985 Equine tracheobronchial lavage: comparison of lavage cytologic and pulmonary histopathologic findings. American Journal of Veterinary Research 46: 144–146

Lasky JA, Brody AR 1997 Interleukins involved in the pathogenesis of chronic airway inflammation. Research in Immunology 148: 39–47

Lavoie JP, Maghni K, Desnoyers M et al 2001 Neutrophilic airway inflammation in horses with heaves is characterized by a Th2-type cytokine profile. American Journal of Respiratory and Critical Care Medicine 164: 1410–1413

Lavoie JP, Pasloske K, Joubert P et al 2002a Comparison of dexamethasone and the PDE IV enzyme inhibitor L826-141 in an animal model of chronic asthma. 98th International Conference, American Thoracic Society, Atlanta, GA

Lavoie JP, Leguillette R, Pasloske K et al 2002b Comparison of dexamethasone and the LTD4 receptor antagonist L-708,738 on lung function and airway cytology in heaves. American Journal of Veterinary Research 63: 579–585

Lavoie JP, Dalle S, Breton L et al 2004 Bronchiectasis in three adult horses with heaves. Journal of Veterinary Internal Medicine 18: 757–760

Lawson GHK, McPherson EA, Murphy JR et al 1979 The presence of precipitating antibodies in the sera of horses with chronic obstructive pulmonary disease (COPD). Equine Veterinary Journal 11: 172–176

Leguillette R, Désévaux C, Lavoie JP 2002 Effects of pentoxifylline on pulmonary function and results of cytologic examination of bronchoalveolar lavage fluid in horses with recurrent airway obstruction. American Journal of Veterinary Research 63: 459–463

Lorch G, Hillier A, Kwochka RW et al 2001 Comparison of immediate intradermal test reactivity with serum IgE quantitation by use of a radioallergosorbent test and two ELISA in horses with and without atopy. Journal of the American Veterinary Medical Association 218: 1314–1322

Lowell F 1964 Observations on heaves: an asthma-like syndrome in the horse. Journal of Allergy 35: 322–330

Mair TS 1996a Bacterial pneumonia associated with corticosteroid therapy in three horses. Veterinary Record 138: 205–207

Mair TS 1996b Obstructive pulmonary disease in 18 horses at summer pasture. Veterinary Record 138: 89–91

Malikides N 2004 The epidemiology of inflammatory airway disease in young thoroughbred racehorses in Australia. PhD thesis, University of Sydney, Australia

Mansmann RA, Osburn BI, Wheat JD et al 1975 Chicken hypersensitivity pneumonitis in horses. Journal of the American Veterinary Medical Association 166: 673–677

Marr KA, Foster AP, Lees P et al 1997 Effect of antigen challenge on the activation of peripheral blood neutrophils from horses with chronic obstructive pulmonary disease. Research in Veterinary Science 62: 253–260

Marr KA, Lees P, Page CP et al 1998a Effect of the 5-lipoxygenase inhibitor, fenleuton, on antigen-induced neutrophil accumulation and lung function changes in horses with chronic obstructive pulmonary disease. Journal of Veterinary Pharmacology and Therapeutics 21: 241–246

Marr KA, Lees P, Page CP et al 1998b Inhaled leukotrienes cause bronchoconstriction and neutrophil accumulation in horses. Research in Veterinary Science 64: 219–224

Marti E, Gerber H, Essich G et al 1991 The genetic basis of equine allergic diseases. 1. Chronic hypersensitivity bronchitis. Equine Veterinary Journal 23: 457–460

Mazan MR, Hoffman AM, Manjerovic N 1999 Comparison of forced oscillation with the conventional method for histamine bronchoprovocation testing in horses. American Journal of Veterinary Research 60: 174–180

Mazan MR, Deveney EF, DeWitt S et al 2004 Energetic cost of breathing, body composition, and pulmonary function in horses with recurrent airway obstruction. Journal of Applied Physiology 97: 91–97

McGorum BC, Dixon PM, Halliwell RE et al 1993a Comparison of cellular and molecular components of bronchoalveolar lavage fluid harvested from different segments of the equine lung. Research in Veterinary Science 55: 57–59

McGorum BC, Dixon PM, Halliwell REW 1993b Quantification of histamine in plasma and pulmonary fluids from horses with chronic obstructive pulmonary disease, before and after natural (hay and straw) challenges. Veterinary Immunology and Immunopathology 36: 223–237

McKiernan BC, Koritz GD 1990 Plasma theophylline concentration and lung function in ponies with recurrent obstructive lung disease. Equine Veterinary Journal 22: 194–197

McPherson EA, Lawson GHK, Murphy JR et al 1978 Chronic obstructive pulmonary disease (COPD): identification of affected horses. Equine Veterinary Journal 10: 47–53

McPherson EA, Lawson GH, Murphy JR et al 1979a Chronic obstructive pulmonary disease (COPD): factors influencing the occurrence. Equine Veterinary Journal 11: 167–171

McPherson EA, Lawson GHK, Murphy JR et al 1979b Chronic obstructive pulmonary disease (COPD) in horses: aetiological studies: responses to intradermal and inhalation antigenic challenge. Equine Veterinary Journal 11: 159–166

Murphy JR, McPherson EA, Dixon PM 1980 Chronic obstructive pulmonary disease (COPD): Effects of bronchodilator drugs on normal and affected horses. Equine Veterinary Journal 12: 10–14

Naylor JM, Clark EG, Clayton HM 1992 Chronic obstructive pulmonary disease: usefulness of clinical signs, bronchoalveolar lavage, and lung biopsy as diagnostic and prognostic aids. Canadian Veterinary Journal 33: 591–598

Nuytten J, Deprez P, Picavet T et al 1988 Comparison of different pulmonary function tests and their prognostic value in horses affected with COPD. Equine Veterinary Science 8: 361–364

Nyman G, Linderg R, Weckner D et al 1991 Pulmonary gas exchange correlate to clinical signs and lung pathology in horses with chronic bronchiolitis. Equine Veterinary Journal 23: 253–260

Obel N, Schmiterlöw C 1948 The action of histamine and other drugs on the bronchial tone of horses suffering from alveolar emphysema (heaves). Acta Pharmacologica Sinica 4: 71–80

Olszewski MA, Robinson NE, Zhu FX et al 1999 Mediators of anaphylaxis but not activated neutrophils augment cholinergic responses of equine small airways. American Journal of Physiology 276: L522–L529

Pearson EG, Riebold TW 1989 Comparison of bronchodilators in alleviating clinical signs in horses with chronic obstructive pulmonary disease. Journal of the American Veterinary Medical Association 194: 1287–1291

Pellegrini A, Kalkinc M, Hermann M et al 1998 Equinins in equine neutrophils: quantification in tracheobronchial secretions as an aid in the diagnosis of chronic pulmonary disease. Veterinary Journal 155: 257–262

Peroni DL, Stanley S, Kollias-Baker C et al 2002 Prednisone *per os* is likely to have limited efficacy in horses. Equine Veterinary Journal 34: 283–287

Picandet V, Leguillette R, Lavoie JP 2003 Comparison of efficacy and tolerability of isoflupredone and dexamethasone in the treatment of horses affected with recurrent airway obstruction ("heaves"). Equine Veterinary Journal 35: 419–424

Picandet V, Jean D, Lavoie JP 2004 Acid base balance in arterial blood of horses with heaves. 22nd Veterinary and Comparative Respiratory Symposium, Montreal, Canada

Pirie RS, Dixon PM, Collie DD et al 2001 Pulmonary and systemic effects of inhaled endotoxin in control and heaves horses. Equine Veterinary Journal 33: 311–318

Pirie RS, Collie DD, Dixon PM et al 2002 Evaluation of nebulised hay dust suspensions (HDS) for the diagnosis and investigation of heaves. 2: Effects of inhaled HDS on control and heaves horses. Equine Veterinary Journal 34: 337–342

Robinson NE 2001 International Workshop on Equine Chronic Airway Disease. Michigan State University 16–18 June 2000. Equine Veterinary Journal 33: 5–19

Robinson NE, Derksen FJ, Berney C et al 1993 The airway response of horses with recurrent airway obstruction (heaves) to aerosol administration of ipratropium bromide. Equine Veterinary Journal 25: 299–303

Robinson NE, Boehler D, Berney C et al 1998 Failure of a FLAP antagonist to prevent airway obstruction in heaves-susceptible horses. World Equine Airways Symposium, Guelph, Canada

Robinson NE, Olszewski MA, Boehler D et al 2000 Relationship between clinical signs and lung function in horses with recurrent airway obstruction (heaves) during a bronchodilator trial. Equine Veterinary Journal 32: 393–400

Robinson NE, Jackson C, Jefcoat A et al 2002 Efficacy of three corticosteroids for the treatment of heaves. Equine Veterinary Journal 34: 17–22

Robinson NE, Berney C, Eberhart S et al 2003 Cough, mucus, airway obstruction, and inflammation in heaves-affected and control horses. American Journal of Veterinary Research 64: 550–557

Rush BR, Flaminio MJ, Matson C et al 1997 Inhaled beclomethasone dipropionate for the treatment of heaves. 15th Symposium of the Veterinary Comparative Respiratory Society, University of Liege, Belgium

Rush BR, Flaminio MJ, Matson CJ et al 1998a Cytologic evaluation of bronchoalveolar lavage fluid from horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively. American Journal of Veterinary Research 59: 1033–1038

Rush BR, Raub ES, Rhoads WS et al 1998b Pulmonary function in horses with recurrent airway obstruction after aerosol and parenteral administration of beclomethasone dipropionate and dexamethasone, respectively. American Journal of Veterinary Research 59: 1039–1043

Schmallenbach KH, Rahman I, Sasse HHL et al 1998 Studies on pulmonary and systemic *Aspergillus fumigatus*-specific IgE and IgG antibodies in horses affected with chronic obstructive pulmonary disease (COPD). Veterinary Immunology and Immunopathology 66: 245–256

Seahorn TL, Beadle RE 1993 Summer pasture-associated obstructive pulmonary disease in horses – 21 cases (1983–1991). Journal of the American Veterinary Medical Association 202: 779–782

Seahorn TL, Groves MG, Harrington KS et al 1996 Chronic obstructive pulmonary disease in horses in Louisiana. Journal of the American Veterinary Medical Association 208: 248–251

Seahorn TL, Beadle RE, McGorum BC et al 1997 Quantification of antigen-specific antibody concentrations in tracheal lavage fluid of horses with summer pasture-associated obstructive pulmonary disease. American Journal of Veterinary Research 58: 1408–1411

Soma LR, Beech J, Gerber NH 1987 Effects of cromolyn in horses with chronic obstructive pulmonary disease. Veterinary Research Communications 11: 339–351

Stamper AJ, Rush BR, Wilkerson MJ et al 2002 Glucocorticoid resistance in a horse with heaves. 20th Symposium of the Veterinary Comparative Respiratory Society, Boston, MA

Thomson JR, McPherson EA 1981 Prophylactic effects of sodium cromoglycate on chronic obstructive pulmonary disease in the horse. Equine Veterinary Journal 13: 243–246

Thomson JR, McPherson EA 1983 Chronic obstructive pulmonary disease in the horse 2: Therapy. Equine Veterinary Journal 15: 207–210

Thomson JR, McPherson EA 1984 Effects of environmental control on pulmonary function of horses affected with chronic obstructive pulmonary disease. Equine Veterinary Journal 16: 35–38

Thurlbeck WM, Lowell FC 1964 Heaves in horses. American Journal of Respiratory Diseases 89: 82–88

Torneke MK, Ingvast-Larsson JC, Johansson JM et al 2000 Pharmacokinetics and pharmacodynamics of terbutaline

in healthy horses. American Journal of Veterinary Research 61: 761–765

Traub-Dargatz JL, McKinnon AO, Thrall MA et al 1992 Evaluation of clinical signs of disease, bronchoalveolar and tracheal wash analysis, and arterial blood gas tensions in 13 horses with chronic obstructive pulmonary disease treated with prednisone, methyl sulfonmethane, and clenbuterol hydrochloride. American Journal of Veterinary Research 53: 1908–1916

Tremblay GM, Ferland C, Lapointe J-M et al 1993 Effect of stabling on bronchoalveolar cells obtained from normal and COPD horses. Equine Veterinary Journal 25: 194–197

Turlej RK, Fievez L, Sandersen CF et al 2001 Enhanced survival of lung granulocytes in an animal model of asthma: evidence for a role of GM-CSF activated STAT5 signalling pathway. Thorax 56: 696–702

van den Hoven R, Zappe H, Zitterl-Eglseer K et al 2003 Study of the effect of Bronchipret on the lung function of five Austrian saddle horses suffering recurrent airway obstruction (heaves). Veterinary Record 152: 555–557

Vandenput S, Istasse L, Nicks B et al 1997 Airborne dust and aeroallergen concentrations in different sources of feed and bedding for horses. Veterinary Quarterly 19: 154–158

Vandenput S, Votion D, Duvivier DH et al 1998a Effect of a set stabled environmental control on pulmonary function and airway reactivity of COPD affected horses. Veterinary Journal 155: 189–195

Vandenput S, Duvivier DH, Votion D et al 1998b Environmental control to maintain stabled COPD horses in clinical remission: effects on pulmonary function. Equine Veterinary Journal 30: 93–96

van Erck E, Votion DM, Kirschvink N et al 2003 Use of the impulse oscillometry system for testing pulmonary function during methacholine bronchoprovocation in horses. American Journal of Veterinary Research 64: 1414–1420

Viel L 1983 Structural–functional correlations of the lung in horses with small airway disease. PhD thesis, University of Guelph

Votion DM, Vandenput SN, Duvivier DH et al 1999 Alveolar clearance in horses with chronic obstructive pulmonary disease. American Journal of Veterinary Research 60: 495–500

Vrins A, Doucet M, Nunez-Ochoa L 1991 A retrospective study of bronchoalveolar lavage cytology in horses with clinical findings of small airway disease. Zentralblatt fur Veterinarmedizin A 38: 472–479

Watson ED, Sweeney CR, Steensma KA 1992 Arachidonate metabolites in bronchoalveolar lavage fluid from horses with and without COPD. Equine Veterinary Journal 24: 379–381

Willoughby RA, McDonnel WN 1979 Pulmonary function testing in horses. Veterinary Clinics of North America; Large Animal Practice 1: 171–196

Winder NC, von Fellenberg R 1987 Chronic small airway disease in horses slaughtered in Switzerland. Schweizer Archiv fur Tierheilkunde 129: 585–593

Winder NC, Hermann M, Grünig G et al 1990 Comparison of bronchoalveolar lavage and respiratory secretion cytology in horses with clinically diagnosed chronic pulmonary disease. Schweizer Archiv fur Tierheilkunde 132: 505–510

Woods PS, Robinson NE, Swanson MC et al 1993 Airborne dust and aeroallergen concentration in a horse stable under two different management systems. Equine Veterinary Journal 25: 208–213

Yang L, Cohn L, Zhang DH et al 1998 Essential role of nuclear factor kappaB in the induction of eosinophilia in allergic airway inflammation. Journal of Experimental Medicine 188: 1739–1750

Yu MF, Wang ZW, Robinson NE et al 1994 Modulation of bronchial smooth muscle function in horses with heaves. Journal of Applied Physiology 77: 2149–2154

42

Inflammatory Airway Disease

Robert Christley and Bonnie R Rush

The terms "inflammatory airway disease" (IAD) and "small-airway inflammatory disease" (SAID) have been used to describe a syndrome of respiratory tract inflammation in racehorses. Typically, diagnosis of IAD is made on the basis of clinical signs (poor performance and cough) and endoscopic findings (nasopharyngeal exudate, pharyngeal lymphoid hyperplasia, and tracheal exudate). This condition has been reported to occur in 11–50% of racehorses in training.

Definition of the Syndrome

Any discussion of IAD is complicated by the lack of a consistent and precise definition of the syndrome. In 2002 the International Workshop on Inflammatory Airway Disease summarized much of what is known about IAD and attempted to define the syndrome (Anon 2003). Broadly, IAD is a clinical syndrome typified by some or all of the following:

- cough
- accumulation of secretions in the trachea
- cytological evidence of airway inflammation
- nasal discharge
- poor exercise performance
- delayed recovery following exercise
- absence of changes in the pattern, rate or depth of respiration
- absence of clinical signs of systemic illness (such as depression, inappetence or pyrexia)
- absence of hematological or serum biochemical evidence of illness.

Given this broad definition and the plethora of potential causes for such changes, it seems likely that IAD represents the common manifestation of a range of etiological pathways. As such, causes of IAD include non-infectious causes (such as airborne allergens, aerotoxins, and particulates) and infectious causes (including viruses, bacteria, and mycoplasma). Eventually, IAD may be divided into a number of subcategories reflecting these different etiologies. Such subcategories likely have different prevalences in different horse populations, and this may account for some of the current confusion regarding IAD in the literature.

Epidemiology

Until a formal definition of IAD is agreed and adhered to, understanding this condition will be complicated by the use of varying case definitions. Despite this, useful information regarding the basic epidemiology of IAD-related conditions is available, and should be considered in the light of the differences in case definitions, target populations, and methodologies. Amongst young racehorses, the prevalence of IAD is undoubtedly high, with estimates in different populations ranging from 11 to 50%. The incidence of IAD is also high, with one study identifying IAD 2 weeks after entering training in 41% of horses that had been free of evidence of respiratory disease when they entered the racing yard (Malikides 2003).

Numerous risk factors for IAD in racehorses have been identified. These include animal, environmental and management factors. Studies in Australia and the UK have found that, among young racehorses, the risk of IAD (or signs related to IAD) decreases with age (Burrell et al 1996, Chapman et al 2000, Christley et al 2001b). It is well recognized that allergens and aerotoxins are important in the development of recurrent airway obstruction (RAO), but recent epidemiological studies have found that poor ventilation and bedding type also influence the risk of IAD in young racehorses (Clarke et al 1987, Burrell et al 1996, Malikides 2003). Furthermore, the percentage of neutrophils in airway fluid samples is significantly associated with the concentration of airborne endotoxin in the environment, suggesting a role for poor air hygiene (Malikides et al 2003). Interestingly, the risk of IAD decreases with the length of time spent in the stable environment (Christley et al 2001b), perhaps because of the development of tolerance to aerotoxins (including endotoxin; Schwartz et al 1994) or to infectious agents. However, the risk of IAD subsequently increases following the commencement of racing.

Relationship Between IAD and RAO

The relationship between IAD and RAO is unclear. Some authors suggest that IAD represents a milder or earlier phase of RAO while others contest that the etiopathogenesis of IAD differs fundamentally from that of RAO. Inflammatory airway disease occurs in racehorses, and so

Table 42.1. Comparison of recurrent airway obstruction (RAO) and inflammatory airway disease (IAD), based on criteria determined by the International Workshop on Inflammatory Airway Disease (2002) and Davis & Rush (2002)

	RAO (symptomatic)	IAD
Signalment	Older (> 5 years old)	Any age, including young racehorses
Clinical signs	Increased rate and depth of breathing	Absence of change in rate, depth or pattern of breathing
Radiographic features	Variable bronchointerstitial pattern	Less severe bronchointerstitial changes, less air trapping
BAL and tracheal cytology	Marked neutrophilia	Fewer inflammatory cells in BAL fluid. Mild neutrophilia, lymphocytosis and monocytosis
Response to environmental control	Good	Variable to poor
Predominant BAL T-lymphocyte phenotype	CD4$^+$	CD8$^+$

BAL, bronchoalveolar lavage.

affects animals at an earlier age than is characteristic for RAO (Table 42.1). In addition, IAD differs from RAO in terms of the clinical signs (milder, subclinical or only evident at maximal exercise, lack of dyspnea), radiographic changes (less severe bronchointerstitial changes, less air trapping), and cytology [fewer inflammatory cells in bronchoalveolar lavage (BAL) fluid]. Inflammatory airway disease results in cytological changes in the BAL fluid (mild neutrophilia, lymphocytosis, and monocytosis) that are reported to be distinguishable from those seen with RAO (marked neutrophilia, lymphopenia, monocytopenia, and normal total cell counts). Furthermore, IAD-affected horses have increased numbers of CD8-positive lymphocytes in their airways, in contrast to RAO-affected horses, which are characterized by the presence of CD4-positive cells. For this reason, Moore et al (1995) concluded that IAD does not have an allergic etiology, is not an early stage of RAO and is probably a response to infection or environmental factors. However, airway cytological findings in horses with RAO are labile and reflect the variable levels of exposure to allergens. Also, when relative cell counts (rather than absolute counts) are used, the presence of a marked relative neutrophilia in acute bouts of RAO necessitates a relative reduction in other cell types and this may account, at least in part, for the relative lymphopenia and monocytopenia which occur in RAO. In addition, whilst relative eosinophil counts are usually reported to be within normal limits in RAO, four of 32 standardbred racehorses with putative IAD had markedly elevated relative eosinophil counts in BAL fluid, suggesting that the clinical signs of IAD can result from a range of etiopathogenic responses (Moore et al 1995).

Etiology

The etiology of IAD likely varies between horses and among horse populations. The numerous potential causes of IAD are listed in Table 42.2. Effective prevention and treatment of this syndrome will be impossible until the underlying causes are elucidated. However, based on the contrasting plausible explanations of the etiology of this syndrome, and the variability of research findings, it is likely that several, or all, of the postulated etiologies are involved, independently or in combination.

Infection with bacteria and mycoplasmas

There is growing evidence that bacteria and mycoplasma may play a role in the etiology of IAD. Tracheal inflammatory changes are positively correlated with the number of bacterial colony-forming units in tracheal washes (Wood et al 1993, Burrell et al 1996). In particular, the aerobic bacteria *Streptococcus zooepidemicus*, *S. pneumoniae* and *Pasteurella* spp., and the mycoplasmas *Mycoplasma felis* and *M. equirhinis*, are significantly associated with lower airway inflammation. However, other workers suggest caution when interpreting these results, because inflammation of the tracheobronchial tree for any reason may leave it more susceptible to colonization by oropharyngeal bacteria (Robinson 1997, Viel 1997). These bacteria may, therefore, be a consequence of lower airway inflammation, rather than its cause. Nonetheless, because they are potential pathogens, it is likely that they may contribute to the worsening and prolongation of IAD.

Table 42.2. Putative causes of IAD

Infection with bacteria and/or mycoplasma
A sequel to exercise-induced pulmonary hemorrhage
Inhalation of airborne pro-inflammatory agents, such as endotoxin in stables with suboptimal air hygiene
Exposure to noxious gases such as H_2S, NH_3, ozone, SO_2, NO_2, and CO
Deep inhalation of particulate matter associated with training and racing
Recurrent or persistent viral infection
A type-I hypersensitivity reaction to environmental molds

Inhalation of particulate matter and airborne pro-inflammatory agents

In horse stables, the concentration of airborne particles may be up to ten times that of outdoor air and 30–40% of the total mass of these particles are sufficiently small to reach the pulmonary alveoli. Furthermore, activities such as cleaning stable bedding and feeding can greatly increase the levels of airborne dust. However, the movements and feeding behavior of horses may be more important in increasing the concentrations of airborne particulate matter in the horse's breathing zone, which may be 30–40 times higher than in other parts of the stall.

Stabling can result in an increase in the number of neutrophils in the BAL fluid of otherwise healthy horses that have no overt signs of pulmonary dysfunction, suggesting a non-specific response to exposure to stable dust. Furthermore, the association between bedding type and poor ventilation and the occurrence of IAD suggests a role for noxious gases or particulate matter in the etiology of IAD.

Recent studies have suggested that the major pro-inflammatory component of particulate matter is adherent endotoxin. Airborne endotoxin concentrations in some horse stables have been reported to exceed those that can induce pulmonary inflammation in humans. Inhaled endotoxin can induce pulmonary inflammation in otherwise normal horses (Pirie et al 2001) and the concentration of endotoxin in the breathing space is associated with the development of IAD in young racehorses (Malikides 2003).

Exercise

The prevalence of IAD is higher in horses undergoing exercise training, compared to non-exercised stablemates. The cause of this effect is not known but exercise may result in deep inhalation of particulate matter, exposure of the small airways to cold unconditioned air, and exercise-induced pulmonary hemorrhage (EIPH). Increased minute ventilation and straightening of the airways during exercise decrease deposition of particles in the upper airways and large lower airways, leading to greater deposition in the small airways. As instillation of blood into the airways is known to induce airway neutrophilia, it is likely that EIPH, a common and repetitive problem in racehorses, plays a role in the development of IAD in some horses. The increased risk of IAD with commencement of racing that is reported in some studies supports a role for deep inhalation of particulate matter during exercise and/or EIPH in the etiology of IAD.

Exposure to noxious gases

Horses may be exposed to common airborne pollutants, such as sulfur dioxide, nitrogen dioxide, ozone, and carbon monoxide. Furthermore, housed animals may be exposed to a range of accumulated noxious gases, including ammonia, hydrogen sulfide, and methane. Such gases may act as respiratory irritants, provoking acute inflammatory responses with injury to the epithelial cells of the lungs and alterations in respiratory function. Many studies in humans and animals have demonstrated an association between these air pollutants and impaired lung function, coughing, and infections of the lower respiratory tract. However, the precise role of these gases in the etiology of IAD is currently unknown.

Recurrent or persistent viral infection

A number of studies suggest a role for viruses in the etiology of IAD. Oral administration of interferon-α (IFN-α), an endogenous immunostimulant with antiviral activity, reduces lower respiratory tract inflammation in racehorses with chronic IAD. Also, equine herpesviruses (EHV) 1 and 4 have been isolated from nasal swabs from a small proportion of racehorses with signs of IAD. Similarly, antibodies to EHV-1 and EHV-4 and to equine influenza virus have been detected in the tracheal secretions of horses with chronic pulmonary inflammation. However, a number of reports indicate that viruses do not have a major role in the etiology of IAD. For example, Burrell et al (1996) and Christley et al (2001a) found no association between IAD and viral seroconversion. In addition, if persistent equine influenza virus infection or postinfection damage to the airways is an important cause of IAD, one might expect a lower incidence of this syndrome in areas that are free of equine influenza, such as Australia. Unfortunately, there is little information available on the incidence of these conditions in different countries, and any comparison would be difficult because of potential confounding by management and housing effects.

Type I hypersensitivity reaction

The etiopathology of eosinophilic pulmonary inflammation may differ from that of neutrophilic pulmonary inflammation, and could represent an immune-mediated response consistent with a type I hypersensitivity reaction. Studies from North America have found that horses affected with eosinophilic pulmonary inflammation typically have a moderate to prominent interstitial pattern on thoracic radiography, and eosinophilic pulmonary granulomas can be identified at post-mortem examination of some horses with pulmonary eosinophilia.

Diagnosis

The diagnostic approach to horses with suspected IAD should aim to confirm this diagnosis and to provide information regarding likely etiological factors so as to

guide therapy. In most cases, this should include a full general physical examination (with or without hematology and serum biochemistry) to rule out concomitant diseases. A thorough examination of the respiratory tract, including auscultation, endoscopy, and cytology and bacteriology of the airway lining fluids should also be performed. Further tests, such as thoracic radiographs and pulmonary function testing, are performed under specific circumstances.

History and clinical signs

A detailed history and physical examination should be performed on all suspected cases of IAD. Together these help to rule out alternative diagnoses and may identify potential etiological factors. Coughing during exercise and/or eating is the most common presenting complaint. Information regarding the clinical history, effects on performance, management and presence of disease in the in-contact horses should be determined. Evidence of systemic disease, such as depression, pyrexia or inappetance, will rule out IAD, or indicate the presence of concomitant disease. If the presenting complaint includes a history of poor performance, an exhaustive examination should rule out alternative causes such as musculoskeletal disease, upper airway dysfunction, and cardiovascular disease before the poor performance is ascribed to IAD.

The respiratory examination should evaluate both the upper and lower respiratory tracts. The presence and character of nasal discharge should be assessed. Careful thoracic auscultation using a rebreathing bag in a quiet environment is recommended. Abnormalities on thoracic auscultation are rarely present in IAD and identification of these should prompt consideration of other pulmonary pathological disorders.

Endoscopy

Endoscopy of the lower airways is a fundamental component of the diagnostic work-up of horses with suspected IAD. In many cases, a presumptive diagnosis of IAD can be made on the basis of endoscopic findings. In addition, endoscopy can facilitate the collection of tracheal fluid samples for cytology and bacteriology and bronchial samples for cytology. Increased amounts of mucus are a common finding in cases of IAD; however, the quantity that constitutes an abnormality is not clear. Grading scores exist to help reduce the subjectivity of the assessment of mucus quantity (see Chapter 5). Generally, healthy horses will have no mucus, or only a few mucus droplets, evident. Increased volumes, however, are consistent with airway inflammation. Caution is required, however, because the range of scores observed for normal horses overlaps with those of horses with IAD. Furthermore, pooling of mucus in the trachea may occur whenever a horse is prevented from lowering its head, such as occurs during

transportation. Provided this restriction is not prolonged, the tracheal mucus volume rapidly returns to normal. Hence, information regarding the immediate history of the horse before examination should be considered when evaluating endoscopic findings.

Airway cytology

An increased proportion of inflammatory cells in airway secretions is one of the principal diagnostic criteria for IAD. Typically, neutrophils predominate, although, less frequently, there may also be increases in the numbers of mast cells and/or eosinophils. As the proportion of neutrophils, and other cells, in the airway secretions of apparently normal horses depends on numerous factors (including type of housing and bedding and methods of sample collection), it is difficult to be too prescriptive about cut-off values for disease. The cut-off values suggested here are for guidance, and clinicians should assess the results for each case in light of other findings. Particular care should be taken when considering results near the cut-off values.

It is believed that horses with different cytological profiles develop a similar range of clinical signs. Some studies have associated eosinophilic IAD with poor exercise performance and airway hyperresponsiveness. However, BAL eosinophilia can also be observed in subclinical or mild disease, indistinguishable from the typical signs of IAD.

If BAL eosinophilia is identified, the clinician should investigate parasitic pulmonary disease, including ascarid migration and lungworm infection, in addition to hypersensitivity pneumonitis. Aerosolized corticosteroid administration is recommended for horses with BAL eosinophilia; however, this treatment has not yet been investigated under conditions of a controlled, randomized clinical trial. In the experience of one author (B.R.R.), eosinophilic pneumonitis responds slowly and incompletely to immunosuppressive therapy. However, the other author (R.M.C.) observed cases of marked eosinophilic IAD that presented as neutrophilic IAD within days to weeks, before resolving, as have other clinicians (Bruce McGorum, personal communication).

Diagnosis of metachromatic cell inflammation requires identification of >2% of the total BAL cell count as mast cells. Clinical signs of metachromatic cell inflammation include poor race performance, chronic cough, and airway hyperreactivity. Identification of mast cells in BAL fluid cytology preparations is facilitated by the use of cationic dyes such as toluidine blue stain, because mast cells are not evident on preparations stained with many of the conventional stains. Mast cells are thought to play an important role in the pathophysiology of early-stage allergic lung disease in humans through release of inflammatory mediators following antigen exposure. Metachromatic cell inflammation likely represents a local pulmonary hypersensitivity response and may represent an early form of RAO. Horses with clinically relevant metachromatic cell

inflammation demonstrated marked bronchoconstriction during bronchoprovocation challenge with histamine. Some clinicians use a bronchoprovocation challenge to determine the clinical relevance of cytological findings and to monitor the response to treatment.

Comparison of tracheal aspirate and BAL cytology

The cytological results of simultaneously collected tracheal aspirate (TA) and BAL samples are often poorly correlated. In one study in which both TA and BAL were performed following exercise, the diagnosis (IAD or normal) was the same in approximately 60% of cases. In almost three-quarters of cases where discrepancy occurred, IAD was diagnosed by TA but not by BAL. Unfortunately, there is still controversy as to whether IAD is a localized or generalized respiratory condition; most likely it may be both. The aforementioned studies comparing the results of TA and BAL suggest that in some cases the cytological changes associated with IAD may be localized. Hence, absence of evidence of inflammation on a single BAL result should be treated with caution if the index of suspicion for IAD is high.

The potential occurrence of localized and generalized changes in IAD has clinical implications. Clinicians may consider performing cytology on both TA and BAL samples. Collection of one type of sample only should be considered an incomplete clinical database, and the conclusion of freedom from IAD should be made with caution.

The effect of exercise on tracheal and bronchoalveolar secretions

Recent exercise (within 30–60 min) may affect the volume of airway secretions and the proportion of cell types recovered by tracheal washes or BAL. Post-exercise TA samples contain more airway secretions than pre-exercise washes and specimens obtained after exercise differ in cytological variables in a proportion of horses. In one study, the proportion of neutrophils in BAL samples from apparently normal horses almost doubled following intense (race speed) exercise. The reasons for the effect of exercise are uncertain. Increased lung movement and airflow during exercise may mechanically force redistribution of existing secretions, in which case sampling post exercise may maximize the chance of detecting an abnormality. However, exercise may also expose the lower airways to inhaled environmental agents that are normally removed in the upper airways, and to blood arising from EIPH, and hence the cytological changes may represent a new insult rather than a pre-existing pathology. The potential effect of recent exercise should be considered when results are evaluated.

Airway bacteriology

The lower respiratory tract of the horse is normally sterile, although it may transiently contain small numbers of bacteria. The bacterial population of the lower respiratory tract of normal horses reflects the balance between the ingress of bacteria from the nasopharynx and environment, and the clearance of these bacteria via the pulmonary defense mechanisms. In normal horses the small numbers of bacteria are effectively transient, being efficiently cleared by normal pulmonary defenses. The frequency of isolation of bacteria from the lower respiratory tract can increase under some circumstances, such as following coughing during sampling, or following exercise or prolonged head elevation. Therefore, the lower respiratory tract is frequently exposed to potentially pathogenic bacteria. Whether or not disease occurs as a result of this contamination depends on the efficacy of the pulmonary defense mechanisms.

Nasopharyngeal contamination of samples

Many organisms isolated from the lower respiratory tract following nasopharyngeal contamination are similar to those thought to be involved in IAD. Therefore, the types of bacteria isolated do not give an indication of the likelihood of contamination. The presence of squamous epithelial cells (SECs) and food material on microscopic examination of samples has been used as evidence of nasopharyngeal contamination. Several protocols for the quantification of SECs have been suggested, including the relative percentage of SECs in the sample and the number of SECs per low-power field. While estimating the number of SECs/ml appears to provide useful information as to the likelihood of contamination, no strict guidelines or cut-off values have been established. Furthermore, such information should always be interpreted in the light of clinical, cytological, and bacteriological findings.

Classification of IAD on the basis of airway cytology and microbiology

Although subcategories of IAD are not well defined, differentiation of cases into a number of groups may facilitate a rational approach to therapy. We currently recommend the following classifications:

- *Bacterial IAD* – identification of known pathogenic bacteria (or *Mycoplasma* spp.), in numbers greater than 10^2 colony-forming units/ml of tracheal aspirate, in the presence of neutrophilic inflammation and the absence of evidence of upper airway contamination warrants administration of antimicrobial therapy, preferably based on the results of culture and sensitivity tests.

- *Non-bacterial IAD* – if bacteria are not considered to be part of the etiology of the airway inflammation, cytological evaluation of BAL or TA may be used to categorize cases into one of the following inflammatory profiles:
  - Mixed inflammation with high total nucleated cell count, neutrophilia (> 5% of total BAL cells, > 20% of total TA cells), lymphocytosis, and monocytosis
  - Increased metachromatic cells (mast cells > 2% of total cells)
  - Eosinophilic inflammation (> 5% of total cells).

Hematology and biochemistry

By definition, horses with IAD are free from systemic illness; consequently hematological and serum biochemical parameters are usually within normal limits. Identification of abnormalities may suggest alternative or coexisting diagnoses.

Radiography

Generally, radiographic examination of horses with suspected IAD is unrewarding. Radiographic findings are often within normal limits and are of little use for grading the degree of pathological change or lung dysfunction. The exception is eosinophilic pulmonary inflammation, because thoracic radiographs are indicated to identify the severity of interstitial infiltration and to monitor the response to therapy and/or disease progression.

Pulmonary function tests

Conventional measures of pulmonary function, such as maximal changes in pleural pressure, pulmonary resistance, and dynamic compliance, are not altered in horses with IAD. Some horses with IAD demonstrate airway hyperreactivity in response to histamine challenge and lower forced expiratory airflows, compared to control horses (Viel 2003). However, to date, these changes have only been assessed in non-random populations, such as those investigated in referral centers. The prevalence of functional abnormalities in the general population of horses with IAD is unknown, and it is possible that such horses represent a subgroup of IAD. In general, pulmonary function tests are not required to diagnose IAD, although they may help differentiate this syndrome from RAO, which is associated with more severe lung dysfunction.

Breath and breath condensate analysis

Although not widely available at present, the analysis of breath and breath condensate may offer useful diagnostic possibilities in the future (Wyse et al 2004). More than 3,000 gases have been identified in exhaled breath. Ethane and pentane, markers of oxidative stress, have been detected in the exhaled breath of horses and other species with pulmonary inflammatory disease. However, these markers are non-specific and will be elevated in any oxidative disorder. Exhaled breath condensate can be easily collected from unsedated horses. Markers that show some promise as disease indicators include hydrogen peroxide, carbon monoxide, thiobarbituric acid-reactive substances, nitrate, 8-isoprostane, and cytokines and leukotrienes. The physical properties of the condensate, including its pH, may also provide information about respiratory inflammation. As an adjunct to other diagnostic tests, such methods may provide objective measures of inflammation and may permit frequent monitoring of the respiratory tract, which may aid tailoring of treatment protocols and facilitate future field-based studies.

Treatment and Prevention

Choice of treatment options for horses with IAD will depend on the definitive or putative diagnosis of the inciting cause. In reality, choice of therapy is often determined by the previous (perceived) success of particular regimens. However, bacterial, neutrophilic IAD appears to have a relatively short duration (days or a few weeks) in the absence of therapy and resolution may occur naturally rather than as a result of veterinary intervention. Therefore, in the absence of randomized trials, the appropriateness of treatment protocols remains largely speculative.

Given what is currently known regarding the potential causes of IAD, rational therapeutic protocols address some or all of the following:

- provision of an environment with low levels of airborne pro-inflammatory agents
- removal of potential inciting causes
- treatment of pathological sequelae, such as airway inflammation and bronchoconstriction.

Categorization of cases, based on airway bacteriological and cytological findings, may help guide therapy. Treatment of metachromatic cell and eosinophilic pulmonary inflammation in the older (> 6 years) sport horse often requires long-term therapy, and clinical signs frequently recur after discontinuation of therapy.

Environmental management

Environmental management has long been a mainstay of treatment of RAO. However, as increased levels of dust, endotoxin, ammonia and other airborne pollutants can induce inflammatory changes in otherwise normal horses, management of cases of IAD should incorporate the provision of a low-irritant environment, regardless of whether environmental pollutants are considered an inciting cause.

Unfortunately, provision of an ideal environment may require considerable effort and expense to the horse owner and hence may be difficult to achieve.

The pollutants considered most likely to be involved in the development of IAD originate from bedding and feed and from the excreta of the horse itself, and may reach high concentrations in poorly ventilated stables. Where practical, keeping horses with IAD outdoors or in stables with open designs may be beneficial. Frequently, however, such modifications are not possible but ensuring that windows and doors are open and that vents are unobstructed may be helpful. Ideally, ventilation should be sufficient to provide at least eight air changes per hour.

Evidence suggests that modification of a horse's immediate breathing zone should be the main goal of environmental management. Hence provision of low-dust bedding and feed is important. Bedding materials with lower concentrations of respirable dust and other pollutants include shredded paper and cardboard, wood chips and large wood shavings, peat moss, and rubber. Where possible, these should be used in place of straw or deep litter shavings. Regular removal of fecal material and urine-soaked bedding is essential. Low-dust forage sources, including soaked hay or preferably haylage, should be used instead of dry hay. Complete, pelleted diets constitute a low-dust source of concentrated feed.

Corticosteroids

The efficacy of corticosteroids in cases of IAD is poorly documented. However, given the underlying inflammatory process involved, administration of corticosteroids may be beneficial in cases of IAD that are considered not to have an infectious etiology. The most logical indications for corticosteroid administration to horses with IAD are those cases with eosinophilic and metachromatic cell inflammation. Reduction in airway reactivity has been reported following 30-day oral administration of prednisone to horses with IAD (Viel 2003). However, no control group was included, and as prednisone remains largely unabsorbed from the equine gut, other factors were likely to have been responsible for the improvement.

Corticosteroids may be administered orally, intravenously, intramuscularly or via inhalation. Recent advances in the delivery of inhaled agents to horses should make this route more popular in the future. In almost all circumstances, short-acting corticosteroids are preferred to better tailor the dose and to reduce the risk of adverse side effects. The use of corticosteroids should be accompanied by efforts to reduce exposure to potential pro-inflammatory mediators, generally through environmental modification; otherwise any beneficial effects may be short-lived.

A range of corticosteroids is available for inhalation therapy (see Chapter 7). It includes beclomethasone dipropionate, budesonide, flunisolide, fluticasone propionate, and triamcinolone acetonide, although only beclomethasone dipropionate and fluticasone propionate have been widely used in horses. Treatment protocols for these agents are currently based on data from horses with RAO, although these should be appropriate for horses with IAD. The dose rate varies with methods of administration. The recommended dose rate for beclomethasone dipropionate is 500–1,500 µg q12h using the 3M Equine Inhaler® or 3,750 µg q12h using the Aeromask®. Administration of the lower dose (500 µg) results in similar clinical efficacy but less adrenal suppression in horses with RAO. Fluticasone propionate, at a dose rate of 2,000 µg q12h via an Aeromask®, has been recommended for horses with RAO. At this dose, adrenal suppression is not observed.

The most appropriate corticosteroid for intramuscular (IM) or intravenous (IV) administration is dexamethasone (0.05–0.1 mg/kg q24h). Dexamethasone (at the same dose) and prednisolone (1–2 mg/kg q12h to q24h) may be administered orally. Typically, these agents are administered at the higher end of the recommended dose rate for 24–48 h, followed by a reduction in the amount and frequency of administration over several days to weeks. However, whether this dose reduction is required for short-term administration (as is appropriate for most cases of IAD) remains uncertain. As noted previously, prednisone is poorly absorbed following oral administration and should not be used.

Bronchodilators

By definition, respiratory dysfunction is not a feature of IAD, and affected horses do not have detectable bronchoconstriction. However, it is possible that judicious use of bronchodilators may be appropriate to reduce minor degrees of small airway obstruction, reduce exercise-induced cough, aid the removal of mucus, and improve the delivery of other agents via the inhalation route. The most commonly used agents are the anticholinergics and β_2-agonists which may be formulated for inhalation or systemic administration. While bronchodilators may provide temporary beneficial effects, they will not address the underlying inflammatory process or airway hyper-reactivity. Furthermore, repeated use of β_2-agonists (without corticosteroids) may result in receptor down-regulation, reducing their efficacy.

Currently, the most common approach to bronchodilation is the systemic administration of clenbuterol, a β_2-agonist. There is a risk of adverse reactions so the recommended dosing regimen is 0.8 µg/kg q12h *per os*, followed, if necessary, by incremental increases of about 0.8 µg/kg every 2 or 3 days. Although doses up to 3.2 µg/kg q12h have been administered in horses with RAO, such doses should not be needed in horses with IAD, which have no clinically detectable bronchoconstriction. The presence of clinically detectable airway obstruction,

such as respiratory distress at rest, should prompt consideration of an alternative diagnosis and the initiation of alternative diagnostic testing.

A number of β_2-agonists are available for inhalation administration. These include albuterol, fenoterol, pirbuterol, formoterol, and salmeterol. The first three of these have rapid onset of action (5 min) and short duration of activity (approximately 1–3 h). Formoterol and salmeterol have a slower onset of action, but can provide up to 8–12 h relief in horses with RAO. As such, salmeterol has been recommended for maintenance therapy for RAO. As salmeterol has much greater β_2 selectivity than albuterol, this agent has reduced risk of adverse (β_1) effects at therapeutic dosages. Occasionally in human asthma patients, the administration of β_2-agonists, particularly albuterol and salmeterol, may be followed by bronchoconstriction. This effect has not yet been noted in horses.

The anticholinergic agent ipratropium bromide induces bronchodilation, attenuates cough, and protects against bronchoconstrictive stimuli. Like atropine, ipratropium bromide is a non-selective muscarinic antagonist, but is poorly absorbed following inhalation (approximately 6%) because of its quaternary ammonium structure. Therefore, ipratropium bromide has few systemic adverse effects and does not inhibit gastrointestinal motility. In RAO-affected horses, the onset of action following inhalation is 15–30 min, and the duration of action is between 4 and 6 h. The dose rate is dependent on the method of administration, ranging from 180 to 360 µg/horse (or 0.5–1 µg/kg) for the Aeromask®, 2 to 3 µg/kg for ultrasonic nebulizers and 200 µg/100 kg (or 2,400 µg/horse) for dry powder inhalers. The effects of anticholinergic agents are additive to those of the β_2-agonists, with the former acting principally on the more central airways and the latter on the smaller peripheral airways. Hence, combination therapy has been recommended.

Sodium cromoglycate

In IAD-affected horses with elevated numbers of metachromatic cells in BAL fluid, aerosol administration of sodium cromolyn (200 mg q12h to q24h) or nedocromil sodium may improve the clinical signs of respiratory disease. Sodium cromolyn can be administered via a nebulizer or metered dose inhaler using a facemask (e.g. Aeromask® or EquineHaler®).

Interferon-α

In horses with a mixed inflammatory cytological profile, the administration of low-dose natural human IFN-α reduces the volume of exudate in the respiratory tract, lowers the total cell counts in BAL fluid, and converts the differential cell count to a non-inflammatory cytological

profile. In contrast, horses with eosinophilic IAD do not respond favorably to IFN-α therapy. IFN-α is a proximal mediator of immunomodulation and has antiviral activity. The specific mechanism of therapeutic benefit of IFN-α in horses with IAD is unknown. The pathway for dissemination of its biological effects following oral administration may be the activation of natural defense systems originating in oropharyngeal-associated lymphoid tissue that involves cellular communication and amplification of the immune response. Lymphocytes exposed to IFN-α can transfer enhanced biological effects to naive lymphocytes in the absence of IFN-α. It is hypothesized that lymphocytes recruited to antiviral activity by IFN-α in the oral cavity can enter the circulation and rapidly confer antiviral capability to cells at distant sites. The process would not require the continued presence of IFN-α and may represent a major amplification mechanism. This mechanism allows the biological effects of IFN-α to reach tissues that are accessible to mobile lymphocytes, in which penetration of IFN-α might be poor, such as the surface of the respiratory tract, gastrointestinal tract, and the eye. The current high cost and limited availability of IFN-α is likely to restrict the use of this drug in horses in the near future.

Antibiotics

Up to 50% of horses with IAD may have evidence of bacterial infection. Despite evidence of spontaneous resolution of IAD in many cases, antimicrobial therapy may be rationally used in selected cases of IAD. The bacteria most commonly involved include *Streptococcus* spp., *Pasteurella* spp., and *Actinobacillus* spp. Other organisms, such as *Bordetella bronchiseptica*, are also occasionally isolated and *Mycoplasma* spp. have been isolated in outbreaks of IAD. Anaerobic bacteria are rarely, if ever, involved.

Penicillin G has good efficacy against the most common isolates. Penicillin may be administered as procaine penicillin (22 mg/kg q12h IM) or sodium or potassium penicillin (22 mg/kg q6h IV). Procaine penicillin is often avoided with race or competition horses because of the restrictions on the detection of procaine in these horses. In addition, intramuscular administration, particularly over long periods, may result in muscle pain. Whilst aqueous preparations of penicillin avoid these problems, the requirement for frequent administration makes its use inconvenient in non-hospitalized horses.

Ceftiofur sodium, a third-generation cephalosporin, is a popular alternative to penicillin. The recommended dose rate is 2.2–5.0 mg/kg q12h to q24h. Although only licensed for IM use in the horse, some clinicians have recommended IV administration. At North American racetracks, peracute clostridial colitis has been anecdotally associated with intravenous administration of ceftiofur sodium. The spectrum of activity of ceftiofur sodium encompasses the bacteria commonly isolated from horses with IAD, and muscle

soreness following IM administration tends to be less of a problem than following procaine penicillin.

Trimethoprim and sulfonamide combinations have a broad spectrum of activity and the added convenience of oral and, in some countries, intravenous administration. However, in North America, a considerable proportion of *Streptococcus* spp. isolated from horses are resistant to trimethoprim–sulfonamide combinations. This does not yet appear to be such a problem in other areas. The recommended oral dose rate for trimethoprim–sulfonamide combinations is 15 mg/kg q12h *per os*; however, some clinicians recommend double this dosage at the same dose interval.

In some areas, the use of oxytetracycline is popular for the treatment of IAD, particularly in racehorses. Although only bacteriostatic at normal dose rates (5–10 mg/kg q12h IV), some clinicians report good results, even with a 24-h dosing interval. Benefits of oxytetracyclines include their intravenous administration and short or absent withdrawal times. Additionally, tetracyclines have direct anti-inflammatory effects, although the efficacy of this action in cases of IAD is unknown.

Effects on Performance

Anecdotally, IAD is frequently diagnosed as a cause of poor exercise performance. However, the true effect of IAD on performance is incompletely documented. Many horses undergoing investigation for poor performance have signs of IAD. However, given the high prevalence of IAD, the clinical relevance of this finding is uncertain. Few studies have investigated the physiological effects of IAD. In one study, submaximal exercise in standardbred trotters failed to identify an obvious difference in IAD-affected horses, compared to healthy horses, in regard to arterial blood gas changes (Nyman 2003). In contrast, others have found that IAD-affected horses develop greater exercise-induced hypoxemia than healthy controls during submaximal exercise (Couëtil & DeNicola 1999). In another study, horses with IAD had increased airway reactivity (Hoffman & Mazan 2003). Furthermore, although only approaching significance ($P = 0.055$), airway resistance was increased in horses with IAD, similar to previous studies (Hoffman 1999), suggesting a degree of airway obstruction in some horses with IAD, although not to the same degree as horses with RAO. Post exercise, horses with IAD have higher blood lactate concentrations, lower bicarbonate concentrations, and lower pH than control horses (Couëtil & DeNicola 1999). Hence there is experimental evidence that IAD induces physiological changes in exercising horses that may result in alterations in performance. It is likely that the clinical impact of IAD on exercise performance is dependent upon the nature, duration, and magnitude of pulmonary inflammation, as well as the nature of the athletic activity of the patient.

REFERENCES

Anon 2003 Workshop summary. In: Hoffman A, Robinson NE, Wade JF (editors) Havemeyer Foundation Monograph Series No. 9, Workshop on Inflammatory Airway Disease: Defining the Syndrome. R & W Publications, Newmarket, pp.89–91

Burrell MH, Wood JLN, Whitwell KE et al 1996 Respiratory disease in thoroughbred horses in training: the relationship between disease and viruses, bacteria and environment. Veterinary Record 139: 308–313

Chapman PS, Green C, Main JPM et al 2000 Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. Veterinary Record 146: 91–95

Christley RM, Hodgson DR, Rose RJ et al 2001a A case–control study of respiratory disease in Thoroughbred racehorses in Sydney, Australia. Equine Veterinary Journal 33: 256–264

Christley RM, Wood JLN, Reid SWJ et al 2001b Coughing in thoroughbred racehorses: risk factors and tracheal endoscopic and cytological findings. Veterinary Record 148: 99–104

Clarke AF, Madelin T, Allpress RG 1987 The relationship of air hygiene in stables to lower airway disease and pharyngeal lymphoid hyperplasia in two groups of Thoroughbred horses. Equine Veterinary Journal 19: 524–530

Couëtil LL, DeNicola BD 1999 Blood gas, plasma lactate and bronchoalveolar lavage cytology analysis in racehorses with respiratory disease. Equine Veterinary Journal Supplement 30: 77–82

Davis E, Rush BR 2002 Equine recurrent airway obstruction: pathogenesis, diagnosis, and patient management. Veterinary Clinics of North America; Equine Practice 18: 453–468

Hoffman AM 1999 Programme of lung function testing horses with suspected small airway disease. Equine Veterinary Education 11: 322–328

Hoffman AM, Mazan M 2003 Airway obstruction and hyper-reactivity in horses with signs of inflammatory airway disease. In: Hoffman A, Robinson NE, Wade JF (editors) Havemeyer Foundation Monograph Series No. 9, Workshop on Inflammatory Airway Disease: Defining the Syndrome. R & W Publications, Newmarket, pp.9–12

Malikides N 2003 Epidemiological studies on inflammatory airway disease (IAD) in young Thoroughbred racehorses. PhD Thesis, University of Sydney

Malikides N, Christley RM, Pirie RS et al 2003 Effect of respirable and total endotoxin on neutrophilic inflammation of lower airways in young Thoroughbred racehorses in Sydney, Australia. CD-ROM Proceedings of International Society for Veterinary Epidemiology and Economics, Vina Del Mar, Chile

Moore BR, Krakowka S, Robertson JT et al 1995 Cytologic evaluation of bronchoalveolar lavage fluid obtained from Standardbred racehorses with inflammatory airway disease. American Journal of Veterinary Research 56: 562–567

Nyman G 2003 Inflammatory airway disease and gas exchange. In: Hoffman A, Robinson NE, Wade JF (editors) Havemeyer Foundation Monograph Series No. 9, Workshop on Inflammatory Airway Disease: Defining the Syndrome. R & W Publications, Newmarket, pp.75–80

Pirie RS, Dixon PM, Collie DD, McGorum BC 2001 Pulmonary and systemic effects of inhaled endotoxin in horses with heaves. Equine Veterinary Journal 33: 311–318

Robinson NE 1997 Pathogenesis and management of airway disease. In: Norwood G (editor) Proceedings of the 43rd Annual Convention of the American Association of Equine Practitioners. American Association of Equine Practitioners, Lexington, Kentucky, pp.106–115

Schwartz DA, Thorne PS, Jagielo PJ et al 1994 Endotoxin responsiveness and grain dust-induced inflammation in the lower respiratory tract. American Journal of Physiology 267: L609–L617

Viel L 1997 Small airway disease as a vanguard for chronic obstructive pulmonary disease. Veterinary Clinics of North America; Equine Practice 13: 549–560

Viel L 2003 Airway obstruction and hyper-reactivity in horses with signs of inflammatory airway disease. In:

Hoffman A, Robinson NE, Wade JF (editors) Havemeyer Foundation Monograph Series No. 9, Workshop on Inflammatory Airway Disease: Defining the Syndrome. R & W Publications, Newmarket, pp.71–74

Wood JLN, Burrell MH, Roberts CA et al 1993 Streptococci and *Pasteurella* spp. associated with disease of the equine lower respiratory. Equine Veterinary Journal 25: 314–318

Hoffman A, Robinson NE, Wade JF (editors) 2003 Havemeyer Foundation Monograph Series No. 9, Workshop on Inflammatory Airway Disease: Defining the Syndrome. R & W Publications, Newmarket.

Wyse CA, Yam PS, Sutton DGM et al 2004 Current and future uses of breath analysis as a diagnostic tool. Veterinary Record 154: 353–360

Miscellaneous Pulmonary Disorders

Tim Mair

Pulmonary Neoplasia

Tumors in the thoracic cavity may occur as primary thoracic neoplasms or tumors that metastasize to the chest from a primary site elsewhere in the body. The lungs, mediastinum and lymph nodes, and pleural cavity may be affected. Thoracic neoplasia is uncommon in the horse, and surveys of necropsy examinations indicate a very low incidence. Cotchin and Baker-Smith (1975) reviewed 1,308 equine post-mortem examinations in an abattoir survey, and recorded only two cases of thoracic neoplasia (one granular cell myoblastoma and one bronchiolar adenoma). Two other surveys of necropsy examinations failed to record any cases of thoracic neoplasia in a total of 931 horses (Baker & Leyland 1975, Sundberg et al 1977). Despite the fact that they are rare, thoracic tumors need to be considered in the differential diagnosis of any horse presenting with signs of chronic pulmonary disease.

The clinical manifestations of thoracic neoplasia are variable, but generally include many non-specific signs such as depression, inappetence, weight loss, and pyrexia. The identification of such systemic signs in horses presenting with chronic pulmonary disease should raise the index of suspicion of thoracic neoplasia. Specific respiratory signs such as cough, dyspnea, and pulmonary hemorrhage vary, depending on the location of the tumor(s) and the extent of the pathology. Coughing is most pronounced in cases where a tumor mass compresses an airway, and dyspnea is particularly marked in cases that have a pleural effusion. Mediastinal tumors often present with clinical signs that reflect a large-volume pleural effusion such as rapid, shallow breathing and pectoral edema. Intrapulmonary tumors, on the other hand, tend to present with exercise intolerance, weight loss, cough, and occasionally epistaxis. Lameness, as a result of either bone infiltration or hypertrophic osteopathy (Marie's disease), is occasionally observed in horses with metastatic pulmonary neoplasia. Hypertrophic osteopathy is a rare syndrome characterized by symmetrical proliferation of subperiosteal bone along the diaphyses and metaphyses of the long bones of the appendicular skeleton. Affected horses have symmetrical, firm swelling of all four limbs and shifting leg lameness (Mair et al 1996). The exact pathophysiology of this bony proliferation is unclear; however, hormonal, hemodynamic, and neurogenic theories have been proposed. Intrathoracic disease is present in the majority of cases of hypertrophic osteopathy and should be investigated in horses displaying these unusual clinical signs.

Lymphoma (lymphosarcoma)

Lymphoma involving the mediastinum is the commonest type of thoracic neoplasia identified in horses (Mair et al 1985). In most cases, the lymphoma is multicentric, but tends to be present in either mediastinal, alimentary, cutaneous or generalized forms (or combinations of these forms) (Mair and Hillyer 1992). In addition to the nonspecific clinical signs of inappetence, including weight loss, most horses with mediastinal lymphosarcoma develop a large-volume pleural effusion that results in dyspnea and tachypnea. Ventral thoracic and pectoral edema often occurs in these cases (Fig. 43.1). Auscultation of the chest will reveal muffling of the lung sounds in the ventral thorax (usually bilaterally), and the cardiac sounds may be audible over a larger than normal area. Confirmation of a pleural effusion can be achieved by thoracic ultrasonography, radiography, and thoracocentesis.

The mass, and any associated pleural effusion, acts as a space-occupying lesion that results in compression of other organs in the chest, and may cause jugular venous distension and pulsation, and dysphagia. The neoplastic mass sometimes protrudes through the thoracic inlet and can be seen or palpated as a unilateral or bilateral mass at the base of the jugular furrow (Fig. 43.1). These masses may be amenable to surgical biopsy, although a deep surgical cut-down may be necessary to reach neoplastic tissue. Generalized enlargement of the peripheral lymph nodes is rarely present in cases of mediastinal lymphoma.

The diagnosis of thoracic lymphoma is based on the clinical signs, identification of pleural effusion, pleural fluid cytology and biopsy of any accessible tumor masses. A mediastinal mass may also be identifiable by ultrasonography (Garber et al 1994). Non-specific hematological changes include leukocytosis, neutrophilia and hyperfibrinogenemia. In a minority of cases, there may be lymphocytosis in the peripheral blood, possibly with the presence of abnormal circulating lymphoblastic cells; however, true leukemias are very rare in the horse. The effusion in cases of lymphoma
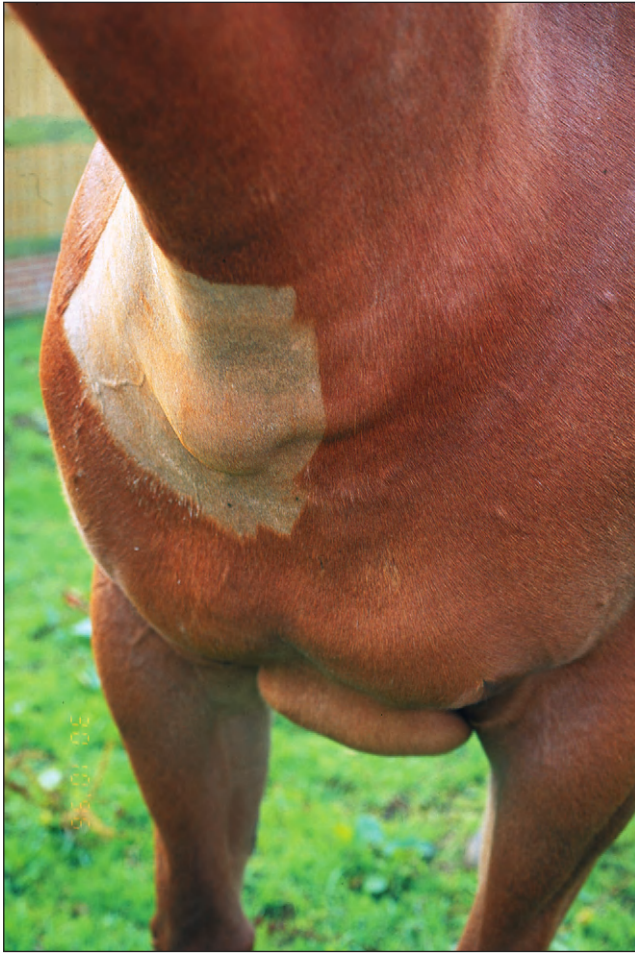
Fig. 43.1. Ventral pectoral edema and mass at the base of the jugular groove in a 7-year-old mare affected by mediastinal lymphoma.

is typically a modified transudate or non-septic exudate, and neoplastic cells are frequently, but not always, present in the fluid. A large number of the cells in such effusions will be mononuclear, mainly lymphocytic. Lymphoblasts with mitoses may be present.

Thoracoscopy can be used to examine the pleural cavity and cranial mediastinum in horses with suspected mediastinal lymphoma (Vachon & Fischer 1998), as well as other forms of thoracic neoplasia (Ford et al 1987, Rossier et al 1990, Mueller et al 1992). Biopsy of suspect lesions can be undertaken during this procedure (see Chapter 20).

Primary pulmonary and pleural tumors

Primary pulmonary neoplasia is very rare. In one review of 38 cases of thoracic neoplasia in horses, only 7.9% involved primary neoplasms (Mair & Brown 1993), whereas another review of 35 cases revealed no primary lung tumors (Sweeney & Gillette 1989). Examples of primary equine lung tumors include pulmonary and bronchial carcinomas and adenocarcinomas (Dill et al 1986, Uphoff & Lyncoln 1987, Van Rensburg et al 1989, Mair & Brown 1993); bronchogenic squamous cell carcinoma (Schultze et al 1988); pulmonary granular cell tumor (Alexander et al 1965, Misdorp & van Gelder 1968, Parker et al 1979, Nickels et al 1980, Turk & Breeze 1981, Sutton & Coleman 1995, Facemire et al 2000, Pusterla et al 2003a); bronchial myxoma (Murphy et al 1978); and pulmonary chondrosarcoma (Sullivan 1960, Clem et al 1986).

Pulmonary granular cell tumor

Pulmonary granular cell tumor (also known as granular cell myoblastoma or putative Schwann cell tumor) appears to be the most common primary equine intrapulmonary tumor. This tumor is of mesenchymal origin, probably derived from myoblasts. The mean age of reported cases is 13 years (Pusterla et al 2003a), and there appears to be no breed predisposition. The majority of reported cases have involved mares. Presenting clinical signs generally include paroxysmal cough, exercise intolerance, and weight loss. Hypertrophic osteopathy has been recorded in a small number of affected horses (Alexander et al 1965, Sutton & Coleman 1995). Some affected horses may survive for several years with this tumor, although progressive respiratory distress and weight loss are likely to ensue. The chronic nature of the clinical presentation can lead to an incorrect initial diagnosis of recurrent airway obstruction (Pusterla et al 2003a).

Diagnosis of pulmonary granular cell tumor is achieved by a combination of the clinical history, physical examination, endoscopy, thoracic radiography and diagnostic ultrasonography, and possibly biopsy. Hematological examination often reveals non-specific changes including hyperfibrinogenemia and leukocytosis with neutrophilia. Pulmonary auscultation is frequently unremarkable, although reduced breath sounds may be identified over one hemithorax because of the occlusion of one main-stem bronchus by the tumor. The tumor usually consists of multiple, well-defined nodules associated with a major bronchus, and a portion of the mass is often seen on endoscopic examination of the carina or bronchi (this examination requires the use of an endoscope longer than 1 m). The majority of the mass is intrapulmonary and often involves the cranial lung lobes. Thoracic radiographs may reveal cranioventral opacity, or single or multiple pulmonary nodules. Usually only one lung is affected. Ultrasonography may show reduced movement of the pleural surface on the affected side of the thorax; pleural thickening and "comet-tail" irregularities may also be apparent. Confirmation of the diagnosis may be achieved by transendoscopic biopsy of a bronchial mass; however neoplastic tissue may not be present in small mucosal pinch biopsies obtained in this

way. An alternative way to obtain larger tissue biopsies (with a greater chance of achieving a positive diagnosis) is to pass a biopsy instrument (such as uterine biopsy forceps) through a small tracheotomy incision performed at the level of the thoracic inlet (Facemire et al 2000). Although the prognosis for this disease is generally very poor, granular cell tumors may be amenable to surgical treatment by lung lobe resection (Facemire et al 2000). Laser ablation of the intrabronchial mass may be possible, and may result in temporary clinical improvement.

Mesothelioma

Mesotheliomas are rare malignant tumors that arise from the mesothelial lining of the pleura, pericardium, and peritoneum. There have been a limited number of reports of pleural mesothelioma in the horse (Straub et al 1974, Kramer et al 1976, Wallace et al 1987, Colbourne et al 1992, Mair et al 1992, Fry et al 2003). In humans, many cases of mesothelioma have been associated with prior exposure to asbestos dust, but this association has not been recognized in the small number of documented equine cases. The tumor is invariably associated with a pleural effusion, and clinical signs include weight loss and progressive respiratory distress. The presence of a pleural effusion can be confirmed by careful thoracic auscultation and diagnostic ultrasonography. Thoracocentesis yields a modified transudate, and neoplastic cells may be identified in fluid samples (Mair et al 1992); however, distinguishing mesothelioma from mesothelial reactivity on the basis of cytology can be difficult. Thoracoscopy and pleural biopsy may provide a positive diagnosis (Fry et al 2003). The malignant nature of the tumor and its rapid spread preclude any effective treatment, and affected horses invariably require euthanasia.

Metastatic thoracic neoplasia

The lungs, mediastinal/thoracic lymph nodes, and pleural cavity may be affected by neoplasia caused by metastatic spread from a primary site elsewhere in the body. There are many single case studies reporting a wide variety of metastatic thoracic neoplasms; however, renal carcinoma, squamous cell carcinoma (Fig. 43.2), melanoma, fibrosarcoma, and hemangiosarcoma appear to be the most common (Prater et al 1989, Sweeney & Gillette 1989, Mair & Brown 1993, Basher et al 1997, Jorgenson et al 1997, Murray et al 1997, Duncan 1998, East et al 1998, Scarratt & Crissman 1998, McConkey et al 2000).

Pleural and mediastinal neoplasms usually produce a significant volume of malignant pleural effusion. Therefore, presenting clinical signs are likely to reflect this large-volume effusion, such as rapid shallow respiration, tachycardia, weight loss, pectoral edema, and distended jugular

veins. Thoracic auscultation reveals muffled heart and lung sounds in the ventral lung fields, and ultrasonographic examination reveals a large volume of hypoechoic fluid with minimal cellularity and few fibrin tags. Pleural fluid recovered from horses with mediastinal neoplasia will appear straw-colored and translucent to slightly serosanguineous, with the exception of hemangiosarcoma, in which the pleural fluid is often hemorrhagic. Neoplastic effusions typically have low to moderate cellularity (total nucleated cell count from 5×10^9/liter to 40×10^9/liter) characterized by reactive mesothelial cells, lymphocytes and macrophages, and a high total protein (3.5–6.5 mg/dl; 35–65 g/liter). Some mediastinal tumors may be exfoliative, and neoplastic cells can be identified on cytological evaluation (melanoma, squamous cell carcinoma, hemangiosarcoma); however, the absence of neoplastic cells in malignant effusions is not uncommon.

The commonest tumors that metastasize to the lungs include adenocarcinoma (renal, ovarian, thyroid), squamous cell carcinoma (Fig. 43.2), malignant melanoma, hemangiosarcoma, or undifferentiated sarcoma (Sweeney & Gillette 1989, Mair & Brown 1993, Jean et al 1994, Scarrat & Crissman 1998). The clinical features of these tumors are generally non-specific and often relate more to the primary site of tumor formation than to thoracic involvement. Examination of other body systems may therefore reveal evidence of multisystemic infiltration or may identify the primary neoplastic mass. Radiography, ultrasonography, thoracocentesis, and pleuroscopy can all be helpful in the diagnosis of these conditions. Definitive diagnosis of intrapulmonary neoplasia may be determined by cytological evaluation of bronchoalveolar lavage (BAL) fluid or histopathological evaluation of endoscopic or percutaneous lung biopsy. In many cases, however, thoracic involvement by tumor is not confirmed in life, and the diagnosis is made at post-mortem.

Fig. 43.2. Metastatic tumor deposit in the lung as a result of squamous cell carcinoma.

Inhaled Tracheobronchial Foreign Bodies

The commonest forms of foreign body that can lodge in the distal trachea and bronchial tree are thorned twigs or brambles (Urquhart & Gerring 1981, Brown & Collier 1983, Duckett et al 1983). The thorns act as barbs that allow the foreign body to progress distally but prevent it from being coughed up. Clinical signs include a chronic cough and malodorous breath. A mucopurulent or blood-stained nasal discharge may be present.

Diagnosis is confirmed by endoscopic examination. Treatment involves removal of the foreign body, which can often be accomplished using a snare passed through the endoscope. Alternatively a distal cervical tracheotomy may be made to allow insertion of grasping forceps or a snare. The foreign body may break up as it is being removed, and several separate procedures may be necessary to remove all of the foreign material. Broad-spectrum antibiotic therapy should be administered postoperatively. Tracheal foreign bodies are further discussed in Chapter 40.

Pulmonary Edema

The fluid fluxes across the pulmonary vascular endothelium are influenced by the same pressure relationships as in the systemic capillaries. The movement of fluid across the capillary membrane is defined by the Starling–Landis equation:

$$J_v = K_f \left[(P_c - P_i) - \sigma(\pi_p - \pi_i) \right]$$

where:

J_v = net capillary (microvascular) filtration rate
K_f = capillary filtration coefficient
P_c = capillary hydrostatic pressure
P_i = interstitial hydrostatic pressure
σ = osmotic reflection coefficient
π_p = plasma colloid osmotic pressure
π_i = interstitial colloid osmotic pressure.

Thus, fluid flux across the capillary is dependent on the capillary hydrostatic pressure being higher than the interstitial hydrostatic pressure, which drives fluid out of the vessel, and oncotic pressure within the capillary lumen being higher than that in the interstitial fluid, which counteracts the hydrostatic pressure. Colloid osmotic pressure, therefore, opposes the drive of fluid out of the capillary (Magdesian 2003).

The hydrostatic pressure in the pulmonary microvessels exceeds the interstitial hydrostatic pressure. This effect favors microfiltration. The interstitial fluid protein osmotic pressure is approximately two-thirds that in the vessel; thus the net osmotic force is absorptive and inward. The components of this equation make it convenient to categorize abnormal fluid flux into the lung into two broad types (Culver et al 2004):

- Hydrostatic edema – this results when there is a net increase in the difference between the hydrostatic pressure in the microvessels and the hydrostatic pressure in the interstitium.
- Permeability edema – this results when endothelial injury increases fluid conductivity across the membrane and decreases the osmotic reflection coefficient and osmotic gradient.

The terms *cardiogenic* and *non-cardiogenic* are commonly used to describe these two forms of edema formation (Table 43.1).

Fluid flux is sensitive to small intravascular or perivascular pressure changes. Intravascular pressure rises may originate downstream (left heart failure) or may follow overall vascular volume increases (overhydration) or redistribution of blood from the systemic to the pulmonary vessels. Normally, a net outflow of fluid from the upstream capillaries is reabsorbed into the downstream capillaries, where the intravascular pressure is lower. When capillary endothelium is injured, locally or through the effect of circulating mediators, the vascular permeability to fluids and solutes is increased resulting in fluid leakage. The ability to retain large molecules is lost, protein-rich plasma leaks out, and the osmotic pressure in the tissues approaches that in the vessels, so that the osmotic force opposing intravascular hydrostatic pressure is lost. This high-permeability or "leaky capillary" state leads to interstitial edema, which can be a fulminant process leading to severe abnormalities of gas exchange.

The epithelial cells that line the alveoli have tight junctions along their apical surface, so this membrane is normally much less permeable than the endothelial

Table 43.1. Causes of pulmonary edema
Cardiogenic edema Mitral valve rupture Chordae tendineae rupture Myocarditis Ventricular tachycardia

Non-cardiogenic edema
Acute upper respiratory tract obstruction
Acute pulmonary injury
  – acute alveolitis and interstitial disease
  – aspiration – near drowning, inadvertent administration of medications into the lungs
  – smoke inhalation
  – oxygen toxicity
  – infectious agents
  – anaphylaxis
  – idiosyncratic drug reactions
  – endotoxemia and systemic inflammatory response syndrome
  – embolism
  – hypertransfusion

membrane, protecting the alveolar spaces as interstitial edema increases. However, as interstitial edema increases, so a structural failure of the epithelial cells occurs, allowing edema to appear within the alveolar spaces ("alveolar edema"). As fluid accumulates, the alveoli can rapidly become completely filled because of surface tension effects. As a result of these effects, alveoli tend to fill with fluid in an "all-or-none" fashion.

The term "acute respiratory distress syndrome" (ARDS) was introduced into human medicine over 25 years ago (Gattinoni et al 2004). The syndrome consists of acute severe alteration in lung structure and function, characterized by hypoxemia, low respiratory compliance, low functional residual capacity, and diffuse radiographic infiltrates, along with increased lung endothelial and alveolar epithelial permeability. Two interrelated pathways can lead to the development of ARDS, namely direct lung injury and indirect injury (resulting from an acute systemic inflammatory response). Similar mechanisms can lead to pulmonary injury in horses (Table 43.1), with pulmonary edema being an important consequence. Thus, primary lung injury can result from aspiration (including near drowning and iatrogenic administration of fluids or medication into the lungs), inhalation of toxic gases (smoke inhalation), oxygen toxicity or infectious agents. Secondary lung injury may be a sequel to anaphylaxis, endotoxemia and systemic inflammatory response syndrome, embolism, and hypertransfusion (Ainsworth & Hackett 2004). Although

many different insults may lead to ARDS, a final common pathway leads to alveolar damage.

Acute life-threatening pulmonary edema, with or without concomitant pulmonary hemorrhage, may also develop as a consequence of severe upper respiratory tract (URT) obstruction. This complication may further compromise gas exchange, and must be addressed when treating URT obstructions. This complication has multi-factorial pathogenesis (Fig. 43.3), including:

- The excessively subatmospheric pressures which develop within the thorax as the horse attempts to inspire against the URT obstruction alter the capillary trans-mural pressure favoring extravasation of fluid from the pulmonary capillaries into the pulmonary interstitium and air spaces.
- The excessively subatmospheric inspiratory intrathoracic pressure also increases venous return while impairing left ventricular function, resulting in pulmonary hypertension and cardiogenic pulmonary edema.
- The hypoxemia resulting from URT obstruction may cause hypoxic pulmonary vasoconstriction which leads to pulmonary hypertension.
- Sympathetic overstimulation and release of vasoactive substances may increase pulmonary capillary permeability.

Pulmonary edema (Fig. 43.4) results in respiratory failure that clinically presents as respiratory distress. Radiography, ultrasonography, endoscopy, tracheal aspirates
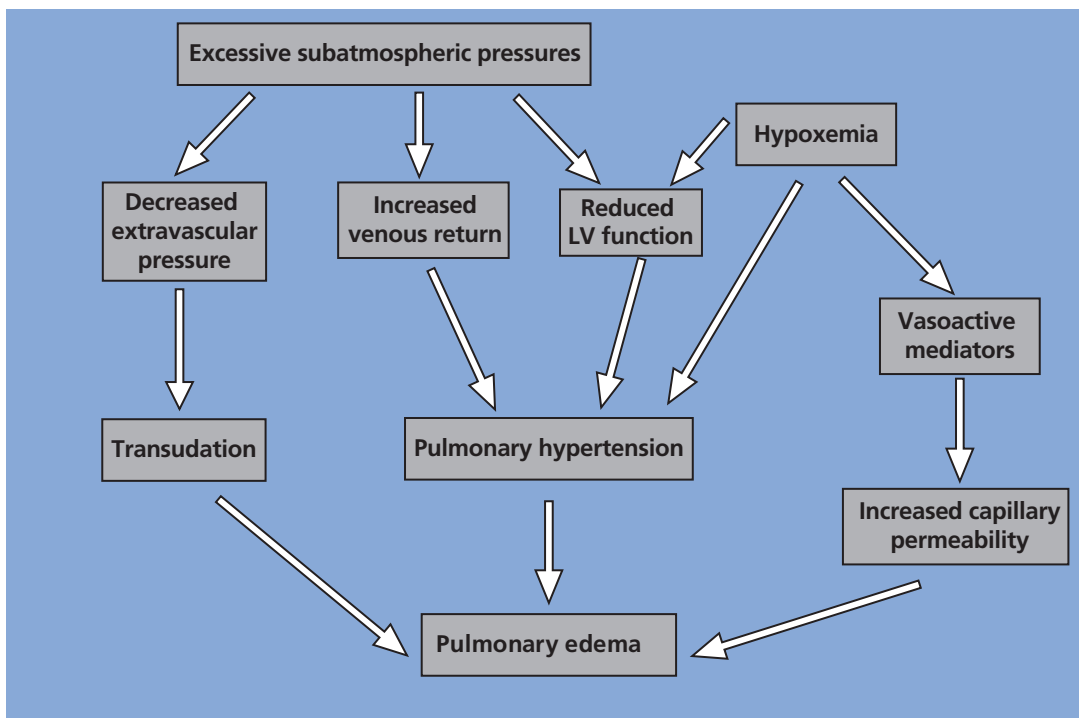
Fig. 43.3. Pathogenesis of pulmonary edema secondary to URT obstruction.
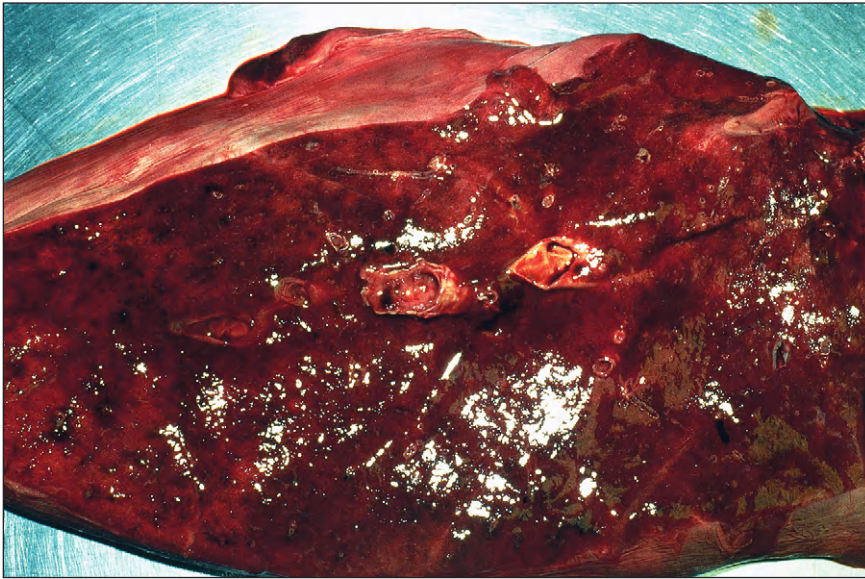
Fig. 43.4. Post-mortem appearance of the lung of a 15-year-old gelding affected by acute respiratory distress syndrome secondary to endotoxemia and gram-negative sepsis. The lungs are diffusely congested and edematous.

or BAL, and arterial blood gas analysis are all helpful in diagnosing and monitoring the disease process. Where possible, differentiation between cardiogenic and non-cardiogenic forms, and diagnosis of the underlying cause should be made. Non-cardiogenic edema should be treated with furosemide, oxygen, and corticosteroids and non-steroidal anti-inflammatory agents (if the pulmonary edema is inflammatory) (Chevalier & Divers 2003). Any underlying systemic inflammatory disease will also need to be treated (including by antibiotics, fluid therapy, and colloid support). URT obstruction should be corrected, such as by temporary tracheostomy. Cardiogenic pulmonary edema is most commonly the result of mitral valve rupture, chordae tendineae rupture, myocarditis or ventricular tachycardia, and should be treated with furosemide [1 mg/kg intravenous (IV)]. Hydralazine (0.5–1.5 mg/kg orally or 0.5 mg/kg IV) can be administered when there is severe mitral regurgitation. Lidocaine (0.25–0.5 mg/kg slowly IV) or $MgSO_4$ (1–2.5 g/450 kg horse/min IV up to 25 g total dose) can be used to treat ventricular tachycardia. Myocarditis should be treated with dexamethasone (0.05–0.22 mg/kg IV).

Alveolitis and Interstitial Pneumonias

The interstitial pneumonias are a poorly defined, heterogeneous group of sporadic respiratory diseases in adult horses. Interstitial disorders of foals are discussed in Chapter 45. The etiology is undetermined in the majority of cases; however, infectious agents, hypersensitivity pneumonitis, plant toxicity, inhaled chemicals, silicosis, and drug reaction have been confirmed to cause interstitial disease in horses (Bruce 1995). *Perilla frutescens* causes

interstitial pneumonia experimentally in ponies (Breeze et al 1984), and croton weed (*Eupatorium adenophorum*) causes chronic interstitial pneumonia in horses in Australia and Hawaii (O'Sullivan 1979).

The pathogenesis of interstitial pneumonia involves four phases: acute alveolitis; proliferation of cellular and connective tissue compartments; irreversible interstitial fibrosis; and irreparable generalized pulmonary fibrosis (Bruce 1995). The clinical signs include weight loss, exercise intolerance, fever, and progressive respiratory distress. Cyanosis may be apparent in the terminal stages. Depending on the stage of disease, most horses with interstitial pneumonia have leukocytosis characterized by mature neutrophilia and hyperfibrinogenemia (Turk et al 1981, Derksen et al 1982, Kelly et al 1995). Pulmonary auscultation reveals crackles and wheezes, although breath sounds may be inaudible in severely affected horses. Interstitial pneumonia is a restrictive pulmonary disease, and affected horses tend to have a prolonged inspiratory phase of respiration and an abbreviated expiratory phase, and they breathe rapidly at low lung volumes. Pulmonary compliance is reduced as a result of pulmonary fibrosis and interstitial inflammation ("stiff lungs"). Pulmonary hypertension and cor pulmonale may occur with end-stage disease.

Cytological evaluation of BAL fluid from most horses with interstitial pneumonia is non-specific and includes high cellularity and chronic, non-septic mixed inflammation (neutrophilia, lymphocytosis, monocytosis) (Derksen et al 1982). Occasional horses may have BAL fluid eosinophilia. Nonetheless, cytological evaluation of BAL fluid may be indicated to rule out potential differential diagnoses including infectious and neoplastic disorders. Bacterial culture of transtracheal aspirates and BAL generally reveals no significant growth (Buergelt et al 1986).
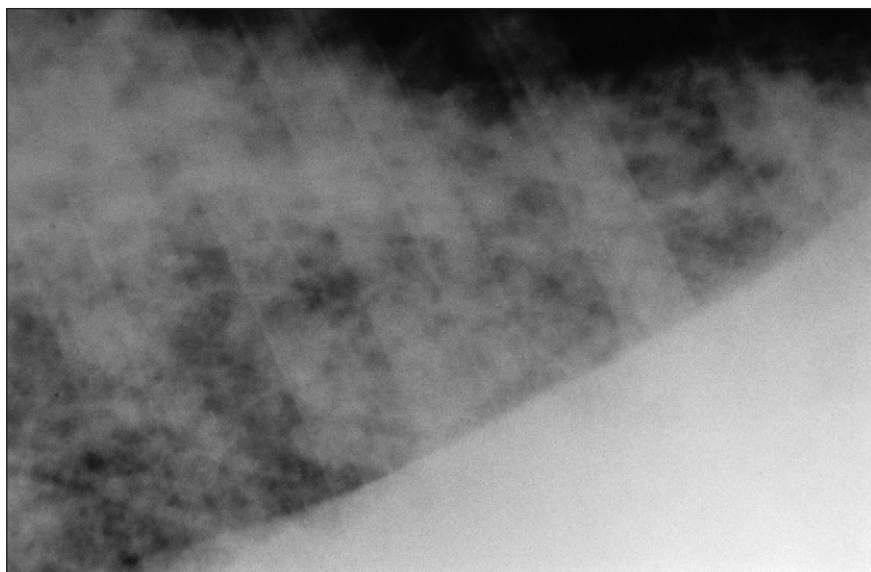
Fig. 43.5. Lateral thoracic radiograph of 8-year-old gelding with chronic progressive respiratory distress. The radiograph demonstrates a nodular/miliary interstitial infiltrate throughout the lungs. Lung biopsy confirmed chronic interstitial pneumonia and fibrosis.
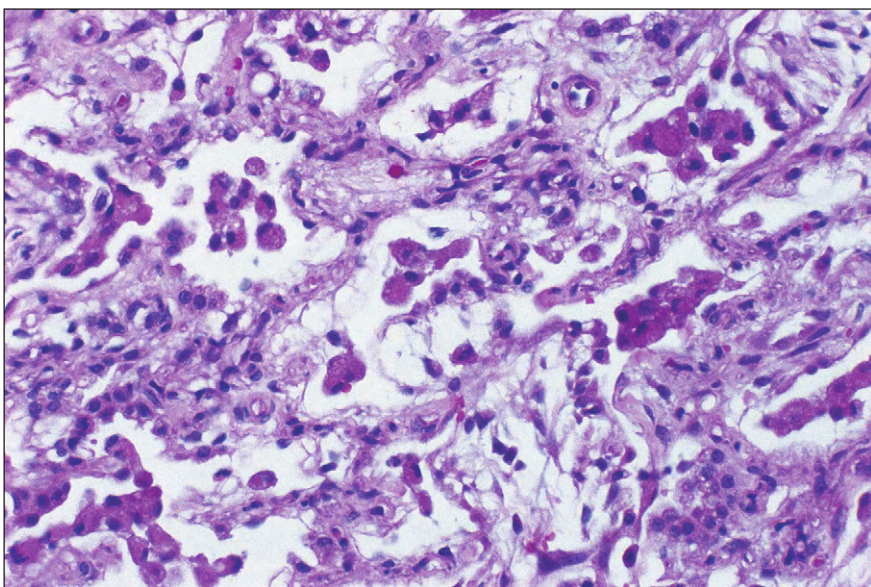
Fig. 43.6. Lung biopsy of an 11-year-old Hackney stallion with a 4-month history of weight loss and progressive respiratory distress. An interstitial infiltrate (similar to that in Fig. 43.5) was found on thoracic radiography. There is fibrosis and thickening of the alveolar walls, with a mixed macrophage and neutrophil infiltrate within the alveolar spaces and interstitium. Hematoxylin and eosin stain.

Thoracic radiography is the most valuable non-invasive tool for diagnosis and monitoring of disease in most instances of interstitial pneumonia. Characteristic radiographic findings include diffuse interstitial infiltration with discrete and diffuse nodularity (Buergelt et al 1986, Berry et al 1991) (Fig. 43.5). The degree of radiographic abnormalities may not correlate with the severity of respiratory compromise, and prognosis should ultimately be determined via histopathological evaluation of a lung biopsy.

Lung biopsy is very helpful to diagnose interstitial pneumonia and determine the extent of pulmonary fibrosis. A percutaneous lung biopsy is recommended (Raphel & Gunson 1981). In the acute phase of disease, there is alveolar septal necrosis, fibrin exudation, and hyaline membrane formation within alveolar spaces. With chronicity, the alveolar walls and supporting stroma become affected, and type II pneumocytes proliferate, producing loss of the functional alveolar–capillary unit (Fig. 43.6). Interstitial inflammation is often granulomatous in the subacute stages of disease and eventually progresses (irreversibly) to pulmonary fibrosis.

Horses with interstitial pneumonia are generally unresponsive to antimicrobial and non-steroidal anti-inflammatory therapy. Treatment with corticosteroids, dimethyl sulfoxide, and furosemide is also ineffective unless instituted early in the disease process (Bruce 1995).

Treatment with corticosteroids and non-steroidal anti-inflammatory therapy should be attempted in horses with acute or subacute interstitial pneumonia based on lung biopsy. The prognosis for return to athletic function is very poor, regardless of the etiology or stage of disease. The prognosis for survival is poor in horses with cyanosis, cor pulmonale, and/or pulmonary fibrosis.

Silicosis

Silicosis is a form of interstitial pneumonia in horses that is caused by inhalation of particulate inorganic silicon dioxide or quartz particles. Human silicosis is a major occupational hazard for those involved in mining, quarrying, metal casting, sandblasting, and the pottery industry. The majority of horses with documented pulmonary silicosis originate from the Monterey-Carmel Peninsula of mid-coastal California (Schwartz et al 1981). Silicon dioxides are cytotoxic to macrophages as well as being fibrogenic. When inhaled, the particles are ingested by alveolar macrophages, causing lysis of the cells, alveolitis, granulomatous change, and subsequent fibrosis. Definitive diagnosis is determined by identification of intracytoplasmic silicate crystals in alveolar macrophages on cytological evaluation of respiratory secretions. Transtracheal aspiration has been used more frequently than BAL to diagnose silicosis in horses; however, serial transtracheal aspirates may be required to identify the characteristic intracellular crystals. Cytological evaluation of BAL fluid has been used for identification of silicate crystals, and is a more sensitive procedure for the diagnosis of pulmonary silicosis. Thoracic radiographic examination reveals a marked interstitial pattern with miliary, reticulonodular, or linear patterns (Berry et al 1991). Lung biopsy or necropsy evaluation is characterized by multifocal, granulomatous pneumonia with areas of pulmonary fibrosis. The prognosis for survival is poor.

Granulomatous Pneumonia

Granulomatous pneumonia is a rare and sporadic disease in horses. Known causes include fungal, bacterial or parasitic agents, silicate pneumoconiosis, disseminated neoplasia, and idiopathic causes (Pusterla et al 2003b). Horses with granulomatous pneumonia usually have a history of weight loss, exercise intolerance, depression, anorexia, fever, tachypnea, cough, nasal discharge, and abnormal lung sounds. Thoracic radiography is particularly useful in helping to differentiate granulomatous pneumonia from other chronic pulmonary diseases. Confirmation of granulomatous change requires evaluation of a lung biopsy.

Some of the underlying diseases that can cause granulomatous pneumonia occur in specific geographical locations, and this can be helpful in reaching a diagnosis (e.g. silicosis and coccidioidomycosis) (Schwartz et al 1981, Ziemer et al 1992). Otherwise, the diagnosis is based on the results of specific tests including the cytological evaluation and culture of tracheal aspirates or BAL fluid, serology, and lung biopsy.

Although tuberculosis is rare in horses, mycobacterial infection should be considered as a potential cause of granulomatous pneumonia. Currently, *Mycobacterium avium* is the commonest form identified in horses. Mycobacterial infections in horses are generally multisystemic, affecting predominantly the lung, cervical vertebrae, and gastrointestinal tract (Mair et al 1986, Buergelt et al 1988). Tissues obtained by lung biopsy should be submitted for specific mycobacterial culture in suspected cases.

Chronic fungal infections, including coccidioidomycosis, histoplasmosis, cryptococcosis, and aspergillosis may be rare causes of chronic granulomatous pneumonia. Coccidioidomycosis appears to be the most commonly reported of these diseases in the USA. This condition is seen in the desert areas of southwestern USA and also in areas of South America. In addition to chronic weight loss and chronic respiratory signs, affected horses may demonstrate musculoskeletal pain, superficial abscessation, intermittent fever, and abdominal pain (Ziemer et al 1992).

Granulomatous pneumonia may occur as part of the generalized equine granulomatous disease syndrome (Pusterla et al 2003b). This is an idiopathic, systemic condition that resembles sarcoidosis in humans. A similar condition has also been reported in horses after the ingestion of hairy vetch (Anderson & Divers 1983). The principal clinical sign in most reported cases of equine idiopathic systemic granulomatous disease is skin lesions (Stannard 1987), but most cases also have lung lesions, as identified by thoracic radiography or post-mortem examination. Typical clinical signs in addition to skin lesions include chronic weight loss, depression, anorexia, tachycardia, tachypnea, and abnormal lung sounds. Thoracic radiographs reveal an interstitial pattern with an underlying miliary or nodular pattern. Hematological data usually reveal anemia, neutrophilia, hyperfibrinogenemia, and hyperglobulinemia (Pusterla et al 2003b). Diagnosis is confirmed by biopsy (skin and lung). Response to systemic corticosteroid therapy is often poor, although some cases can be brought into remission with prolonged treatment.

Development of interstitial pneumonia after intravenous administration of purified mycobacterial cell wall extract (non-specific immunostimulant) has been reported in four horses (Viel & Kenney 1993). The clinical signs included cough, fever, tachypnea, lethargy, and leukocytosis. The pulmonary lesions included progressive, multifocal, granulomatous pneumonitis, bronchiolitis, and pulmonary fibrosis. Thoracic radiographic examination revealed diffuse interstitial infiltrate and cytological examination of BAL fluid

revealed lymphocytic inflammation. A marked local reaction can be elicited by intradermal injection of mycobacterial cell wall extract in affected horses.

Pulmonary Fibrosis

Pulmonary fibrosis represents the end stage of a number of different interstitial lung diseases (Fig. 43.7). It is likely that all of the various forms of alveolitis, interstitial pneumonia, and granulomatous pneumonia could progress eventually to pulmonary fibrosis. In humans, the term "idiopathic pulmonary fibrosis" (also known as cryptogenic fibrosing alveolitis) is used to describe all patients with pulmonary fibrosis of unknown cause, regardless of differences in histological patterns (Costabel & Britton 2004). On occasion, horses with similar clinical and pathological signs are identified.

The term "idiopathic pulmonary fibrosis" should only be used in horses with chronic interstitial fibrosis (confirmed by lung biopsy or post-mortem examination) where the disease is limited to the lung, and where other known causes of interstitial lung disease have been excluded.

The clinical features generally include chronic and progressive respiratory failure, often of insidious onset. Tachypnea and inspiratory dyspnea are present, with or without a mild cough. Since the disease causes a restrictive defect, affected horses tend to have a prolonged inspiratory phase of respiration and an abbreviated expiratory phase, and they breathe rapidly at low lung volumes. Chronic weight loss and general depression are present. Thoracic radiographs reveal a diffuse interstitial pattern, and lung biopsy confirms interstitial fibrosis. Treatment with corticosteroids may be attempted but the prognosis is generally hopeless.

Idiopathic pulmonary fibrosis appears to be commoner in the donkey than the horse. Chronic fibrosis of the subpleural and interstitial lung tissues is commonly found at post-mortem examination of donkeys, often as an incidental finding. The etiology is unknown. Most affected donkeys remain bright and alert, with a normal appetite. The sedentary nature of most donkeys means that pulmonary fibrosis may be advanced before clinical signs are obvious. Early clinical signs may include tachypnea and occasional coughing. An episode of acute, severe respiratory distress with dyspnea and tachypnea may also be recognized. Following resolution of this acute respiratory distress, chronic and progressive dyspnea develops. Occasional coughing is sometimes observed, and a nasal discharge may be present in some cases. Stertor may arise if there is concomitant tracheal collapse. There is no known effective treatment.

Smoke Inhalation

Smoke inhalation produces edema, congestion, and necrosis of both the upper and lower respiratory tract as a result of both thermal and chemical injury (Trunkey 1978). Upper respiratory tract injury primarily results from thermal injury as a result of inhalation of hot air and particles. Thermal damage to the lower respiratory tract is less common but can occur with the inhalation of superheated particles ($< 5\,\mu$m). The majority of lower respiratory tract damage results from the inhalation of toxic chemicals. Highly water-soluble gases, such as aldehydes, ammonia, chlorine, hydrogen chloride, and sulfur dioxide, produce rapid pulmonary injury, whereas insoluble gases, such as nitrogen and phosgene oxide, cause delayed pulmonary injury. Carbon monoxide and cyanide toxicities,

Fig. 43.7. Post-mortem appearance of the lung of a 9-year-old thoroughbred mare with pulmonary fibrosis. The normal lung tissue is largely replaced by firm, pale fibrous tissue.

common with smoke inhalation, do not cause direct airway damage, but exacerbate tissue hypoxia by impairing oxygen delivery and utilization. Carbon monoxide toxicity is a common cause of mortality associated with fire (Cahalane & Demling 1984). Carbon monoxide has a greater affinity than oxygen for hemoglobin, causing the formation of carboxyhemoglobin, tissue hypoxia, and death. Cyanide is inhaled in the form of hydrogen cyanide, commonly in the fumes from burning plastic. The clinical features of cyanide toxicity are those related to severe hypoxia and metabolic acidosis, causing cardiovascular collapse, respiratory arrest, and coma.

Clinical signs of respiratory injury are often immediate; however, some horses may not exhibit signs for 2 to 3 days and should remain under close observation for at least 72 h (Geor & Ames 1991).

There are three stages of smoke inhalation injury, namely:

- acute pulmonary insufficiency
- pulmonary edema
- bronchopneumonia.

Acute pulmonary insufficiency occurs during the first 36 h, and is attributed to thermal injury to the upper respiratory tract (6–18 h after exposure), carbon monoxide and cyanide toxicities (immediate), and chemical injury to the tracheobronchial tree and lung parenchyma. Thermal injury to the upper respiratory tract peaks at 18–24 h after exposure, and may result in upper airway edema to the point of obstruction necessitating an emergency tracheotomy. Carbon monoxide toxicity is difficult to detect clinically because the presence of carboxyhemoglobin in the blood gives it a bright red color; therefore, the clinician must anticipate carbon monoxide toxicity in smoke inhalation patients. Irritation from soluble gases causes bronchoconstriction, mucosal edema of central airways, and degradation of pulmonary surfactant.

The second stage of inhalation injury begins at approximately 24 h, and is characterized by pulmonary edema, which develops as a result of the inflammatory response initiated by chemical injury. Clinical signs include tachypnea, dyspnea, paroxysmal coughing, and nasal discharge. In instances of severe mucosal injury, a pseudomembranous cast, consisting of cellular debris, fibrin, and proteinaceous exudate, may form in large to medium-sized airways at 24–72 h. Necrotizing bronchiolitis, intra-alveolar hemorrhage, thrombus formation, pneumothorax, and massive pulmonary edema are potential sequelae. The presence of subcutaneous emphysema indicates pneumothorax and/or pneumomediastinum as the result of ruptured pulmonary bullae.

The third stage of smoke inhalation injury is bronchopneumonia, which may develop as early as 5–7 days after exposure. However the development of bacterial infection may occur up to 2 weeks after exposure. The primary predisposing factor is impaired pulmonary defense mechanisms including loss of mucociliary clearance, impaired alveolar macrophage function, and denuded respiratory epithelium. Clinical signs include continued cough and nasal discharge and development of fever and depression.

Endoscopic examination of the upper and lower respiratory tract is the most sensitive indicator of smoke inhalation injury. Thoracic radiography may be useful to monitor the progression of pulmonary edema and consolidation, detect the development of bronchopneumonia, and identify pneumothorax or pneumomediastinum. Thoracic radiography may also identify pulmonary fibrosis in patients that have suffered progressive, severe inhalation injury several months after recovery. Serial blood gas analysis can be performed to monitor respiratory function and carboxyhemoglobin concentrations should be determined to detect carbon monoxide toxicity. Transtracheal wash may be performed during the bronchopneumonia stage of smoke inhalation injury to identify opportunistic pathogens.

Oxygen therapy is indicated in horses with smoke inhalation injury to facilitate the removal of carbon monoxide and improve pulmonary gas exchange (Kemper et al 1993). Fractional concentrations of 40% oxygen can be achieved with flow rates of 50–100 ml/kg. Administration of >50% oxygen for more than 24 h may exacerbate pulmonary damage. Tracheostomy may be necessary in horses with severe injury/edema of the upper airway. Indication for tracheostomy is determined by the presence of a loud inspiratory stridor. In addition to providing a patent airway, tracheostomy may facilitate the removal of fibrinous casts from the lower respiratory tract.

Bronchodilator therapy should be administered as early as possible (stage 1 bronchoconstriction) to horses with smoke inhalation injury. Aerosolized albuterol is the most powerful, rapid-acting bronchodilator and should be paired with a long-acting aerosolized (salmeterol) or oral (clenbuterol) β_2-agonist. The majority of bronchoconstriction associated with smoke inhalation injury is vagally mediated, so addition of a parasympatholytic agent is valuable. Atropine can be administered on an emergency, single-dose basis and aerosolized ipratropium can be used for subsequent maintenance therapy.

Furosemide (1–2 mg/kg) will reduce pulmonary edema via diuretic and non-diuretic mechanisms and is indicated upon the development of clinical signs of pulmonary edema. Fluid, electrolyte, and acid–base balance should be monitored when furosemide is administered repeatedly.

Corticosteroid therapy has been used successfully in horses with smoke inhalation injury to combat pulmonary edema and maintain surfactant production. However, the immunosuppressive effects of corticosteroids may unnecessarily predispose patients to bacterial bronchopneumonia and studies in humans and animals with smoke
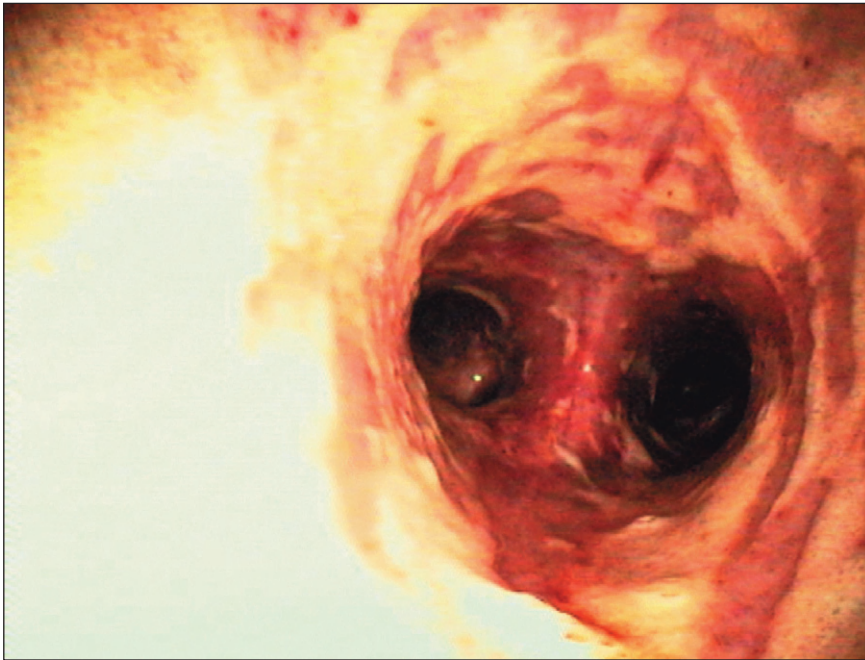
Fig. 43.8. Endoscopic appearance of the distal trachea and main-stem bronchi of an 8-year-old pony gelding with aspiration bronchopneumonia following an episode of esophageal obstruction (choke). The airways are inflamed with exudate and food material.

inhalation have failed to identify the therapeutic benefit of corticosteroid administration. Corticosteroids appear to be particularly detrimental in patients with surface burns, and therefore their use should be limited to patients with smoke inhalation injury alone. Dimethyl sulfoxide has demonstrated therapeutic benefit in some smoke inhalation patients and is thought to reduce inflammation and edema via free-radical scavenging. Non-steroidal anti-inflammatory drugs may be an alternative to corticosteroids for the reduction of pulmonary inflammation.

The use of intravenous fluid therapy in patients with smoke inhalation is controversial. Clinicians must strike a careful balance between maintaining adequate plasma volume for the shocked patient and exacerbating pulmonary edema, during phases 1 and 2 of injury. Intravenous fluid therapy is now considered an essential component of treatment in human patients with severe pulmonary injury and cardiovascular shock, and intravenous fluid therapy has been successfully used in horses with severe smoke inhalation injury.

Prophylactic antibiotic therapy is particularly controversial. Indiscriminate prophylactic use of antibiotics in human patients leads to the selection of resistant strains of bacteria and has not reduced mortality in burn patients. Nonetheless, most equine clinicians consider administration of prophylactic antibiotics to be standard care in equine patients with severe pulmonary injury, and particularly in cases requiring tracheostomy.

The long-term prognosis for horses with smoke inhalation injury is uncertain. Some horses appear to recover uneventfully without detectable evidence of pulmonary impairment, whereas other horses suffer from chronic exercise intolerance because of pulmonary fibrosis and bronchoconstriction.

Aspiration Pneumonia

Horses are unusual in that they tolerate the entry of foreign material into the trachea without it inducing the severe cough response seen in most other species. This poorly developed protective response to aspiration may predispose the horse to aspiration pneumonia.

Many diseases that result in pharyngeal and esophageal dysphagia can result in the aspiration of food and saliva into the trachea and lower respiratory tract (Fig. 43.8). Examples of such conditions include esophageal obstruction (choke) and pharyngeal paralysis/paresis secondary to guttural pouch mycosis. In addition, aspiration of food and saliva can arise following treatment of recurrent laryngeal neuropathy by laryngeal prosthesis surgery.

The nature of the pulmonary injury occurring after aspiration of foreign material depends on the nature of the material. Thus the aspiration of gastric contents causes severe injury resulting in pulmonary edema and hemorrhagic pneumonia (Epstein 1980). Inadvertent deposition of mineral oil into the lungs can occur when a nasogastric tube is misplaced, or if the oil is administered as an oral drench. Mineral oil induces a chronic and progressive granulomatous pneumonia that has a poor long-term prognosis (Scarratt et al 1998, Bos et al 2002).

In most cases, aspiration into the lungs causes pulmonary infection and the development of pneumonia

and/or lung abscesses. The bacterial flora of the oropharynx is mixed, and a variety of organisms can be responsible for the pulmonary infection. Anaerobic infections are common. The cranioventral portions of the lungs are predominantly affected by aspiration pneumonia. Clinical signs are similar to those seen in other forms of bacterial pneumonia (fever, tachypnea, dyspnea, nasal discharge, coughing, inappetence, weight loss, exercise intolerance, etc.). Malodorous breath is common in cases of anaerobic infection, abscess formation or lung necrosis. Diagnosis is achieved by a combination of the history, clinical signs, endoscopy (identification of mucopurulent exudate with or without traces of blood in the lower airways), clinical pathology (leukocytosis, neutrophilia, hyperfibrinogenemia, hyperglobulinemia), thoracic radiography (radiodensity in the cranioventral lung fields), ultrasonography (consolidation of ventral lung lobes with possible pleural effusion), and cytology of tracheobronchial aspirates. Culture of aspirates should include incubation under both aerobic and anaerobic conditions.

Treatment of aspiration pneumonia depends on preventing further aspiration, and treating the pneumonia as for the more common forms of pneumonia and pleuropneumonia in the adult horse (see Chapter 46).

Drowning and Near Drowning

Drowning and near drowning are very rare in horses, although several cases have been reported (Austin et al 1988, Humber 1988). Submersion in water can lead to asphyxiation caused by laryngeal obstruction from glottal spasm. Aspiration of water into the lower airways leads to dilution of the surfactant levels and resultant atelectasis and hypoxemia. If salt water is aspirated, the hypertonic fluid draws fluid from the pulmonary interstitium and circulation, leading to alveolar edema. Aspiration pneumonia may be a sequel to near drowning, and can be detected by auscultation, thoracic radiography, and cytological evaluation of tracheal aspirates.

The immediate treatment of near drowning should include oxygen therapy (if practical). Arterial blood gas analysis is helpful in determining the need for oxygen and in monitoring the course of the condition. Bronchodilators may be helpful to counteract bronchospasm. The use of diuretics such as furosemide is controversial, but may be helpful to reduce pulmonary edema. Broad-spectrum antimicrobials (such as penicillin, gentamicin, and metronidazole) are indicated if aspiration pneumonia is diagnosed, and should be considered for prophylaxis against infection even if the infection is not established. Pulmonary surfactant therapy might also be considered.

The prognosis for near drowning depends on the duration of immersion, the water temperature, the amount and type of fluid aspirated, whether foreign material and/or infectious agents are aspirated, the general health and immune status of the horse, and how soon appropriate treatment is instituted (Beech 1991).

Eosinophilic Pneumonia

Eosinophilic pneumonia (eosinophilic pneumonitis) is an uncommon syndrome characterized by (a typically interstitial) eosinophilic infiltrate into the lungs. The term probably encompasses a group of diverse pulmonary diseases that all result in eosinophilia in BAL fluid and eosinophil infiltrates in the lung tissues. There may or may not be peripheral blood eosinophilia.

The clinical signs associated with eosinophilic pneumonia are similar to many other pulmonary diseases, including chronic coughing, purulent nasal discharge, and dyspnea.

The cause of eosinophilic pneumonia is frequently difficult to establish, although allergic, fungal, and parasitic causes must all be considered.

Parasitic diseases

Infection by the lungworm *Dictyocaulus arnfieldi* can result in eosinophilic bronchopneumonia. Lungworm infestation should be suspected in cases of a "herd outbreak" of heaves in horses with historical exposure to donkeys and/or donkey crosses (Klei 1986). Mules, asses, and donkeys are the natural hosts of *D. arnfieldi*, but they rarely develop clinical evidence of infestation. Coughing is a prominent feature of the disease and clinical evidence of lower airway obstruction (expiratory difficulty) is common. Clinical signs of disease are typically observed in late summer and early fall in geographic areas with cold winter weather. Horses with lungworm infestation may have peripheral eosinophilia, and eosinophils are the predominant inflammatory cell type in BAL fluid or transtracheal aspirates (MacKay & Urquhart 1979). Eosinophilic BAL fluid is not pathognomonic for lungworm infections; however, identification of eosinophilia should prompt the clinician to determine exposure to donkeys and mules and perform a Baermann fecal examination on the patient and potential reservoir hosts. Larvae may be observed in respiratory secretions from horses with lungworms and provide definitive evidence of infestation. Adult horses rarely develop a patent infection (2%), so negative findings on Baermann fecal examination do not preclude a diagnosis of lungworms. Foals may develop a patent lungworm infection, in the absence of clinical signs. Ivermectin (200 µg/kg *per os*) is effective against both mature and immature stages of the parasite and is the drug of choice for treatment of donkeys and horses with lungworm infection (Britt & Preston 1985).

Several nematode parasites can infect the lung. These include the migrating larval stages of *Parascaris equorum*

and *Strongyloides westeri*. Aberrant migration of other parasites, such as *Strongylus vulgaris* and *Habronema* sp., can also occur. Although such parasitic migration is often asymptomatic or is associated with very mild signs of respiratory disease (intermittent cough, mild nasal discharge), it may play an important role in the development of secondary bacterial pneumonia in foals and yearlings. Pulmonary migration of *Parascaris equorum* occurs 7–14 days after larval ingestion, and can induce inflammatory changes characterized by eosinophilic and lymphoid infiltrates. These inflammatory reactions may represent an allergic response to parasitic antigens. Between 10 and 12 weeks are required for detection of a patent *Parascaris equorum* infection via fecal flotation tests. Peripheral blood eosinophilia may be noted in foals with heavy parasite loads and may reflect parasitic migration.

Hydatid cysts represent a stage of the metacestode *Echinococcus granulosus*, the tapeworm of domestic carnivores. Horses and donkeys can become infected when grazing on pastures that are contaminated by the feces of dogs and foxes. The horse acts as an intermediate host in which hydatid cysts containing fertile protoscolices develop, usually in the liver and lungs. The cysts can reach 6–7 cm in diameter, but are usually asymptomatic (identified at post-mortem examination). However, in large numbers, cysts can affect lung and liver function, and rupture of the pulmonary or pleural hydatid cyst may result in pleural effusion. Ante-mortem diagnosis can be achieved by diagnostic ultrasonography and laparoscopy, or by thoracic radiography.

Other causes of eosinophilic pneumonia

Eosinophilic pneumonia can be one manifestation of the group of conditions generally referred to as inflammatory airway diseases (IAD) (Rush et al 1995, Hoffman et al 1998). Diagnosis is achieved by cytological examination of BAL samples. Clinical signs observed in horses with eosinophilic BAL fluid are indistinguishable from those in other IAD-affected horses. Eosinophilic BAL fluid is associated with poor exercise performance, high total nucleated BAL fluid cell counts, airway hyperresponsiveness, and may or may not be accompanied by systemic eosinophilia (Hare & Viel 1998).

The pathophysiology of pulmonary eosinophilic inflammation likely represents an immune-mediated pulmonary response consistent with a type I hypersensitivity reaction. Affected horses typically have a moderate to strong interstitial pattern on thoracic radiography, and eosinophilic pulmonary granulomas are often identified at post-mortem examination of horses with pulmonary eosinophilia. If eosinophilic BAL fluid is identified, the clinician should investigate parasitic pulmonary disease, including ascarid migration and lungworm infection, in addition to hypersensitivity pneumonitis. Aerosolized corticosteroid adminis-

tration is recommended for horses with eosinophilic BAL fluid; however, this treatment has not yet been investigated in a controlled, randomized clinical trial in racehorses. Eosinophilic pneumonitis tends to respond slowly and incompletely to immunosuppressive therapy.

Hemorrhagic Pneumonia (Acute Hemorrhagic Pulmonary Infarction and Necrotizing Pneumonia)

Acute hemorrhagic pneumonia represents a form/type of bacterial pleuropneumonia in which there is evidence of hemorrhagic pulmonary infarction, necrotizing pneumonia, and pulmonary thromboembolism (Carr et al 1997). These horses respond poorly to treatment compared to horses with other forms of bacterial pleuropneumonia.

Affected horses present with an acute or peracute onset of severe respiratory distress with a bilateral serosanguineous or hemorrhagic nasal discharge. Thoracic radiography and ultrasonography reveal evidence of severe pulmonary consolidation and pleural effusion. A serosanguineous suppurative pleural effusion is identified by thoracocentesis. Treatment should include the same therapies as for other cases of bacterial pleuropneumonia, but the prognosis for recovery is poor.

At post-mortem examination, there is a sharp line of demarcation between grossly normal and grossly abnormal lung, which is palpably firm and dark red to black on the cut surface. Thrombosis of large vessels supplying the affected areas is frequently found. Necrotic areas surrounded by a fibrous capsule or pulmonary abscesses are characteristic of more chronic lesions.

REFERENCES

Ainsworth DM, Hackett RP 2004 Disorders of the respiratory system. In: Reed SM, Bayly WM, Sellon DC (editors) Equine Internal Medicine, 2nd edn. Saunders, St Louis, pp.289–353

Alexander JE, Keown GH, Palotay JL 1965 Granular cell myoblastoma with hypertrophic pulmonary osteoarthropathy in a mare. Journal of the American Veterinary Medical Association 146: 703–708

Anderson CA, Divers TJ 1983 Systemic granulomatous inflammation in a horse grazing hairy vetch. Journal of the American Veterinary Medical Association 183: 569–570

Austin SM, Foreman JH, Goetz TE 1988 Aspiration pneumonia following near drowning in a mare: a case report. Equine Veterinary Science 8: 313–316

Baker JR, Leyland A 1975 Histological survey of tumours of the horse with particular reference to those of the skin. Veterinary Record 96: 419–422

Basher AWP, Severin GA, Chavkin MJ et al 1997 Orbital neuroendocrine tumours in three horses. Journal of the American Veterinary Medical Association 210: 668–671

Beech J 1991 Miscellaneous lung and pleural injuries. In: Beech J (editor) Equine Respiratory Disorders. Lea & Febiger, Philadelphia, pp.213–222

Berry CR, O'Brien TR, Madigan JE et al 1991 Thoracic radiographic features of silicosis in 19 horses. Journal of Veterinary Internal Medicine 5: 248–256

Bos M, De Bosschere H, Deprez P et al 2002 Chemical identification of the (causative) lipids in a case of exogenous lipoid pneumonia in a horse. Equine Veterinary Journal 34: 744–747

Breeze R G, Laegreid W W, Bayly W M et al 1984 Perilla ketone toxicity: a chemical model for the study of equine restrictive lung disease Equine Veterinary Journal 16: 180–184

Britt DP, Preston JM 1985 Efficacy of ivermectin against *Dictyocaulus arnfieldi* in ponies. Veterinary Record 116: 343–345

Brown CM, Collier MA 1983 Tracheobronchial foreign body in a horse. Journal of the American Veterinary Medical Association 182: 280–281

Bruce EH 1995 Interstitial pneumonia in horses. Compendium on Continuing Education for the Practicing Veterinarian 17: 1145–1153

Buergelt CD, Hines SA, Cantor G et al 1986 A retrospective study of proliferative interstitial lung disease of horses in Florida. Veterinary Pathology 23: 750–756

Buergelt CD, Green SL, Mayhew IG et al 1988 Avian mycobacteriosis in three horses. Cornell Veterinarian 78: 365–380

Cahalane M, Demling RH 1984 Early respiratory abnormalities from smoke inhalation. Journal of the American Veterinary Medical Association 251: 771–773

Carr EA, Carlson GP, Wilson WD et al 1997 Acute hemorrhagic pulmonary infarction and necrotizing pneumonia in horses: 21 cases (1967–1993). Journal of the American Veterinary Medical Association 210: 1774–1778

Chevalier H, Divers TJ 2003 Pulmonary dysfunction in adult horses in the intensive care unit. Clinical Techniques in Equine Practice 2: 165–177

Clem MF, O'Brien TD, Christopher MM et al 1986 Pulmonary chondrosarcoma in a horse. Compendium on Continuing Education for the Practicing Veterinarian 8: S964–969

Colbourne CM, Bolton JR, Mills JN et al 1992 Mesothelioma in horses. Veterinary Pathology 14: 513–515

Costabel U, Britton JR 2004 Idiopathic pulmonary fibrosis and other idiopathic interstitial pneumonias. In Albert RK, Spiro SG, Jett JR (editors) Clinical Respiratory Medicine, 2nd edn. Mosby, Philadelphia, pp.551–558

Cotchin E, Baker-Smith J 1975 Tumours in horses encountered in an abattoir survey. Veterinary Record 97: 339

Culver, BH, Sevransky J, Brower R 2004 Pulmonary circulation. In Albert RK, Spiro SG, Jett JR (editors) Clinical Respiratory Medicine, 2nd edn. Mosby, Philadelphia, pp.85–98

Derksen FJ, Slocombe RF, Brown CM et al 1982 Chronic restrictive pulmonary disease in a horse. Journal of the American Veterinary Medical Association 181: 887–889

Dill S, Moise NS, Meschter CL 1986 Cardiac failure in a stallion secondary to metastasis of an anaplastic pulmonary carcinoma. Equine Veterinary Journal 18: 414–417

Duckett WM, Baum JL, Cook W 1983 Bronchial foreign body in a horse. Equine Practice 5: 8

Duncan RB 1998 Malignant sertoli cell tumour in a horse. Equine Veterinary Journal 30: 355–357

East LM, Steyn PE, Dickinson CE, Frank AA 1998 Occult osseous metastasis of a colonic adenocarcinoma visualized with technetium Tc[99m] hydroxymethylene diphosphate scintigraphy in a horse. Journal of the American Veterinary Medical Association 213: 1167–1170

Epstein PE 1980 Aspiration diseases of the lungs. In Fishman AP (editor) Pleural Diseases and Disorders. McGraw-Hill, New York pp.1327–1340

Facemire PR, Chilcoat CD, Sojka J et al 2000 Treatment of granular cell tumor via complete right lung resection in a horse. Journal of the American Veterinary Medical Association 217: 1522–1525

Ford TS, Vaala WE, Sweeney CR et al 1987 Pleuroscopic diagnosis of gastroesophageal squamous cell carcinoma in a horse. Journal of the American Veterinary Medical Association 190: 1556–1558

Fry MM, Magdesian KG, Judy CE et al 2003 Antemortem diagnosis of equine mesothelioma by pleural biopsy. Equine Veterinary Journal 35: 723–727

Garber JL, Reef VB, Reimer JM 1994 Sonographic findings in horses with mediastinal lymphosarcoma: 13 cases (1985–1992). Journal of the American Veterinary Medical Association 205: 1432–1436

Gattinoni L, Pelosi P, Brazzi L, Valenza F 2004 Acute respiratory distress syndrome. In Albert RK, Spiro SG, Jett JR (editors) Clinical Respiratory Medicine, 2nd edn. Mosby, Philadelphia, pp.743–758

Geor RJ, Ames TR 1991 Smoke inhalation injury in horses. Compendium on Continuing Education for the Practicing Veterinarian 13: 1162–1169.

Hare JE, Viel L 1998 Pulmonary eosinophilia associated with increased airway responsiveness in young racing horses. Journal of Veterinary Internal Medicine 12: 163–170

Hoffman AM, Mazan RM, Ellenberg BS 1998 Association between bronchoalveolar lavage cytologic features and airway reactivity in horses with a history of exercise intolerance. American Journal of Veterinary Research 59: 176–181

Humber KA 1988 Near drowning of a gelding. Journal of the American Veterinary Medical Association 192: 377–378

Jean D, Lavoie JP, Nunez L, Legace A et al 1994 Cutaneous hemangiosarcoma with pulmonary metastasis in a horse. Journal of the American Veterinary Medical Association 60: 776–777

Jorgensen JS, Geoly FJ, Berry CR et al 1997 Lameness and pleural effusion associated with an aggressive fibrosarcoma in a horse. Journal of the American Veterinary Medical Association 210: 1328–1331

Kelly DF, Newsholme SJ, Baker JR et al 1995 Diffuse alveolar damage in the horse. Equine Veterinary Journal 27: 76–78

Kemper T, Spier S, Barratt-Boyes DM et al 1993 Treatment of smoke inhalation in five horses. Journal of the American Veterinary Medical Association 202: 91–94

Klei TR 1986 Other parasites. Recent advances. Veterinary Clinics of North America; Equine Practice 2: 329–336

Kramer JW, Nickels FA, Bell T 1976 Cytology of diffuse mesothelioma in the thorax in the horse. Equine Veterinary Journal 8: 81–83

MacKay RJ, Urquhart KA 1979 An outbreak of eosinophilic bronchitis in horses possibly associated with *Dictyocaulus arnfieldi* infection. Equine Veterinary Journal 11: 110–112

Magdesian KG 2003 Colloid replacement in the ICU. Clinical Techniques in Equine Practice 2: 130–137

Mair TS, Hillyer MH 1992 Clinical features of lymphosarcoma in the horse: 77 cases. Equine Veterinary Education 4: 108–113

Mair TS, Brown PJ 1993 Clinical and pathological features of thoracic neoplasia in the horse. Equine Veterinary Journal 25: 220–223

Mair TS, Lane JG, Lucke VM 1985 Clinicopathological features of lymphosarcoma involving the thoracic cavity in the horse. Equine Veterinary Journal 17: 428–433

Mair TS, Taylor FGR, Gibbs C et al 1986 Generalized avian tuberculosis in a horse. Equine Veterinary Journal 18: 226–230

Mair TS, Hillyer, MH, Brown PJ 1992 Mesothelioma of the pleural cavity in a horse: diagnostic features. Equine Veterinary Education 4: 59–61

Mair TS, Dyson SJ, Fraser JA et al 1996 Hypertrophic osteopathy (Marie's disease) in Equidae: a review of twenty-four cases. Equine Veterinary Journal 28: 256–262

McConkey S, Lopez A, Pringle J 2000 Extramedullary plasmacytoma in a horse with ptyalism and dysphagia. Journal of Veterinary Diagnostic Investigation 12: 282–284

Misdorp W, van Gelder NHL 1968 Granular cell myoblastoma in the horse. A report of 4 cases. Pathologia Veterinaria 5: 385–394

Mueller POE, Morris DD, Carmichael P et al 1992 Antemortem diagnosis of cholangiocellular carcinoma in a horse. Journal of the American Veterinary Medical Association 201: 899–901

Murphy JR, Breeze RG, McPherson EA 1978 Myxoma of the equine respiratory tract. Modern Veterinary Practice 59: 529–532

Murray MJ, Cavey DM, Feldman BF et al 1997 Signs of sympathetic denervation associated with a thoracic melanoma in a horse. Journal of Veterinary Internal Medicine 11: 199–203

Nickels FA, Brown CM, Breeze RG 1980 Equine granular cell tumour. Modern Veterinary Practice 61: 593–596

O'Sullivan BM 1979 Croton weed (*Eupatorium adenophorum*) toxicity in horses. Australian Veterinary Journal 55: 19–21

Parker GA, Novilla MN, Brown AC et al 1979 Granular cell tumour (myoblastoma) in the lung of a horse. Journal of Comparative Pathology 89: 421–430

Prater PE, Patton CS, Held JP 1989 Pleural effusion resulting from malignant hepatoblastoma in a horse. Journal of the American Veterinary Medical Association 194: 383–385

Pusterla N, Norris AJ, Stacy BA et al 2003a Granular cell tumours in the lungs of three horses. Veterinary Record 153: 530–532

Pusterla N, Pesavento PA, Smith P et al 2003b Idiopathic granulomatous pneumonia in seven horses. Veterinary Record 153: 653–655

Raphel CF, Gunson DE 1981 Percutaneous lung biopsy in the horse. Cornell Veterinarian 71: 439–448

Rossier Y, Sweeney CR, Heyer G et al 1990 Pleuroscopic diagnosis of hemangiosarcoma in a horse. Journal of the American Veterinary Medical Association 196: 1639–1640

Rush BR, Krakowka S, Robertson JT 1995 Cytologic evaluation of bronchoalveolar lavage fluid obtained from Standardbred racehorses with inflammatory airway disease. American Journal of Veterinary Research 56: 562–568

Scarratt WK, Crissman MV 1998 Neoplasia of the respiratory tract. Veterinary Clinics of North America; Equine Practice 14: 451–474

Scarratt WK, Moon ML, Sponenberg DP et al 1998 Inappropriate administration of mineral oil resulting in lipoid pneumonia in three horses. Equine Veterinary Journal 30: 85–88

Schultze AE, Sonea I, Bell TG 1988 Primary malignant pulmonary neoplasia in two horses. Journal of the American Veterinary Medical Association 193: 477–480

Schwartz LW, Knight HD, Whittig LD et al 1981 Silicate pneumoconiosis and pulmonary fibrosis in horses from the Monterey-Carmel peninsula. Chest 80: 82S–85S

Stannard AA 1987 Generalised granulomatous disease. In: Robinson NE (editor) Current Therapy in Equine Medicine, 2nd edn. WB Saunders, Philadelphia, pp.645–646

Straub R, von Tscharner C, Pauli B et al 1974 Pleural mesothelioma in a horse. Schweizer Archiv fur Tierheilkunde 116: 207–211

Sullivan DJ 1960 Cartilaginous tumors (chondroma and chondrosarcoma) in animals. American Journal of Veterinary Research 21: 531–535

Sundberg JP, Burnstein T, Page EH et al 1977 Neoplasms of equidae. Journal of the American Veterinary Medical Association 170: 150–152

Sutton RH, Coleman GT 1995 A pulmonary granular cell tumour with associated hypertrophic osteopathy in a horse. New Zealand Veterinary Journal 43: 123

Sweeney CR, Gillette DM 1989 Thoracic neoplasia in equids: 35 cases (1967–1987). Journal of the American Veterinary Medical Association 195: 374–377

Trunkey DD 1978 Inhalation injury. Surgical Clinics of North America 58: 1133–1140

Turk JR, Brown CM, Johnson GC 1981 Diffuse alveolar damage with fibrosing alveolitis in a horse. Veterinary Pathology 18: 560–561

Turk MA, Breeze RG 1981 Histochemical and ultrastructural features of an equine pulmonary granular cell tumour (myoblastoma). Journal of Comparative Pathology 91: 471–481

Uphoff CS, Lyncoln JA 1987 A primary pulmonary tumour in a horse. Equine Practice 9: 19–20

Urquhart KA, Gerring EL 1981 Tracheobronchial foreign body in a pony. Equine Veterinary Journal 13: 262–264

Vachon AM, Fischer AT 1998 Thoracoscopy in the horse: diagnostic and therapeutic indications in 28 cases. Equine Veterinary Journal 30: 467–475

Van Rensburg IBJ, Stadler P, Soley J 1989 Bronchiolo-alveolar adenocarcinoma in a horse. Journal of the South African Veterinary Association 60: 212–214

Viel L, Kenney D 1993 Suspected adverse pulmonary reactions to Equimune I.V. in four horses presented to the Ontario Veterinary College. 12th Veterinary Respiratory Symposium. Kennett Square, PA.

Wallace SS, Jayo MJ, Maddux JM et al 1987 Mesothelioma in a horse. Compendium on Continuing Education for Practicing Veterinarians 9: 210–216

Ziemer EL, Pappagianis D, Madigan JE et al 1992 Coccidioidomycosis in horses: 15 cases (1975–1984). Journal of the American Veterinary Medical Association 201: 910–916

44 Exercise-induced Pulmonary Hemorrhage

Kenneth W Hinchcliff

Exercise-induced pulmonary hemorrhage (EIPH) occurs in horses throughout the world and does not appear to have any geographic distribution. It is a disorder of horses that run at high speed, such as thoroughbred or standardbred racehorses. The disorder is uncommon in endurance horses or draft breeds, although it does occur in horses used for these activities. As a general rule, the more intense the exercise or higher the speed attained, the greater the proportion of horses with EIPH.

The prevalence of EIPH varies with the method used to detect it and the frequency with which horses are examined, as discussed later in this section. Almost all thoroughbred racehorses in active training have hemosiderophages in bronchoalveolar lavage fluid, indicating that all have some degree of EIPH (McKane et al 1993). The prevalence of EIPH decreases when diagnosis is based on endoscopic examination of horses after exercise or racing.

EIPH is very common in thoroughbred racehorses; estimates of prevalence based on a single endoscopic examination of the trachea and bronchi are 43–75% (Pascoe et al 1981a, Raphel & Soma 1982, Mason et al 1983). The prevalence increases with the frequency of examination, with over 80% of horses having evidence of EIPH on at least one occasion following examination after each of three consecutive races (Sweeney et al 1990). The prevalence of EIPH in standardbred racehorses is assumed to be lower, with 26–34% of horses reported to have blood in the trachea after racing (Speirs 1982, MacNamara et al 1990). However, these studies were based on a single examination and one study (MacNamara et al 1990) only reported as positive those horses with blood covering more than one half of the tracheobronchial tree. When examined after each of three races, 87% of standardbred racehorses have evidence of EIPH on at least one occasion (Lapointe et al 1994), suggesting that EIPH is as common in standardbred racehorses as it is in thoroughbred racehorses.

EIPH occurs in approximately 62% of racing Quarter horses, and has been observed in Quarter horses used for barrel racing (Hillidge et al 1984). The disorder also occurs in racing Appaloosa horses (Hillidge et al 1986) and approximately 11% of polo ponies are affected with EIPH (Voynick & Sweeney 1986).

Age is considered an important risk factor for EIPH with the prevalence of the disorder being higher in older horses (Pascoe 1981, Raphel & Soma 1982, Mason et al 1983). There is no consistent association of sex with prevalence of EIPH (Pascoe et al 1981a, Raphel & Soma 1982, Speirs 1982, Mason et al 1983).

Among thoroughbred racehorses the prevalence of EIPH increases with increasing speed (Raphel & Soma 1982, Oikawa 1999), being greater in thoroughbreds after racing than after breezing (galloping). Lesions of EIPH are not detected in young thoroughbred racehorses that have trained at speeds less than 7 m/second (Raphel & Soma 1982, Oikawa 1999).

Epistaxis associated with exercise is almost always attributable to EIPH and occurs only in a small proportion of racehorses (Takahashi et al 2001, Williams et al 2001, Weideman et al 2003). The prevalence of epistaxis in racehorses varies between 0.1 and 9.0%, with the frequency depending on the breed, age and sex of horses selected for study, the type of racing, and the timing and frequency of observation of horses after racing. Epistaxis is more common in older horses (Takahashi et al 2001, Weideman et al 2003). There are conflicting reports of a sex predisposition although epistaxis may be more common in female thoroughbreds (Takahashi et al 2001, Weideman et al 2003). Epistaxis is more common after races of < 1600 m than after longer races (Takahashi et al 2001), although not all sources agree on this point (Weideman et al 2003). However, horses in steeplechase races, which are typically longer than 2000 m, are at greater risk of epistaxis than are horses in flat races (Takahashi et al 2001).

Horses that have experienced one episode of epistaxis are more likely to have a second episode. For this reason some racing jurisdictions do not permit horses with epistaxis to race for a period of weeks to months after the initial episode, with more prolonged enforced rest after a subsequent episode of epistaxis and retirement from racing after a third bout. The recurrence rate after one episode of epistaxis in thoroughbred horses is approximately 13.5%, despite affected horses not being permitted to race for 1 month after the initial episode of epistaxis (Takahashi et al 2001). This high rate of recurrence suggests that the inciting pulmonary lesions have not resolved.

Table 44.1. Causes of hemorrhage into airways of horses or epistaxis
Hemorrhage into trachea or bronchi
Exercise-induced pulmonary hemorrhage
Pulmonary abscess
Trauma
Pneumonia
Pulmonary foreign body
Pulmonary neoplasia
Epistaxis
All of the above
Guttural pouch mycosis
Progressive ethmoidal hematoma
Thrombocytopenia
Trauma
Neoplasia

B

Fig. 44.1. Diagrammatic representation of the proximate cause of exercise-induced pulmonary hemorrhage. (A) A normal pulmonary capillary–alveolar unit; (B) rupture of the capillary endothelium with subsequent extravasation of red blood cells and plasma into the interstitial space, rupture of alveolar epithelium and leakage of red blood cells into the alveolus and air spaces.

Pathophysiology and Etiology

Epistaxis and hemorrhage into airways can occur as a result of a number of diseases (Table 44.1). However, the likely proximate cause of EIPH is rupture of alveolar capillary membranes with subsequent extravasation of blood into interstitial and alveolar spaces (Fig. 44.1) (West et al 1993). The source of blood in such instances is the pulmonary circulation. Bleeding from the bronchial circulation during exercise has been suggested, based on histological evidence of bronchial angiogenesis in horses that have experienced previous episodes of EIPH (Pascoe 1996), but contribution of the bronchial circulation to EIPH has not been demonstrated. Regardless of the contribution of bronchial circulation to blood in the airways, the likely initial lesion is in capillaries associated with the pulmonary circulation. Hemorrhage into the interstitial space and alveoli, with subsequent rostral movement of blood in the airways, results in blood in the trachea and bronchi.

Rupture of alveolar capillaries occurs secondary to an exercise-induced increase in transmural pressure (pressure difference between the inside of the capillary and the alveolar lumen). If the transmural stress exceeds the tensile strength of the capillary wall, the capillary ruptures (West & Mathieu-Costello 1994). The proximate cause of alveolar capillary rupture is the high transmural pressure generated by positive intracapillary pressures that are largely attributable to capillary blood pressure, and the lower intra-alveolar pressure generated by the subatmospheric pleural pressures associated with inspiration.

During exercise, the absolute magnitudes of both pulmonary capillary pressure and alveolar pressure increase, with a consequent increase in transmural pressure. Strenuous exercise is associated with marked increases in pulmonary artery pressure in horses (Manohar et al 1993, Birks et al 1997, Langsetmo et al 2000). Values for mean pulmonary arterial pressure at rest of 20–25 mmHg

increase to > 90 mmHg during intense exercise because of the large cardiac output achieved by exercising horses. The increases in pulmonary artery pressure, combined with an increase in left atrial pressure during exercise, likely result in an increase in pulmonary capillary pressure. Combined with the increase in pulmonary capillary pressure is a marked decrease (more negative) in pleural, and therefore alveolar, pressures during exercise. Pleural pressures of normal horses during inspiration decrease from

Table 44.2. Potential factors inducing or contributing to the severity of exercise-induced pulmonary hemorrhage

Pulmonary capillary hypertension
Rheologic properties of blood
Subatmospheric intrapleural (alveolar) pressures
Extrathoracic airway obstruction (e.g. laryngeal hemiplegia)
Intrathoracic airway obstruction (e.g. bronchoconstriction)
Small airway inflammatory disease
    viral or bacterial infections
    allergy
    air pollution (dust, ozone)
Coagulopathy
    abnormal platelet function
    capillary fragility
Bronchial neovascularization
Pulmonary fibrosis and altered compliance
Locomotory forces
    foot strike
    abdominal piston

approximately -0.7 kPa (-5.3 mmHg) at rest to as low as -8.5 kPa (-64 mmHg) during strenuous exercise (Art et al 1990). Together, the increases in pulmonary capillary pressure and the decrease (more negative) in intrapleural (alveolar) pressure contribute to a marked increase in stress in the alveolar wall. Although the alveolar wall and pulmonary capillary of horses are stronger than those of other species, rupture may occur because the wall stress in the alveolus exceeds the mechanical strength of the capillary (Birks et al 1994).

Other factors that could contribute to the pathogenesis of EIPH are listed in Table 44.2 and include small airway disease, upper airway obstruction, hemostatic abnormalities, changes in blood viscosity and erythrocyte shape, intrathoracic shear forces associated with gait, and bronchial artery angiogenesis (Pascoe 1996, Schroter et al 1998). It is likely that the pathogenesis of EIPH involves several processes, including pulmonary hypertension, lower alveolar pressure, and changes in lung structure, that summate to induce stress failure of pulmonary capillaries.

Obstruction of either the upper or lower airways has been proposed as a cause of EIPH (Cook 1988). Inspiratory airway obstruction results in more negative intrapleural, and therefore alveolar, pressures. This effect is exacerbated by exercise with the result that alveolar transmural pressure is greater in horses with airway obstructions during racing (Ducharme et al 1999, Hackett et al 1999). The higher transmural pressure in such horses may increase the severity of EIPH, although this has not been demonstrated. Moreover, while inspiratory airway obstruction may predispose to EIPH, the prevalence of this condition is much less than that of EIPH, indicating that it is not the sole factor inducing EIPH in most horses.

Horses with moderate to severe EIPH have histological evidence of inflammation of the small airways (O'Callaghan et al 1987d, Oikawa 1999), and there is a clear association between presence of EIPH and inflammatory changes in bronchoalveolar or tracheal aspirate fluid (Newton & Wood 2002). However, instillation of autologous blood into the airways induces a marked inflammatory response in normal horses (McKane & Slocombe 1999), and it is therefore unclear if inflammation alone induces or predisposes to EIPH or if the inflammation is a result of EIPH. Theoretically, small airway inflammation and bronchoconstriction have the potential to produce intrathoracic airway obstruction and, therefore, a more negative alveolar pressure. Given that small airway disease is common in horses, there is the potential for an important effect of factors such as viral infections, air pollution and allergic airway disease to contribute to the initiation or propagation of EIPH (Deaton & Marlin 2004).

Exercise is accompanied by marked changes in blood flow characteristics attributable to an increase in hematocrit and a decrease in red cell deformability (Fedde & Erickson 1998, Weiss & Smith 1998). These changes cause an increase in microvascular shear stress and thus could, conceivably, contribute to capillary rupture. However, there is at present no direct evidence that indicates that this is an important feature of EIPH development.

The characteristic location of lesions of EIPH in the caudodorsal lung fields has led to the proposal that hemorrhage is a result of tissue damage occurring when waves of stress, generated by forelimb foot strike, are focused and amplified into the narrowing cross-sectional area of the caudal lung lobes (Schroter et al 1998). According to this theory, the locomotory impact of the forelimbs results in transmission of forces through the scapula to the body wall, from where they pass into the lungs caudally and dorsally. As the wave of pressure passes into the narrower caudodorsal regions of the lungs it generates progressively greater shearing forces that disrupt tissue and cause EIPH. However, studies of intrapleural pressures have not demonstrated the presence of a systemic pressure wave passing through the lung and do not provide support for this hypothesis (Jones et al 2002).

Horses with EIPH have been suspected of having defects in either hemostasis or fibrinolysis. However, while exercise induces substantial changes in blood coagulation and fibrinolysis (McKeever et al 1990), there is no evidence that horses with EIPH have defective coagulation or increased fibrinolysis (Bayly et al 1983, Johnstone et al 1991).

Regardless of the cause, rupture of pulmonary capillaries and subsequent hemorrhage into airways and interstitium causes inflammation of both airways and interstitium with subsequent development of fibrosis and alteration of tissue compliance (Fig. 44.2). Heterogeneity of compliance within the lungs, and particularly at the junction of normal and diseased tissue, results in
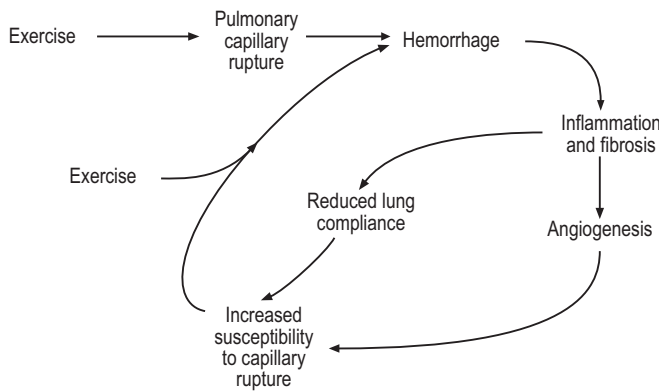
Fig. 44.2. Schematic of proposed mechanism of perpetuation and exacerbation of EIPH with continued exercise. See text for details.

Fig. 44.3. Horse with exercised-induced pulmonary hemorrhage and epistaxis.

development of abnormal shear stress with subsequent tissue damage. These changes are exacerbated by inflammation and obstruction of small airways with resulting uneven inflation of the lungs (Robinson & Derksen 1980). The structural abnormalities, combined with pulmonary hypertension and the large intrathoracic forces associated with respiration during strenuous exercise, cause repetitive damage at the boundary of normal and diseased tissue with further hemorrhage and inflammation. The process, once started, is lifelong and continues for as long as the horse continues to perform strenuous exercise (Pascoe 1996).

Clinical Signs

History and presenting complaint

Poor athletic performance or epistaxis are the most common presenting complaints for horses with EIPH. While poor performance may be attributable to any of a large number of causes, epistaxis associated with exercise is almost always secondary to EIPH (Fig. 44.3).

   Epistaxis as the result of EIPH occurs during or shortly after exercise and is usually first noticed at the end of a race, particularly when the horse is returned to the paddock or winner's circle and is allowed to lower its head. It is usually bilateral and resolves within hours of the end of the race. Epistaxis may occur on more than one occasion, especially when horses are raced or exercised at high speed soon after an initial episode.

EIPH and performance

Failure of racehorses to perform to the expected standard (poor performance) is often, accurately or not, attributed to EIPH. Many horses with poor performance have cytological evidence of EIPH on microscopic examination of tracheobronchial aspirates or bronchoalveolar lavage fluid or

have blood evident on endoscopic examination of the tracheobronchial tree performed 30–90 min after strenuous exercise or racing (McKane et al 1993, Martin et al 1999). However, it is important to recognize that EIPH is very common in racehorses and it should be considered the cause of poor performance only after other causes have been eliminated. Severe EIPH undoubtedly results in poor performance and, on rare occasions, death of thoroughbred racehorses (Gunson et al 1988). However, the effect of less severe EIPH on the race performance of thoroughbred or standardbred horses has not been conclusively determined. A relationship between finishing position and incidence of EIPH, diagnosed by bronchoscopic examination, was not detected for 191 thoroughbred racehorses that finished in first, second, or third place (Pascoe et al 1981b). Furthermore, there was no relationship between the proportion of horses with EIPH and placing (first, second or third versus other) in another 98 horses (Pascoe et al 1981b). Similarly, there was no relationship between finishing position and proportion of horses with EIPH in 191 thoroughbreds examined after racing (Raphel & Soma 1982). There was no relationship between severity of EIPH, assessed on tracheobronchoscopic examination, and race performance in 258 thoroughbreds or 296 standardbred racehorses (Birks et al 2002). Together, these studies do not demonstrate a clear relationship between the presence of EIPH, or its severity, and race performance.

In contrast to the studies discussed above, among 452 thoroughbred horses examined after racing in Hong Kong, those finishing in the first three positions had less severe EIPH than did horses finishing in lower positions (Mason et al 1983). Of horses finishing in the first three places, 43.9% had evidence of EIPH on tracheobronchoscopic examination after racing whereas 55.9% of horses finishing in fourth to 14th place had evidence of EIPH. Thoroughbred horses with EIPH racing in Victoria, Australia have impaired performance compared to unaffected horses. Affected horses have lower likelihood of finishing in the first three places, are less likely to be elite money earners and finish further behind the winner than do unaffected horses (Hinchcliff et al 2004).

Results of studies in standardbred racehorses indicate either a lack of effect of EIPH on performance or an association between EIPH and superior performance. There was no relationship between presence of EIPH and finishing position in 29 standardbred racehorses with intermittent EIPH examined on at least two occasions (Lapointe et al 1994), nor in 92 standardbred racehorses examined on one occasion (Speirs 1982). However, of 965 standardbred racehorses examined after racing, those finishing first or second were 1.4 times more likely (95% CI 0.9–2.2) to have evidence of EIPH on tracheobronchoscopic examination than were horses that finished in seventh or eighth position (Rohrbach 1990).

Physical Examination

Apart from epistaxis in a small proportion of affected horses, there are few abnormalities detectable on routine physical examination of horses with EIPH. Rectal temperature and heart and breathing rates may be elevated as a consequence of exercise in horses examined soon after exercise, but values of these variables in horses with EIPH at rest are not noticeably different from those in horses with no evidence of EIPH. Affected horses may swallow more frequently during recovery from exercise than do unaffected horses, probably as a result of blood in the larynx and pharynx. Coughing is common in horses recovering from strenuous exercise; after recovery from exercise, horses with EIPH are no more likely to cough than are unaffected horses (Christley et al 2001). Other clinical signs related to respiratory abnormalities are uncommon in horses with EIPH. Respiratory distress is rare in horses with EIPH and when present indicates severe hemorrhage or other serious lung disease such as pneumonia, pneumothorax or rupture of a pulmonary abscess. Lung sounds are abnormal in a small number of EIPH-affected horses and when present are characterized by increased intensity of normal breath sounds during rebreathing examination (O'Callaghan et al 1987a). Tracheal crackles may be present in horses with EIPH but are also heard in unaffected horses (Cook 1974).

Diagnostic Tests

There are a variety of techniques available for determining the presence and severity of EIPH including direct examination of the airways through a flexible endoscope or examination of bronchial lavage fluid or tracheal aspirates for evidence of hemorrhage. The utility of these diagnostic tests varies and choice of examination technique depends on the time between the horse racing and the examination and the desired sensitivity of the test. For instance, tracheobronchoscopic examination is most appropriate if a horse is examined within 1–2 h of exercise whereas examination of airway washings is most appropriate if the examination is days to a week after strenuous exercise. Radiography, pulmonary scintigraphic examination, and lung function tests are useful in eliminating other respiratory diseases as a cause of poor performance, but are minimally useful in confirming a diagnosis of EIPH or in determining the severity of hemorrhage.

Tracheobronchoscopy

Observation of blood in the trachea or large bronchi of horses 30–120 min after racing or strenuous exercise provides a definitive diagnosis of EIPH. The amount of blood in the large airways varies from a few small specks on the airway walls to a stream of blood occupying the ventral one-third of the trachea (Fig. 44.4). Blood may also be present in the larynx and nasopharynx. If there is a strong suspicion of EIPH and blood is not present on a single examination conducted soon after exercise, the examination should be repeated 60–90 min later. Some horses with EIPH do not have blood present in the rostral airways immediately after exercise, but do have blood present when examined 1–2 h later. Blood is detectable by tracheobronchoscopic examination for 1–3 days in most horses, with some horses having blood detectable for up to 7 days.

Bronchoscopic examination can be used to estimate the severity of EIPH through a grading system (Pascoe et al 1981b, Hinchcliff et al 2005). The interobserver repeatability of tracheobronchoscopic assessment of severity of EIPH using a 0–4 grading scale has been demonstrated (Hinchcliff et al 2004b) (Fig. 44.4).

- grade 0 – no blood detected in the pharynx, larynx, trachea or main-stem bronchi
- grade 1 – presence of one or more flecks of blood or ≤ 2 short (less than one-quarter the length of the trachea) narrow (< 10% of the tracheal surface area) streams of blood in the trachea or main-stem bronchi visible from the tracheal bifurcation
- grade 2 – one long stream of blood (more than half the length of the trachea) or > 2 short streams occupying less than one-third of the tracheal circumference
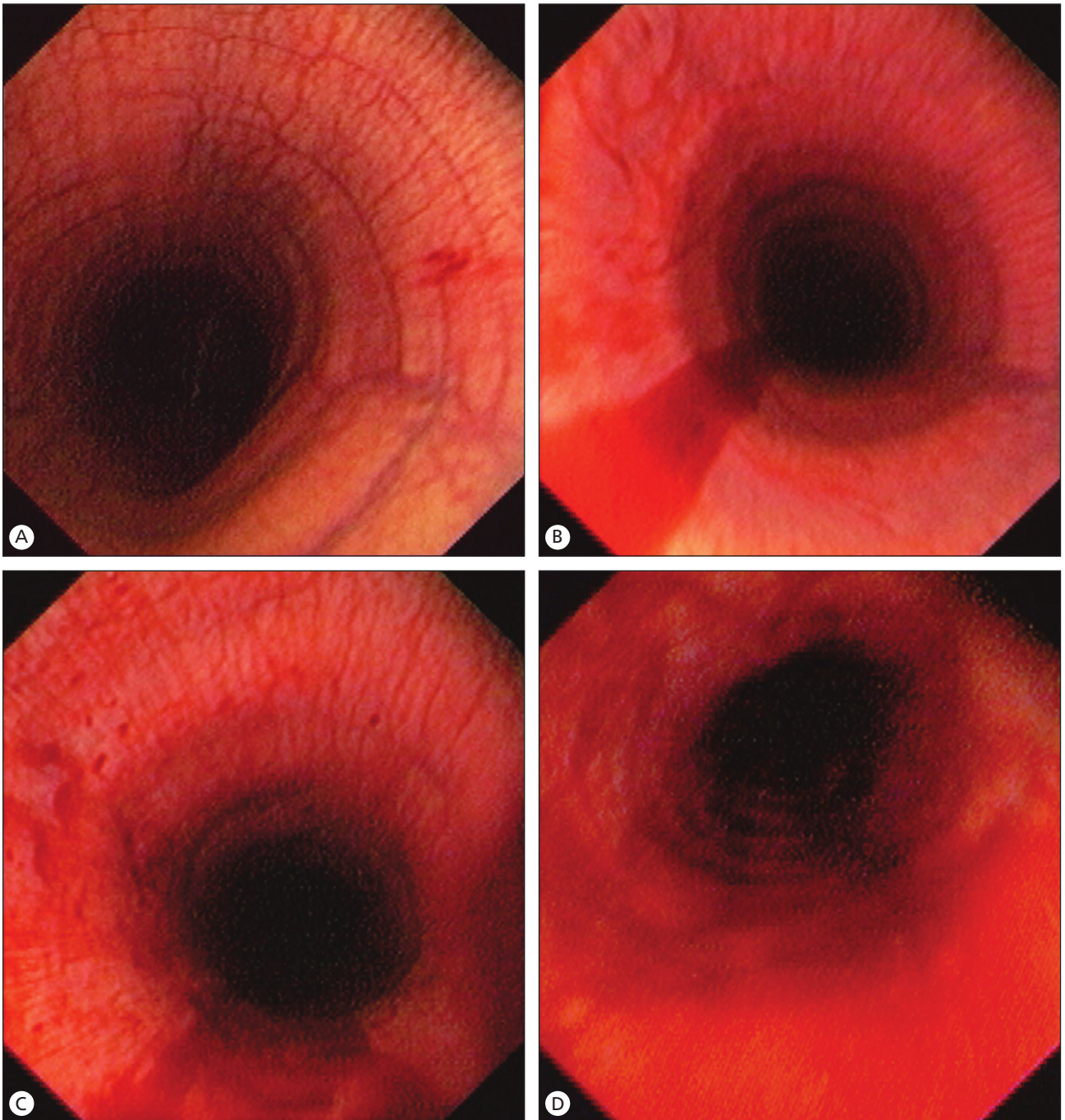
Fig. 44.4. Tracheobronchoscopic findings in horses with EIPH. (A) A horse with Grade 1 hemorrhage; (B) a horse with Grade 2 hemorrhage; (C) a horse with Grade 3 hemorrhage; (D) a horse with Grade 4 hemorrhage. Reproduced from Hinchcliff et al, 2005, Fig. 1, with permission.

- grade 3 – multiple, distinct streams of blood covering more than one-third of the tracheal circumference; no blood pooling at the thoracic inlet
- grade 4 – multiple, coalescing streams of blood covering >90% of the tracheal surface with pooling of blood at the thoracic inlet.

It is assumed that a higher score represents more severe hemorrhage, but while the repeatability of this scoring system has been established, the relationship between the amount of blood in the large airways and the actual amount of hemorrhage has not been established.

Examination of airway secretions or lavage fluid

The presence of red cells or macrophages containing either effete red cells or the breakdown products of hemoglobin (hemosiderophages) in tracheal aspirates or broncho-alveolar lavage fluid provides evidence of EIPH. Detection of red cells or hemosiderophages in tracheal aspirates or bronchoalveolar lavage fluid is believed to be both sensitive and specific in the diagnosis of EIPH (Fogarty & Buckley 1991, McKane et al 1993). Examination of airway fluids indicates the presence of EIPH in a greater proportion of horses than does tracheobronchoscopic examination after strenuous exercise or racing. The greater sensitivity of examination of airway fluid is likely attributable to the ability of this examination to detect the presence of small amounts of blood or its residual products and the longevity of these products, particularly the latter, in the airways. While endoscopic examination may detect blood in occasional horses up to 7 days after an episode of EIPH, cellular evidence of pulmonary hemorrhage persists for weeks after a single episode (Fogarty & Buckley 1991, Step et al 1991, McKane et al 1993). Red blood cells and macrophages containing red cells are present in bronchoalveolar lavage fluid or tracheal aspirates for at least 1 week after strenuous exercise or instillation of autologous blood into airways and hemosiderophages are present for at least 21 days and possibly longer (Step et al 1991, McKane et al 1993).

Recent studies have reported the use of red cell numbers in bronchoalveolar lavage fluid as a quantitative indicator of EIPH (Meyer et al 1998, Langsetmo et al 2000, Geor et al 2001, Kindig et al 2001). However, this indicator of EIPH severity has not been validated nor demonstrated to be more reliable or repeatable than tracheobronchoscopic examination and visual scoring. Furthermore, considerable concern exists over the suitability of red cell counts in bronchoalveolar lavage fluid for the assessment of EIPH severity given that an unknown area, although presumably small, of the lung is examined by lavage and there is a risk that this area of lung may not be representative of the lung as a whole, similar to the situation for examination of bronchoalveolar lavage fluid of horses with pneumonia (Rossier et al 1991). Bronchoalveolar lavage of selected segments of both lungs, achieved using an endoscope, may obviate some of these concerns.

Radiography

Thoracic radiography is of limited use in detecting horses with EIPH. Radiographs may demonstrate the presence of densities in the caudodorsal lung fields of some horses but many affected horses have minimal to undetectable radiographic abnormalities (Pascoe et al 1983) (Figure 10.19). Examination of thoracic radiographs of horses with EIPH may be useful in ruling out the presence of

another disease process, such as a pulmonary abscess, contributing to the horse's pulmonary hemorrhage or poor athletic performance.

Necropsy Examination

Exercise-induced pulmonary hemorrhage is a rare cause of death of racehorses. Necropsy examination of affected horses is usually incidental to examination for another cause of death. Pertinent abnormalities in horses with EIPH are restricted to the respiratory tract. Grossly, horses examined within hours of strenuous exercise, such as horses examined because of catastrophic musculoskeletal injuries incurred during racing, may have severe petechiation in the caudodorsal lung fields. Horses with chronic disease have blue/gray or blue/brown discoloration of the visceral pleural surfaces of the caudodorsal lung fields that is often sharply demarcated, especially on the diaphragmatic surface (Fig. 44.5) (O'Callaghan et al 1987b). The discoloration affects both lungs equally, with 30–50% of

Fig. 44.5. Lungs of a thoroughbred racehorse with exercise-induced pulmonary hemorrhage. The lesions are restricted to the caudodorsal lung fields and cause a blue–gray discoloration of the visceral pleural surface.

the lung fields being discolored in severe cases. Affected areas do not collapse to the same extent as unaffected areas and, in the deflated lung, have a spleen-like consistency. On cut surface, the discolored areas of lung are predominantly contiguous with the dorsal pleural surface and extend ventrally into the lung parenchyma. Areas of affected lung may be separated by normal lung. There is proliferation of bronchial vessels, predominantly arteries and arterioles, in affected areas (O'Callaghan et al 1987c). Histologically, affected areas exhibit bronchiolitis, hemosiderophages in the alveolar lumen and interstitial spaces, and fibrosis of interlobular septa, pleura and around vessels and bronchioles (O'Callaghan et al 1987d).

Treatment

Therapy of EIPH is usually a combination of attempts to reduce the severity of subsequent hemorrhage and efforts to minimize the effect of recent hemorrhage. Treatment of EIPH is problematic for a number of reasons. Firstly, the pathogenesis of EIPH has not been determined although the available evidence supports a role for stress failure of pulmonary capillaries secondary to exercise-induced pulmonary hypertension (see above). Secondly, there is a lack of information using large numbers of horses under field conditions that demonstrates an effect of any medication or management practice (with the exception of bedding) on EIPH. There are numerous studies of small numbers of horses (<40) under experimental conditions, but these studies often lacked the statistical power to detect treatment effects and, furthermore, the relevance of studies conducted on a treadmill to horses racing competitively is questionable. Treatments for EIPH are usually intended to address a specific aspect of the pathogenesis of the disease and will be discussed in that context.

Prevention of stress failure of the pulmonary capillaries

There is interest in reducing the pressure difference across the pulmonary capillary membrane in an effort to reduce EIPH. Theoretically, this can be achieved by reducing the pressure within the capillary or increasing (making less subatmospheric) the pressure within the intrathoracic airways and alveolus.

Reducing pulmonary capillary pressure

Furosemide administration as prophylaxis of EIPH is permitted in a number of racing jurisdictions worldwide, most notably Canada, USA, Mexico, and most South American countries (Anonymous 2002). Within the USA and Canada, almost all thoroughbred, standardbred and Quarter horse racing jurisdictions permit administration

of furosemide before racing. The vast majority (>90%) of thoroughbred horses racing in the USA receive furosemide before racing at an estimated annual cost of between $6,000,000 and $20,000,000 (Heller 2002). Although accurate numbers are not available, it appears that a smaller proportion of standardbred and Quarter horse racehorses receive furosemide before racing. Furosemide is administered to 22–32% of standardbred racehorses and 19% of racing Quarter horses in two racing jurisdictions (Sime et al 1994, Soma et al 1996, 2000).

The efficacy of furosemide in treatment of EIPH is uncertain. While field studies of large numbers of horses do not demonstrate an effect of furosemide on the prevalence of EIPH (Sweeney et al 1990, Birks et al 2002), studies of thoroughbred horses running on a treadmill provide evidence that furosemide reduces the severity of EIPH (Geor et al 2001, Kindig et al 2001, McDonough et al 2004). Under field conditions, based on tracheobronchoscopic evaluation of the severity of bleeding, furosemide has been reported to reduce or have no influence on the severity of bleeding (Pascoe et al 1985, Birks et al 2002). This apparent inconsistency may be attributable to measurement of red blood cell counts in bronchoalveolar lavage fluid of horses that have run on a treadmill not being representative of effects of furosemide under field conditions. The weight of evidence, albeit unconvincing, from field studies does not support a role for furosemide in preventing or reducing the severity of EIPH.

The mechanism by which furosemide may reduce the severity of EIPH is unknown although it is speculated that furosemide, by attenuating the exercise-induced increase in pulmonary artery and pulmonary capillary pressure of horses, reduces the frequency or severity of pulmonary capillary rupture (Manohar 1993, Manohar et al 1993, Gleed et al 1999).

Furosemide administration is associated with superior performance in both thoroughbred and standardbred racehorses (Gross et al 1999, Soma et al 2000). Thoroughbred horses treated with furosemide are 1.4 times as likely to win the race, earn more money and have a standardized 6-furlong race time 0.56–1.09 seconds less than untreated horses (Gross et al 1999). Similarly, furosemide reduces the 1-mile race times of standardbred pacers by 0.31–0.74 seconds (Soma et al 2000).

Enalapril inhibits angiotensin-converting enzyme activity in horses, but does not affect the pulmonary artery pressure of exercising horses (Muir et al 2001). Similarly, the efficacy of enalapril in preventing EIPH has not been demonstrated.

Nitric oxide is a potent vasodilator in many vascular beds. Administration of nitroglycerin (a nitric oxide donor) reduces pulmonary artery pressure of standing horses but does not affect pulmonary artery pressure of horses during intense exercise (Manohar & Goetz 1999). The nitric oxide

donor L-arginine has no demonstrated efficacy in reducing pulmonary capillary pressure or EIPH in horses. The effect of L-NAME, an inhibitor of nitric oxide synthetase, on pulmonary artery pressure during maximal exercise is controversial with either no effect or a decrease in pulmonary artery pressure reported (Manohar & Goetz 1998, Kindig et al 2000). Interestingly, L-NAME administration caused an increase in severity of EIPH (Kindig et al 2000). Sildenafil, a phosphodiesterase inhibitor that accentuates the effect of nitric oxide and is used in the treatment of erectile dysfunction in men, has been administered to horses in an apparent attempt to reduce EIPH. However, its efficacy in preventing EIPH or reducing pulmonary capillary pressure has not been demonstrated.

An increase in pulmonary capillary pressure secondary to altered rheological properties of blood during exercise has been suggested as a possible contributing factor to EIPH (Fedde & Erickson 1998). Furosemide increases blood viscosity whereas pentoxifylline increases red blood cell deformability and may attenuate the increase in blood viscosity that occurs during exercise (Weiss et al 1994, 1996a,b) However, pentoxifylline does not affect the pulmonary capillary pressure of exercising horses and did not affect the prevalence of EIPH in a small experimental study (Manohar et al 2000a).

Increasing alveolar inspiratory pressure

Airway obstruction, either intrathoracic or extrathoracic, increases airway resistance and results in a more subatmospheric intrathoracic (pleural) pressure during inspiration to maintain tidal volume and alveolar ventilation. Causes of extrathoracic airway obstruction include laryngeal hemiplegia and other abnormalities of the upper airway, whereas intrathoracic obstruction is usually a result of bronchoconstriction and inflammatory airway disease. Horses with partial extrathoracic inspiratory obstruction or bronchoconstriction and airway inflammation associated with recurrent airway obstruction (heaves) have pleural (and hence alveolar) pressures that are lower (more negative) than those in unaffected horses or in horses after effective treatment.

Partial inspiratory obstruction, such as that produced by laryngeal hemiplegia, exacerbates the exercise-induced decrease in intrapleural pressures with a consequent increase in transmural capillary pressures (Jackson et al 1997, Ducharme et al 1999, Hackett et al 1999). These changes may exacerbate the severity of EIPH although an association between upper airway obstructive disease and EIPH has not been demonstrated. Surgical correction of airway obstruction is expected to resolve the more negative intrapleural pressure but its effect on EIPH is unknown.

Recently, the role of the nares in contributing to upper airway resistance, and hence lowering inspiratory intrapleural pressure during intense exercise, has attracted the attention of some investigators. Application of nasal dilator bands (Flair® strips) reduces nasal resistance by dilating the nasal valve (Holcombe et al 2002), and reduces red cell count of bronchoalveolar lavage fluid collected from horses after intense exercise on a treadmill (Geor et al 2001, Kindig et al 2001). Furthermore, application of the nasal dilator strips to horses in simulated races reduces red cell count in bronchoalveolar lavage fluid of some, but not all, horses (Valdez et al 2004).

The role of small airway inflammation and bronchoconstriction in the pathogenesis of EIPH is unclear. However, horses with EIPH are often treated with drugs intended to decrease lower airway inflammation and relieve bronchoconstriction. The β-adrenergic bronchodilatory drugs such as clenbuterol and albuterol are effective in inducing bronchodilation in horses with bronchoconstriction but their efficacy in preventing EIPH is either unknown or, in very small studies, is not evident. Clenbuterol does not alter the hemodynamic responses of horses to exertion nor attenuate exercise-induced arterial hypoxemia in normal horses (Slocombe et al 1992, Manohar et al 2000b). A very small study (two horses) of ipratropium, a parasympatholytic drug administered by inhalation, indicated promise in preventing EIPH (Sweeney et al 1984). Corticosteroids, including dexamethasone, fluticasone and beclomethasone administered by inhalation, parenterally or enterally, reduce airway inflammation and obstruction but have no demonstrated efficacy in preventing EIPH. Cromolyn sodium (sodium cromoglycate) has no efficacy in preventing EIPH (Hillidge et al 1987).

Water vapor treatment (inhalation of water-saturated air) has been proposed as a treatment for EIPH because of its putative effect on small airway disease. However, water vapor treatment has no effect on EIPH (Sweeney et al 1988).

The use of bedding with low allergenic potential (shredded paper) to prevent EIPH has no apparent effect on prevalence of the condition (Mason et al 1984). While it is suggested that preventing or minimizing small airway disease may reduce the severity of EIPH, studies to demonstrate such an effect have not been reported. However, optimizing the air quality in barns and stables and preventing infectious respiratory disease appear sensible precautions.

Interstitial inflammation and bronchial angiogenesis

Hemorrhage into interstitial tissues induces inflammation with subsequent development of fibrosis and bronchial artery angiogenesis (O'Callaghan et al 1987c, McKane & Slocombe 1999, 2002). The role of these changes in perpetuating EIPH in horses is unclear, but likely is of

some importance. Treatments to reduce interstitial inflammation and promote healing with minimal fibrosis have been proposed. Rest is an obvious recommendation and many racing jurisdictions have rules regarding enforced rest for horses with epistaxis. While the recommendation for rest is intuitive, there is no information that rest reduces the severity or incidence of EIPH in horses with prior evidence of this disorder.

Similarly, corticosteroids are often administered, by inhalation, enterally or parenterally, in an attempt to reduce pulmonary inflammation and minimize fibrosis. Again, the efficacy of this intervention in preventing or minimizing severity of EIPH has not been documented.

Excessive bleeding

Coagulopathy and fibrinolysis

Exercise induces substantial changes in blood coagulation and fibrinolysis (McKeever et al 1990). However, there is no evidence that horses with EIPH have defective coagulation or increased fibrinolysis (Bayly et al 1983, Johnstone et al 1991). Regardless of this, aminocaproic acid, a potent inhibitor of fibrin degradation, has been administered to horses to prevent EIPH but its efficacy in preventing EIPH has not been demonstrated. Similarly, estrogens are given to horses with the expectation of improving hemostasis although the effect of estrogens on coagulation in any species is unclear. There is no evidence that estrogens prevent EIPH in horses.

Vitamin K is administered to horses with EIPH presumably with the expectation that it will decrease coagulation times. However, as EIPH is not associated with prolonged bleeding times, it is unlikely that this intervention will affect the prevalence or severity of EIPH.

Platelet function

Aspirin inhibits platelet aggregation in horses and increases bleeding time (Kopp et al 1985). Seemingly paradoxically, aspirin is sometimes administered to horses with EIPH because of concerns that increased platelet aggregation contributes to EIPH (Mahony et al 1992). There is no evidence that aspirin either exacerbates or prevents EIPH.

Capillary integrity

Capillary fragility increases the risk of hemorrhage in many species. Various bioflavonoids have been suggested to increase capillary integrity and prevent bleeding. However, hesperidin and citrus bioflavonoids have no efficacy in preventing EIPH in horses (Sweeney & Soma 1984). Similarly, vitamin C is administered to horses with EIPH without scientific evidence of any beneficial effect.

Summary of treatment options

Selection of therapy for horses with EIPH is problematic. Given that most horses have some degree of pulmonary hemorrhage during most bouts of intense exercise, the decision must be made not only as to the type of treatment and its timing but also to which horses to treat. Moreover, the apparent progressive nature of the disease with continued work highlights the importance of early and effective prophylaxis and emphasizes the need for studies of possible factors such as air quality and respiratory infections in inciting the disorder.

The currently favored treatment for EIPH is administration of furosemide before intense exercise. Its use is permitted in racehorses in a number of countries. Increasingly persuasive laboratory evidence of an effect of furosemide to reduce red cell count in bronchoalveolar lavage fluid collected from horses soon after intense exercise supports the contention that furosemide is effective in reducing the severity of EIPH in racehorses. However, it should be borne in mind that neither the relationship between severity of EIPH and red cell count in bronchoalveolar lavage fluid, nor the efficacy of furosemide in reducing severity of EIPH in racehorses in the field has been demonstrated. In fact, there is evidence that furosemide does not reduce the prevalence of EIPH and other evidence that it does not reduce the severity of EIPH under field conditions. The association between furosemide administration and superior performance in standardbred and thoroughbred racehorses should be borne in mind when recommending use of this drug.

Rest is an obvious recommendation for horses with EIPH, but the hemorrhage is likely to recur when the horse is next strenuously exercised. The duration of rest and the optimal exercise program for the return of horses to racing after EIPH is unknown, although some jurisdictions require exercise no more intense than trotting for 2 months. Firm recommendations cannot be made on duration of rest because of a lack of objective information.

Although a role for lower airway disease (either infectious or allergic) in the genesis of EIPH has not been demonstrated, control of infectious diseases and minimization of non-infectious lower airway inflammation appears prudent.

Prognosis

The prognosis for racing for horses with clinically significant EIPH is guarded because of the progressive nature of the disease. Horses that have experienced severe EIPH on one occasion are likely to do so again regardless of treatment. However, the risk of horses experiencing a repeated bout of severe hemorrhage and the effect of EIPH on career longevity are unknown.

REFERENCES

Anonymous 2002 International agreement on breeding and racing and appendices, International Federation of Horse Racing Authorities

Art T, Anderson L, Woakes A J et al 1990 Mechanics of breathing during strenuous exercise in Thoroughbred horses. Respiration Physiology 82: 279–294

Bayly WM, Meyers KM, Keck MT 1983 Effects of furosemide on exercise-induced alterations in haemostasis in Thoroughbred horses exhibiting post-exercise epistaxis. In: Snow DH, Persson SGB, Rose RJ (editors) Equine Exercise Physiology. Granta Editions, Cambridge, pp.64–70

Birks EK, Mathieu-Costello O, Fu Z et al 1994 Comparative aspects of the strength of pulmonary capillaries in rabbit, dog, and horse. Respiratory Physiology 97: 235–246

Birks EK, Mathieu-Costello O, Fu Z et al 1997 Very high pressures are required to cause stress failure of pulmonary capillaries in thoroughbred racehorses. Journal of Applied Physiology 82: 1584–1592

Birks EK, Shuler KM, Soma LR et al 2002 EIPH: postrace endoscopic evaluation of Standardbreds and Thoroughbreds. Equine Veterinary Journal Supplement 34: 375–378

Christley RM, Hodgson DR, Rose RJ et al 2001 Coughing in thoroughbred racehorses: risk factors and tracheal endoscopic and cytological findings. Veterinary Record 148: 99–104

Cook WR 1974 Epistaxis in the racehorse. Equine Veterinary Journal 6: 45–58

Cook WR 1988 Hypotheses on exercise-induced pulmonary hemorrhage in horses. Journal of the American Veterinary Medical Association 193: 8–10

Deaton C, Marlin D 2004 A review of the effects of environmental pollution on the equine respiratory tract: considerations for the 2004 Athens Olympic Games. Journal of Equine Comparative Exercise Physiology 1: 171–176

Ducharme NG, Hackett RP, Gleed RD et al 1999 Pulmonary capillary pressure in horses undergoing alteration of pleural pressure by imposition of various airway resistive loads. Equine Veterinary Journal Supplement 30: 27–33

Fedde MR, Erickson HH 1998 Increase in blood-viscosity in the sprinting horse – can it account for the high pulmonary arterial-pressure? Equine Veterinary Journal 30: 329–334

Fogarty U, Buckley T 1991 Bronchoalveolar lavage findings in horses with exercise intolerance. Equine Veterinary Journal 23: 434–437

Geor RJ, Ommundson L, Fenton G et al 2001 Effects of an external nasal strip and frusemide on pulmonary haemorrhage in Thoroughbreds following high-intensity exercise. Equine Veterinary Journal 33: 577–584

Gleed RD, Ducharme NG, Hackett R 1999 Effects of frusemide on pulmonary capillary pressure in horses exercising on a treadmill. Equine Veterinary Journal Supplement 30: 102–106

Gross DK, Morley PS, Hinchcliff KW et al 1999 Effect of furosemide on performance of Thoroughbreds racing in the United States and Canada. Journal of the American Veterinary Medical Association 215: 670–675

Gunson DE, Sweeney CR, Soma LR 1988 Sudden death attributable to exercise-induced pulmonary hemorrhage in racehorses: nine cases (1981–1983). Journal of the American Veterinary Medical Association 193: 102–106

Hackett RP, Ducharme NG, Ainsworth DM et al 1999 Effects of extrathoracic airway obstruction on intrathoracic

pressure and pulmonary artery pressure in exercising horses. American Journal of Veterinary Research 60: 485–494

Heller B 2002 Run, Baby, Run: What Every Owner, Breeder, and Handicapper Should Know About Lasix in Racehorses. The Russell Meerdink Company, Neenah, WI, p.180

Hillidge CJ, Lane TJ, Johnson E 1984 Preliminary investigations of exercise-induced pulmonary hemorrhage in racing Quarter Horses. Journal of Equine Veterinary Science 4: 21–23

Hillidge CJ, Lane TJ, Whitlock TW 1986 Exercise-induced pulmonary hemorrhage in the racing Appaloosa horse. Journal of Equine Veterinary Science 5: 351–353

Hillidge CJ, Whitlock TW, Lane TJ 1987 Failure of inhaled disodium cromoglycate aerosol to prevent exercise-induced pulmonary hemorrhage in racing quarter horses. Journal of Veterinary Pharmacology and Therapeutics 10: 257–260

Hinchcliff KW, Jackson M, Morley PS et al 2004 Association of exercise-induced pulmonary hemorrhage and performance in racing Thoroughbred horses. Proceedings of the American Association of Equine Practitioners, Denver 50: 276–277

Hinchcliff KW, Jackson M, Morley PS et al (2005) Tracheo-bronchoscopic assessment of the severity of exercise-induced pulmonary hemorrhage in Thoroughbred race-horses. American Journal of Veterinary Research 66: 596–598

Holcombe SJ, Berney C, Cornelisse C J et al 2002 Effect of commercially available nasal strips on airway resistance in exercising horses. American Journal of Veterinary Research 63: 1101–1105

Jackson JA, Ducharme NG, Hackett RP et al 1997 Effects of airway obstruction on transmural pulmonary artery pressure in exercising horses. American Journal of Veterinary Research 58: 897–903

Johnstone IB, Viel L, Crane S et al 1991 Hemostatic studies in racing standardbred horses with exercise-induced pulmonary hemorrhage. Hemostatic parameters at rest and after moderate exercise. Canadian Journal of Veterinary Research 55: 101–106

Jones JH, Cox TS, Takahashi T et al 2002 Heterogeneity of intrapleural pressures during exercise. Equine Veterinary Journal Supplement 34: 391–396

Kindig CA, Erickson BK, Poole DC 2000 Dissociation of exercise-induced pulmonary hemorrhage and pulmonary artery pressure via nitric oxide synthase inhibition. Journal of Equine Veterinary Science 20: 715–721

Kindig CA, McDonough P, Fenton G et al 2001 Efficacy of nasal strip and furosemide in mitigating EIPH in Thoroughbred horses. Journal of Applied Physiology 91: 1396–1400

Kopp K, Moore J, Byars T et al 1985 Template bleeding time and thromboxane generation in the horse: effects of three non-steroidal anti-inflammatory drugs. Equine Veterinary Journal 17: 322–324

Langsetmo I, Meyer MR, Erickson HH 2000 Relationship of pulmonary arterial pressure to pulmonary haemorrhage in exercising horses. Equine Veterinary Journal 32: 379–384

Lapointe JM, Vrins A, McCarvill E 1994 A survey of exercise-induced pulmonary haemorrhage in Quebec standardbred racehorses. Equine Veterinary Journal 26: 482–485

MacNamara B, Bauer S, Iafe J 1990 Endoscopic evaluation of exercise-induced pulmonary hemorrhage and chronic obstructive pulmonary disease in association with poor

performance in racing Standardbreds. Journal of the American Veterinary Medical Association 196: 443–445

Mahony C, Rantanen NW, DeMichael JA et al 1992 Spontaneous echocardiographic contrast in the thoroughbred: high prevalence in racehorses and a characteristic abnormality in bleeders. Equine Veterinary Journal 24: 129–133

Manohar M 1993 Furosemide attenuates the exercise-induced increase in pulmonary artery wedge pressure in horses. American Journal of Veterinary Research 54: 952–958

Manohar M, Goetz TE 1999 Pulmonary vascular pressures of strenuously exercising Thoroughbreds during intravenous infusion of nitroglycerin. American Journal of Veterinary Research 60: 1436–1440

Manohar M, Hutchens E, Coney E 1993 Pulmonary haemodynamics in the exercising horse and their relationship to exercise-induced pulmonary haemorrhage. British Veterinary Journal 149: 419–428

Manohar M, Goetz TE 1998 L-Name does not affect exercise-induced pulmonary hypertension in thoroughbred horses. Journal of Applied Physiology 84: 1902–1908

Manohar M, Goetz T, Rothenbaum P et al 2000a Intravenous pentoxifylline does not affect the exercise-induced pulmonary arterial, capillary or venous hypertension in Thoroughbred horses. Journal of Veterinary Pharmacology and Therapeutics 23: 317–322

Manohar M, Goetz TE, Rothenbaum P et al 2000b Clenbuterol administration does not attenuate the exercise-induced pulmonary arterial, capillary or venous hypertension in strenuously exercising Thoroughbred horses. Equine Veterinary Journal 32: 546–550

Martin BB, Beech J, Parente EJ 1999 Cytologic examination of specimens obtained by means of tracheal washes performed before and after high-speed treadmill exercise in horses with a history of poor performance. Journal of the American Veterinary Medical Association 214: 673–677

Mason DK, Collins EA, Watkins KL 1983 Exercise-induced pulmonary haemorrhage in horses. In: Snow DH, Persson SGB, Rose RJ (editors) Equine Exercise Physiology. Granta Editions, Cambridge, pp.57–63

Mason DK, Collins EA, Watkins KL 1984 Effect of bedding on the incidence of exercise induced pulmonary haemorrhage in racehorses in Hong Kong. Veterinary Record 115: 268–269

McDonough P, Kindig C, Hildreth T et al 2004 Effect of furosemide and the equine nasal strip on exercise-induced pulmonary hemorrhage and time-to-fatigue in maximally exercising horses. Journal of Equine Comparative Exercise Physiology 1: 177–184

McKane SA, Slocombe R 1999 Sequential changes in bronchoalveolar cytology after autologous blood inoculation. Equine Veterinary Journal Supplement 30: 126–130

McKane SA, Slocombe RF 2002 Alveolar fibrosis and changes in lung morphometry in response to intrapulmonary blood. Equine Veterinary Journal Supplement 34: 451–458

McKane SA, Canfield PJ, Rose RJ 1993 Equine bronchoalveolar lavage cytology: survey of thoroughbred racehorses in training. Australian Veterinary Journal 70: 401–404

McKeever KH, Hinchcliff KW, Kociba GJ et al 1990 Exercise-induced changes in clotting time and fibrinolytic activity in horses. American Journal of Veterinary Research 51: 1335–1339

Meyer TS, Fedde MR, Gaughan EM et al 1998 Quantification of exercise-induced pulmonary haemorrhage with bronchoalveolar lavage. Equine Veterinary Journal 30: 284–288

Muir WW, Sams RA, Hubbell JAE et al 2001 Effects of enalaprilat on cardiorespiratory, hemodynamic, and hematologic variables in exercising horses. American Journal of Veterinary Research 62: 1008–1013

Newton JR, Wood JLN 2002 Evidence of an association between inflammatory airway disease and EIPH in young Thoroughbreds in training. Equine Veterinary Journal Supplement 34: 417–424

O'Callaghan MW, Pascoe JR, Tyler WS et al 1987a Exercise-induced pulmonary haemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. I. Clinical profile of horses. Equine Veterinary Journal 19: 384–388

O'Callaghan MW, Pascoe JR, Tyler WS et al 1987b Exercise-induced pulmonary haemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. II. Gross lung pathology. Equine Veterinary Journal 19: 389–393

O'Callaghan MW, Pascoe JR, Tyler WS et al 1987c Exercise-induced pulmonary haemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. III. Subgross findings in lungs subjected to latex perfusions of the bronchial and pulmonary arteries. Equine Veterinary Journal 19: 394–404

O'Callaghan MW, Pascoe JR, Tyler WS et al 1987d Exercise-induced pulmonary haemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. V. Microscopic observations. Equine Veterinary Journal 19: 411–418

Oikawa M 1999 Exercise-induced haemorrhagic lesions in the dorsocaudal extremities of the caudal lobes of the lungs of young thoroughbred horses. Journal of Comparative Pathology 121: 339–347

Pascoe JR 1996 Exercise-induced pulmonary hemorrhage: a unifying concept. Proceedings of the American Association of Equine Practitioners 42: 220–226

Pascoe JR, Ferraro G, Cannon J et al 1981a Exercise-induced pulmonary hemorrhage in racing thoroughbreds: a preliminary study. American Journal of Veterinary Research 42: 703–707

Pascoe JR, Ferraro GL, Cannon JH et al 1981b Exercise-induced pulmonary hemorrhage in racing thoroughbreds: a preliminary study. American Journal of Veterinary Research 42: 703–707

Pascoe JR, O'Brien TR, Wheat JD et al 1983 Radiographic aspects of exercise-induced pulmonary hemorrhage in racing horses. Veterinary Radiology 24: 85–92

Pascoe JR, McCabe AE, Franti CE et al 1985 Efficacy of furosemide in the treatment of exercise-induced pulmonary hemorrhage in Thoroughbred racehorses. American Journal of Veterinary Research 46: 2000–2003

Raphel CF, Soma LR 1982 Exercise-induced pulmonary hemorrhage in Thoroughbreds after racing and breezing. American Journal of Veterinary Research 43: 1123–1127

Robinson NE, Derksen FJ 1980 Small airway obstruction as a cause of exercise-associated pulmonary hemorrhage. Proceedings of the American Association of Equine Practitioners 26: 421–430

Rohrbach BW 1990 Exercise-induced pulmonary hemorrhage, chronic obstructive pulmonary disease, and racing performance. Journal of the American Veterinary Medical Association 196: 1563–1564

Rossier Y, Sweeney CR, Ziemer EL 1991 Bronchoalveolar lavage fluid cytologic findings in horses with pneumonia or pleuropneumonia. Journal of the American Veterinary Medical Association 198: 1001–1004

Schroter RC, Marlin DJ, Denny E 1998 Exercise-induced pulmonary haemorrhage (EIPH) in horses results from locomotory impact induced trauma: a novel, unifying concept. Equine Veterinary Journal 30: 186–192

Sime D, Engen R, Miller-Graber P 1994 Frequency and use of medications in horses racing in Prairie Meadows. Iowa State University Vet 54.

Slocombe R, Covelli G, Bayly W 1992 Respiratory mechanics of horses during stepwise treadmill exercise tests, and the effect of clenbuterol pretreatment on them. Australian Veterinary Journal 69: 221–225

Soma LR, Uboh CE, Nann L 1996 Prerace venous blood acid–base values in Standardbred horses. Equine Veterinary Journal 28: 390–396

Soma LR, Birks EK, Uboh CE et al 2000 The effects of frusemide on racing times of Standardbred pacers. Equine Veterinary Journal 32: 334–340

Speirs VC 1982 Pulmonary hemorrhage in Standardbred race horses. Australian Veterinary Journal 59: 38–40

Step DL, Freeman KP, Gleed RD et al 1991 Cytologic and endoscopic findings after intrapulmonary blood inoculations in horses. Journal of Equine Veterinary Science 11: 340–345

Sweeney CR, Soma LR 1984 Exercise-induced pulmonary hemorrhage in thoroughbred horses: response to furosemide or hesperidin-citrus bioflavonoids. Journal of the American Veterinary Medical Association 185: 195–197

Sweeney CR, Soma LR, Bucan CA et al 1984 Exercise-induced pulmonary hemorrhage in exercising Thoroughbreds: preliminary results with pre-exercise medication. Cornell Veterinarian 74: 263–268

Sweeney CR, Hall J, Fisher JR et al 1988 Efficacy of water vapor-saturated air in the treatment of exercise-induced pulmonary hemorrhage in Thoroughbred racehorses. American Journal of Veterinary Research 49: 1705–1707

Sweeney CR, Soma LR, Maxson AD et al 1990 Effects of furosemide on the racing times of Thoroughbreds. American Journal of Veterinary Research 51: 772–778

Takahashi T, Hiraga A, Ohmura H et al 2001 Frequency of and risk factors for epistaxis associated with exercise-induced pulmonary hemorrhage in horses: 251,609 race starts (1992–1997). Journal of the American Veterinary Medical Association 218: 1462–1464

Valdez S, Nieto J, Spier S et al 2004 Effect of an external nasal dilator strip on cytologic characteristics of bronchoalveolar lavage fluid in Thoroughbred racehorses. Journal of the American Veterinary Medical Association 224: 558–561

Voynick BT, Sweeney CR 1986 Exercised-induced pulmonary hemorrhage in polo and racing horses. Journal of the American Veterinary Medical Association 188: 301–302

Weideman H, Schoeman S, Jordaan G et al 2003 Epistaxis related to exercise-induced pulmonary haemorrhage in South African Thoroughbreds. Journal of the South African Veterinary Association 74: 127–131

Weiss DJ, Smith CM 2nd 1998 Haemorheological alterations associated with competitive racing activity in horses: implications for exercise-induced pulmonary haemorrhage (EIPH). Equine Veterinary Journal 30: 7–12

Weiss DJ, Evanson OA, Geor RJ 1994 The effects of furosemide and pentoxifylline on the flow properties of equine erythrocytes: in vitro studies. Veterinary Research Communications 18: 373–381

Weiss DJ, Geor RJ, Burger K 1996a Effects of furosemide on hemorheologic alterations induced by incremental treadmill exercise in thoroughbreds. American Journal of Veterinary Research 57: 891–895

Weiss DJ, Geor RJ, Burger K 1996b Effects of pentoxifylline on hemorheologic alterations induced by incremental treadmill exercise in thoroughbreds. American Journal of Veterinary Research 57: 1364–1368

West JB, Mathieu-Costello O 1994 Stress failure of pulmonary capillaries as a mechanism for exercise induced pulmonary haemorrhage in the horse. Equine Veterinary Journal 26: 441–447

West JB, Mathieu-Costello O, Jones JH et al 1993 Stress failure of pulmonary capillaries in racehorses with exercise-induced pulmonary hemorrhage. Journal of Applied Physiology 75: 1097–1109

Williams R, Harkins L, Hammond C et al 2001 Racehorse injuries, clinical problems and fatalities recorded on British racecourses from flat racing and National Hunt racing during 1996, 1997 and 1998. Equine Veterinary Journal 33: 478–486

45

Respiratory Diseases of the Foal

Elizabeth A Carr

Respiratory disease is one of the leading causes of morbidity and mortality in neonatal foals. Many factors unique to the neonate make them more susceptible to respiratory disorders. Prematurity, inadequate colostral transfer, meconium aspiration, and neonatal sepsis are just a few of the syndromes that can result in respiratory failure. This chapter reviews the common respiratory syndromes recognized in equine neonatology. Diagnostic evaluation, clinical assessment, treatment, and potential complications will be discussed. The chapter focuses primarily on respiratory disorders of the neonatal period. In addition, lower respiratory tract syndromes seen principally in foals are also discussed. Respiratory disorders common to weanlings and adult horses are discussed elsewhere in this book.

The diagnosis of respiratory disease in the foal can be difficult. Neonatal foals with respiratory disease rarely cough or exhibit nasal discharge. Clinical signs may be non-specific including increased restlessness, agitation, and an elevated respiratory rate. Auscultation is frequently unrewarding because foals often have little to no abnormal lung sounds even with significant pulmonary disease. Consequently, the diagnosis of pulmonary disease can be difficult without additional diagnostic testing and a strong index of suspicion. The decision to perform additional diagnostic testing (including blood gas analysis, thoracic radiography, thoracic ultrasound, and tracheal wash and culture) needs to be made using historical information on the pregnancy, foaling and early postpartum period, coupled with physical examination findings.

History

The goal in obtaining a history is to identify risk factors or events that suggest that the foal may be ill or at risk for illness. Any foal with identifiable risk factors or evidence of underlying illness warrants a higher degree of surveillance, and may require additional diagnostic testing to detect illness and institute treatment at the earliest possible stage. Neonatal foal survival rates are correlated with early detection of illness and therapeutic intervention (Gayle et al 1998). Consequently, when evaluating a foal, it is imperative to obtain a detailed history regarding the pregnancy, parturition, and immediate postpartum period. Information regarding the mare's previous pregnancies

and general health, as well as information regarding the farm's management and overall herd health, is critical in assessing the potential for illness in the neonate.

Prepartum maternal signs

Although the majority of mares have an uneventful gestation and foal with little, if any, intervention, in other mares, premonitory signs, either present as a clinical finding during physical examination or as a component of the history, warrant additional scrutiny for potential problems. Mares that have a history of pregnancy complications such as endometritis, dystocia or abortion should be monitored carefully, and their foals warrant a high degree of surveillance postpartum. Severe maternal illness such as endotoxemia, fever, cardiovascular disease, renal failure of advanced pregnancy, prepartum uterine artery hemorrhage, or conditions requiring general anesthesia and surgical intervention may compromise the fetus and lead to delivery of a weak, sick or premature foal at greater risk for illness.

Parturition

Normal parturition occurs in three stages. Stage 1 can persist for a variable duration and is associated with uterine contractions as the foal moves into position for birth. Stage 2 begins with the rupture of the chorioallantoic membrane and ends with delivery of the foal. Stage 3 is expulsion of the placenta. Stage 2 should be relatively short, lasting approximately 20 min. Because placental attachments begin to separate and oxygen delivery to the foal is compromised during stage 2, delay in delivery of the foal can result in asphyxia. If parturition is prolonged, fetal stress may result in expulsion and aspiration of meconium. Compression of the thorax as it passes through the mare's pelvic canal assists in removal of fluid from the lungs. A foal delivered by Caesarean section does not experience this thoracic compression and needs assistance to remove excess fluid from the lungs. Foals with evidence of placentitis, a history of premature placental separation, dystocia, premature delivery or delivery by Caesarean section are all at greater risk of neonatal illness, sepsis and respiratory failure and should be monitored closely.

Postpartum

The normal foal should achieve sternal recumbency within minutes of birth. Respiratory and heart rates should be 60/min or more during the first few minutes and nasal stimulation should result in a sneeze or cough and obvious avoidance behavior. The foal that cannot achieve sternal recumbency, or that has a respiratory or heart rate <60/min at birth is abnormal and warrants continued surveillance and potential intervention. Meconium staining of the skin is associated with intrauterine stress and is a concern because of the potential for meconium aspiration. The foal should be standing by 2 h of age and nursing soon afterwards. The goal is to ensure colostrum ingestion before exposure to potential pathogens in the immediate environment. A foal that has delayed suckling and spends several hours udder seeking and licking the walls and the mare's perineal area is at greater risk for subsequent infection and illness. Any foal that deviates from normal parameters should be carefully monitored and intervention should be provided as necessary.

Examination of the Foal Respiratory System

Normal vital parameters and behavior patterns have been published for foals from birth to several months of age (Koterba 1990). Deviation from normal, particularly in the early neonatal period, should warrant a careful physical examination. A respiratory rate of 60–80 breaths/min is typical immediately after birth. This rate should decline to 30–40 breaths/min over the first 2 h. Normal foals may have an elevated respiratory rate when lying in lateral recumbency. Persistent tachypnea, although a relatively non-specific clinical finding, warrants a close examination of the respiratory system particularly if the foal appears agitated, restless or unwilling to lie down and sleep. A mildly exaggerated inspiratory and expiratory effort may be the only clinical sign of respiratory disease during the early stages of illness. During nursing, the foal is required to breath-hold, and thus in cases with severe respiratory compromise and hypoxemia the foal may be reluctant to nurse for more than a few seconds at a time.

Neonatal foals have a monophasic breath cycle, unlike adult horses that exhibit a biphasic cycle with both passive and active components to inspiration and expiration (Koterba et al 1995a). In young foals both inspiration and expiration are active but as the foal matures, expiration and then later inspiration become biphasic (Koterba et al 1995b). Long apneustic pauses in breathing, i.e. breath-holds during inhalation, are abnormal and suggest central nervous system derangements such as might be seen with periparturient asphyxia syndrome.

Auscultation of the thorax of the foal should be performed in a quiet place with the foal standing or in sternal recumbency. Because of the relatively compliant chest wall of the neonate, foals will experience some collapse of dependent alveoli when they lie on their sides. Consequently, abnormal lung sounds (crackles and wheezes) may be heard on the dependent side after a period of prolonged lateral recumbency. Use of a rebreathing bag to increase ventilation is generally not necessary when auscultating the neonate given their relatively small size and thin chest wall and may be contraindicated in hypoxic or hypercapnic individuals. While careful auscultation of the thorax is recommended, it can be an insensitive tool for assessing lung disease. Lung auscultatory changes are often subtle, with minimal adventitial sounds even in the face of severe pneumonia. With larger areas of lung consolidation auscultation may reveal a "tubular" or hollow sound as air is heard moving principally through large airways.

Thoracic ultrasound

While the auscultation of abnormal or diminished lung sounds is a clear indication for further diagnostic testing including thoracic ultrasound, radiography, and arterial blood gas analysis, many foals with respiratory disease may have relatively normal lung sounds. Consequently, most foals admitted to the clinic will need additional diagnostic tests.

Abdominal ultrasound is routinely performed in all neonates admitted to the clinic at Michigan State University and it is easy to include the thorax as well. Ideally the foal is evaluated standing or in sternal recumbency and the pleural surface is imaged through the intercostal spaces using a 5- to 7.5-MHz probe. Either a sector or linear probe can be used, depending on the ultrasonographer's preference. Normal thoracic ultrasound should reveal a sharp line at the pleural surface with reverberation artifacts at regularly spaced intervals in the deeper image. These artifactual lines are a repeated reception of the original echo from the visceral surface as it "bounces" between the transducer and the receiver. The pleural surface should appear to glide dorsally and ventrally with breathing so that the diaphragm and abdominal viscera are uncovered during exhalation. Little to no pleural fluid should be evident. Early signs of respiratory disease include the generation of pleural "comet tails" that are the result of irregularities in the normally smooth pleural membrane (Fig. 45.1). These irregularities can be temporarily seen in the normal lung after prolonged recumbency or can signify early inflammatory changes. Moderate to severe alveolar consolidation results in deeper penetration of the ultrasound waves, allowing observation of parenchymal
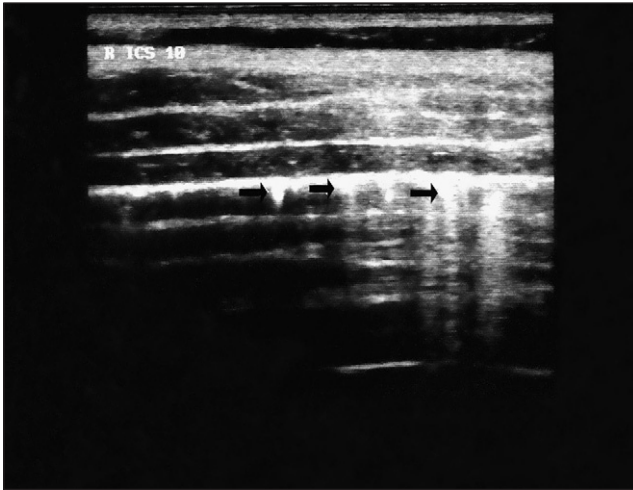
Fig. 45.1. Ultrasound picture of multiple pleural irregularities, "comet tails" (arrows). The finding of these pleural irregularities is consistent with mild or early pneumonia or prolonged lateral recumbency and should alert the clinician to pursue further diagnostic evaluation.
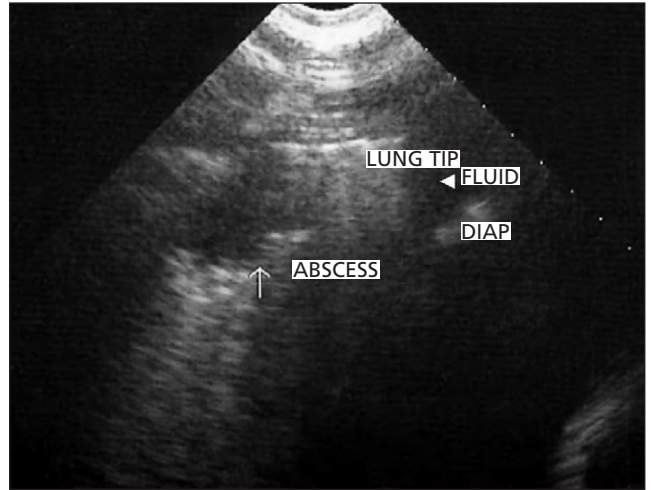
Fig. 45.2. Ultrasound picture of a pulmonary abscess in the right hemithorax at the level of the eighth intercostal space.

changes (Fig. 45.2). Because the ability of ultrasound to image the deeper lung is limited by air in the abaxial lung fields, it is not accurate in assessing solitary, deep pulmonary abscesses. When evaluating a case of foal pneumonia, both radiographic and ultrasonographic examinations of the thorax are recommended to obtain the most accurate information.

Unlike the situation in adult horses, pleural effusion is a relatively uncommon finding in neonatal pneumonia. Pleural effusion occurs secondary to rib fractures (hemothorax) and uroabdomen. In the latter case, the urothorax is the result of transudation across the diaphragm from the uroabdomen.

In addition to evaluation of the lungs, individual ribs should be imaged to assess the presence of rib fractures. The simplest way to image the ribs is to place the index finger and thumb of one hand into the intercostal spaces immediately before and after the rib being imaged, while the other hand holds and moves the transducer longitudinally down the rib. The most common sites of rib fractures are just dorsal to the costochondral junction. The ventral fragment frequently displaces inward and can cause injury to the lung or heart (Fig. 45.3). While rib fractures have been reported in clinically normal foals with no history of previous trauma, they are a cause of concern particularly in recumbent foals requiring assistance to rise (Jean et al 1999, Sprayberry et al 2001). While a normal healthy foal may guard the area of a rib fracture and minimize further injury, a recumbent foal being assisted to stand or restrained for evaluation is at a greater risk of further injury. Foals with rib fractures should be restrained

carefully. Continued injury as evidenced by pulmonary contusion or pleural hemorrhage would be an indication for fracture stabilization (Fig. 45.4) (Bellezzo et al 2004).

Thoracic radiography

Although thoracic radiographs are a useful diagnostic tool to evaluate the severity of respiratory disease and response to treatment, radiographic changes frequently lag behind clinical findings, necessitating careful interpretation of the radiographs. For example, in human infants with acute persistent pulmonary hypertension, patients may exhibit severe hypoxemia and respiratory distress with few radiographic changes. Over time, pulmonary infiltrates become apparent as edema and inflammation progress; however, the early evaluation may be unremarkable. Consequently, pulmonary radiographs should be interpreted carefully and repeat radiographs are indicated in a patient with persistent or deteriorating respiratory function.

The location of pulmonary densities may be helpful in determining the underlying cause of respiratory disease. Alveolar infiltrates and consolidation in the ventral lung fields are frequently associated with pneumonia as a result of milk aspiration (Fig. 45.5). However, meconium aspiration *in utero* frequently results in a more diffuse alveolar infiltrate. Viral infections or hematogenous bacterial pneumonias typically cause more diffuse alveolar infiltrates with varying severity of interstitial and bronchial changes. Pulmonary radiographs of foals with primary surfactant deficiency may appear normal immediately after birth; later radiographic evaluation often reveals diffuse, severe alveolar infiltrates and consolidation (Fig. 45.6). In a study of 128 foals, an attempt was made to correlate pulmonary radiographic findings to survival (Bedenice et al
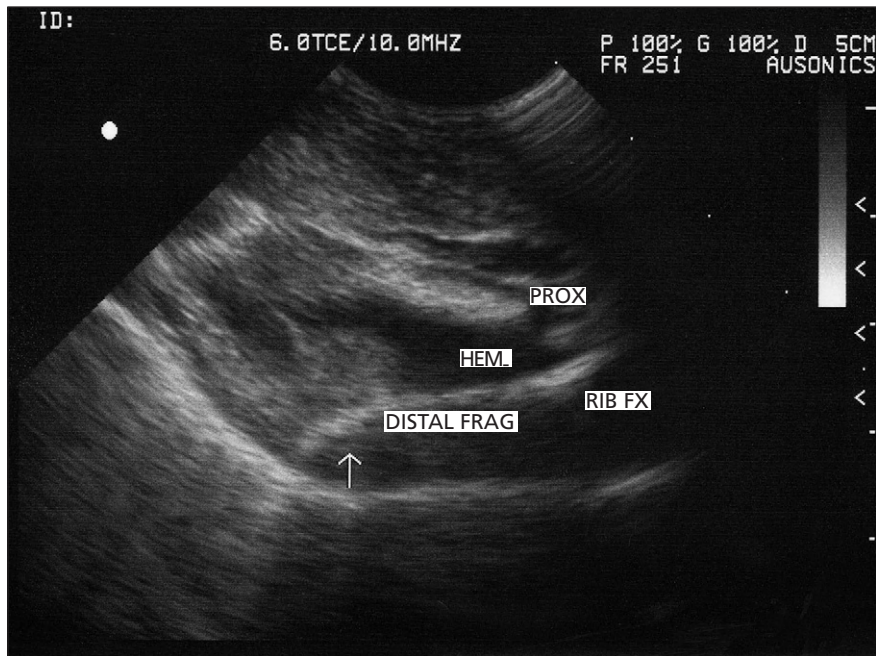
Fig. 45.3. Ultrasound picture of a fractured rib (RIB FX) just proximal (PROX) to the costochondral junction. The distal fragment (FRAG) is displaced medially and has caused contusions to the lung directly beneath it (arrow). Hemorrhage (HEM) can be seen surrounding the fracture site.
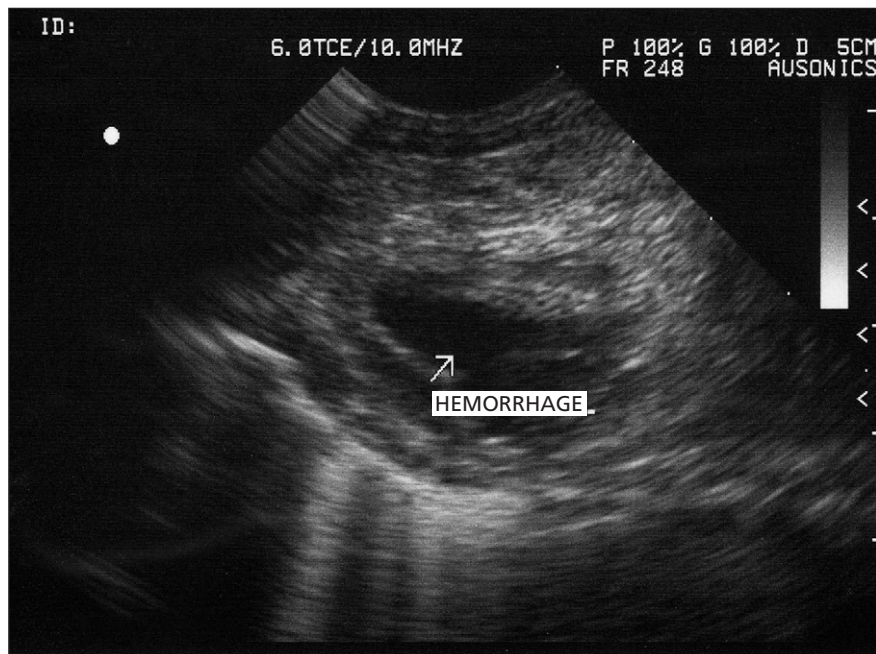
Fig. 45.4. Ultrasound picture of hemorrhage and pulmonary contusions just cranial to a rib fracture. The detection of this hemorrhage and contusion alerted the clinician to the possibility of a rib fracture. Further evaluation revealed the fracture of the rib just caudal to this image (see Fig. 45.3).
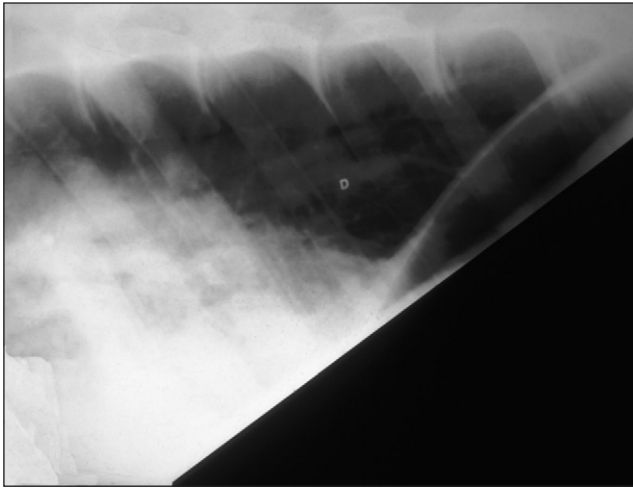
Fig. 45.5. Thoracic radiograph of foal presented with history of dysphagia. The cardiac silhouette is no longer visible because of the severe ventral consolidation that is consistent with aspiration pneumonia.

Fig. 45.6. One-day-old neonatal foal with acute onset of respiratory distress. Radiographs reveal severe, diffuse, alveolar pattern in all lung fields. Air bronchograms can be seen particularly clearly in the dorsocaudal lung field.

2003). Foals with radiographic findings of diffuse pulmonary disease and foals with severe alveolar changes were more likely to die than others. While the results of this study are interesting, radiographic findings remain a single piece of the puzzle and should be interpreted in the light of other diagnostic results.

Table 45.1. Blood gas pH and bicarbonate values in foals						
Age	Position sampled	pH	$P_a co_2$ (mmHg)	$P_A o_2$ (mmHg)	HCO_3^- (mEq/l)	No.
Birth	lateral	7.413 ± 0.019	45.7 ± 1.1	39.7 ± 2.1	26.3 ± 1.1	8
2 min	lateral	7.312 ± 0.016	54.1 ± 2.0	56.4 ± 2.3	24.0 ± 1.2	10
15 min	lateral	7.322 ± 0.025	50.4 ± 2.7	57.5 ± 3.6	24.4 ± 1.6	9
30 min	lateral	7.354 ± 0.010	51.5 ± 1.5	57.0 ± 1.8	25.3 ± 6.7	10
60 min	lateral	7.362 ± 0.013	47.3 ± 2.2	60.9 ± 2.7	25.3 ± 1.0	9
2 h	lateral	7.362 ± 0.012	47.7 ± 1.7	66.5 ± 2.3	25.0 ± 0.9	8
4 h	lateral	7.355 ± 0.017	45.0 ± 1.9	75.7 ± 4.9	23.6 ± 1.1	10
12 h	lateral	7.357 ± 0.024	44.3 ± 1.2	73.5 ± 3.0	23.2 ± 1.3	10
24 h	lateral	7.393 ± 0.012	45.5 ± 1.5	67.6 ± 4.4	26.2 ± 1.1	9
48 h	lateral	7.396 ± 0.008	46.1 ± 1.1	74.9 ± 3.3	25.7 ± 0.6	8
4 days	lateral	7.396 ± 0.012	45.8 ± 1.1	81.2 ± 2.1	23.2 ± 2.1	8

Reproduced from Stewart et al 1984, with permission.

Blood gas analysis

At birth the foal's arterial oxygen tension ($P_A o_2$) is much lower (43 ± 3.9 mmHg) than the adult horse (Table 45.1) (Rose et al 1982). Within the first hour of life, $P_A o_2$ should increase to 65–75 mmHg, and by 24 h of age it should approximate adult values. Arterial oxygen tension also varies with recumbency. When the foal is in lateral recumbency $P_A o_2$ can be up to 14 mmHg lower than when the foal is standing or sternally recumbent (Stewart et al 1984). Consequently the clinician needs to take into account the age and posture of the foal when interpreting arterial blood gas results. Because of this variation and the relatively low arterial oxygen tension on the first day of life, the $P_A o_2$ threshold indicated for institution of oxygen supplementation may vary. A $P_A o_2$ of ≥ 60 mmHg generally correlates with 90% saturation of hemoglobin or greater and for this reason the majority of clinicians institute oxygen therapy when $P_A o_2$ or oxygen saturation is < 60 mmHg or < 90%, respectively.

An arterial blood gas analysis is indicated in any foal with an elevated respiratory rate, dyspnea, cyanosis or other evidence of pulmonary disease to determine the severity of compromise, and to assess the need of adjunctive therapy. If arterial blood gas analysis facilities are not readily available, or a sample cannot be obtained, pulse oximetry can be utilized. Pulse oximetry is less accurate than arterial blood gas analysis in quantifying the severity of hypoxemia, particularly in hypovolemic, poorly perfused individuals, but can be useful to evaluate trends and response to therapy (Chaffin et al 1996).

Lung Development and the Fetal–Neonatal Transition

Understanding neonatal respiratory diseases, their complications and treatment is facilitated by knowledge of the development of the lung and the changes in the lung and circulation that occur at birth (Fig. 45.7). *In utero*, oxygen is delivered to the fetus via the placental circulation. Oxygenated blood enters the fetus via the umbilical vein, through the caudal vena cava to the right atrium. After entering the right chamber of the heart, blood is shunted to the left chamber of the heart, the aorta, and the systemic circulation through the foramen ovale and ductus arteriosus. Before birth, the fetal lung is collapsed and fluid-filled, pulmonary vascular resistance is high as the result of low oxygen tension and there is minimal blood flow through the pulmonary circulatory system (Raj & Shimoda 2002). Pressures in the right heart and pulmonary artery are greater than in the left heart and aorta, respectively, because of this high resistance. The majority of oxygenated blood entering the right atrium is directly shunted across the foramen ovale to the left atrium, thence to the left ventricle, carotid arteries, and brain. The remaining blood entering the right atrium flows via the right ventricle and pulmonary artery to be shunted across the ductus arteriosus to the descending aorta. The two sides of the fetal heart act in parallel and the right ventricle is dominant in both size and load.

Development of the fetal lung begins with the glandular stage when branching and budding of the bronchial tree is occurring. Progression to the canalicular stage occurs at approximately 190–210 days in the equine fetus. During the canalicular stage, the development of acinar structures consisting of respiratory bronchioles, alveolar ducts, and rudimentary alveoli occurs. Surfactant-containing lamellar bodies are visible in type II pneumocytes and differentiation from type II into type I cells occurs. Further differentiation of terminal airways and acinar tubules resulting in a large expansion of the gas exchange surface area occurs during this final phase of development. There is an increase in the number of surfactant-containing lamellar

bodies, and the maturation of type I cells and the closely associated capillaries also occurs during this final developmental stage.

Once the foal's placenta detaches during parturition, hypoxia, hypercapnia, and acidosis drive the respiratory effort to breathe. The first extrauterine breath requires generation of a large subatmospheric pressure to open airways and alveoli for the first time. During this first inhalation, as the alveoli open, surfactant present in the mature fetal lungs is released from the type II alveolar epithelial cells and spreads out along the alveolar surface. As surfactant spreads along the air–fluid interface, it decreases the surface tension, helping to stabilize the alveoli and preventing alveolar collapse during exhalation. The large increase in lung volume (resulting in mechanical stretch of the pulmonary vasculature) and the increased oxygen tension in the alveoli cause passive and active vasodilatation. This results in a decrease in pulmonary vascular resistance and an increase in pulmonary blood flow (Raj & Shimoda 2002). As resistance falls, the pressure across the pulmonary circulation falls below that of the systemic circulation. The increased capacitance of the pulmonary circulation that is associated with pulmonary vasodilatation accommodates the increase in cardiac output directed through the lungs. In addition, the removal of the large placental vasculature results in a decrease in systemic vascular area and systemic resistance rises accordingly. The foramen ovale is a one-way valve that closes when the pressure in the left atrium exceeds the pressure in the right atrium. Similarly, when aortic pressure exceeds pulmonary arterial pressure, blood flow across the ductus arteriosus reverses. The ductus arteriosus physically closes during the ensuing first days of life. Physiological stimuli for closure include increased oxygen tension and decreased levels of circulating prostaglandins. Persistent flow of a small volume of blood through the ductus arteriosus can be auscultated as a systolic murmur for the first few days of life.

To allow functional gas exchange, the neonatal lung must remain inflated and the foal must establish a functional residual capacity that provides a reservoir of air for gas exchange. Pulmonary surfactant is essential for this because it reduces the surface tension of the air–liquid interface that lines the alveoli. Surfactant is a mixture of dipalmitoyl phosphatidylcholine and proteins that is produced by the type II alveolar cells. Surfactant is produced in the equine fetal lung by 100 days of gestation and pulmonary development is considered complete by approximately 300 days' gestation; however, this maturation can vary depending on external factors (Arvidson et al 1975, Pattle et al 1975) (see Prematurity in the following section). Surfactant is continually being turned over within the lung and its concentration on the alveolar surface depends on
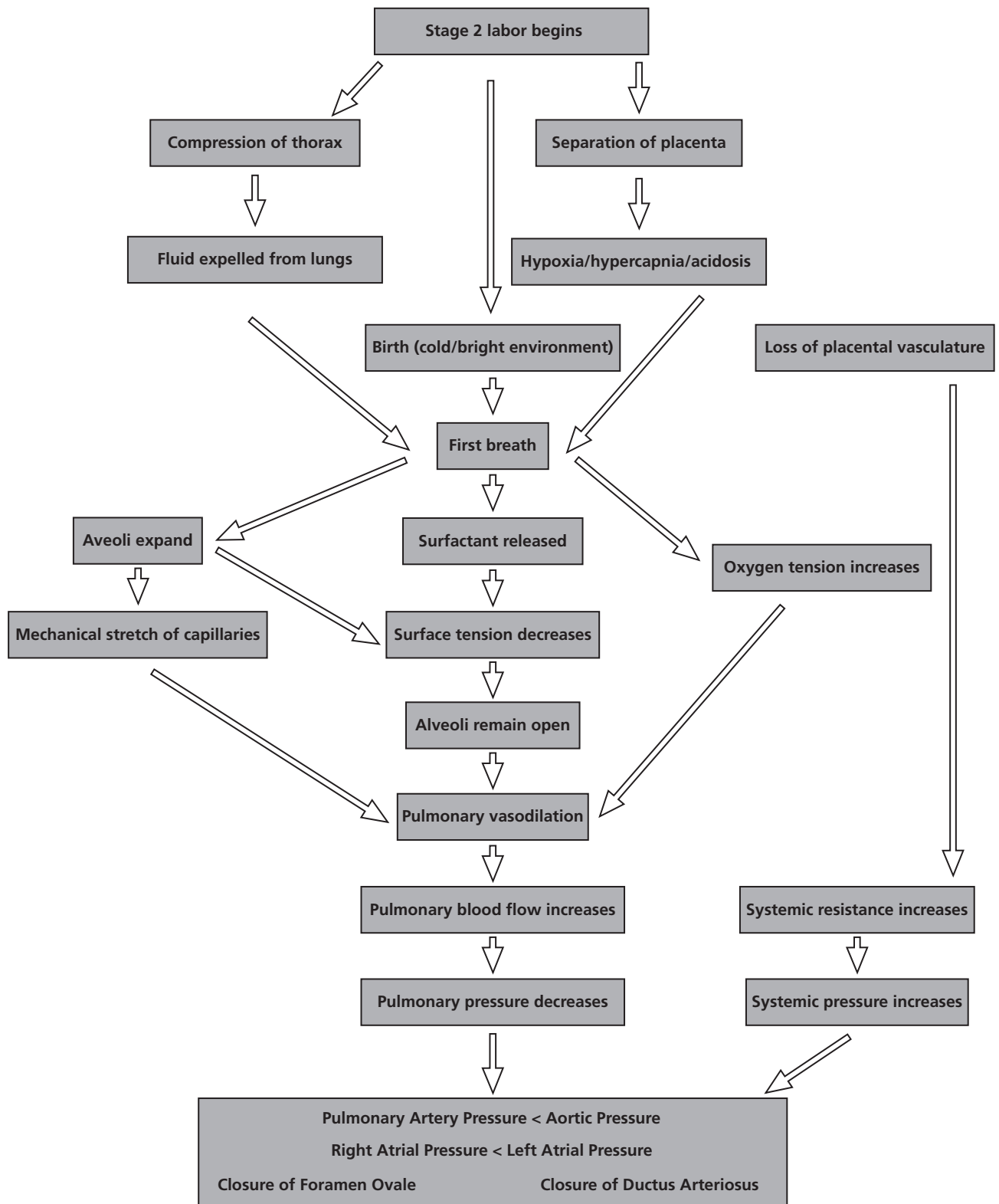
Fig. 45.7. Diagram of cardiopulmonary changes occurring at birth.

its rate of production and breakdown. Many factors that accompany neonatal lung disease facilitate the destruction of surfactant or reduce its production. Meconium is toxic to the type II epithelial cells in which surfactant is produced, whilst inflammatory mediators, reactive oxygen species, and meconium directly destroy or inactivate surfactant (Oelberg et al 1990, Moses et al 1991, Sun 1993b, Holopainen 1999, Dargaville & McDougall 2001, Tollofsrud et al 2003, Fuhrman 2004).

Unlike the adult horse, in which a stiff chest wall prevents collapse of the lung in the face of airway obstruction, the neonate has a compliant chest wall that cannot prevent lung collapse. For this reason, the neonate is prone to develop atelectasis when there is a lack of surfactant or when an intrapulmonary airway becomes obstructed. The compliant chest also tends to facilitate collapse of the dependent lung when foals are laterally recumbent for a prolonged period of time.

Because the neonatal lung retains some of its fetal characteristics, it is prone to some complications that do not occur in adults. The fetal pulmonary circulation constricts in response to pulmonary hypoxia. When the fetus becomes hypoxic, constriction of the pulmonary circulation diverts blood away from the non-essential fetal lung to more vital organs via the ductus arteriosus. This high reactivity of the fetal circulation to pulmonary hypoxia is retained in the neonate. As a consequence, lung disease and its accompanying alveolar hypoxia can lead to constriction of pulmonary arteries and consequently pulmonary hypertension. If pressure in the pulmonary artery rises high enough, blood flow can reverse through the ductus arteriosus thus returning the foal to a fetal circulation.

Specific Respiratory Disorders of the Foal

Prematurity and primary surfactant deficiency

Most equine clinicians define prematurity in foals as a gestation of 320 days or less. However, gestational age alone does not appear to correlate completely with the risk of prematurity or primary surfactant deficiency. Many foals considered premature by gestational duration have normal respiratory function and physical characteristics, while some term foals have respiratory dysfunction and/or physical evidence of prematurity (short, silky hair coat, domed head, floppy ears, tendon laxity, and small body size). As noted, pulmonary development is considered complete by approximately 300 days gestation, however, this maturation can vary depending on external factors (Arvidson et al 1975, Pattle et al 1975). Chronic *in utero* stress hastens maturation of both the lung and hematological systems, although the underlying mechanism is unclear. Consequently, a foal born prematurely to a mare with a chronic illness may be developmentally more

mature than a foal born prematurely in a mare with no previous history of illness or problems. Chronic placentitis or other maternal factors may retard fetal development resulting in a dysmature foal with inadequately developed pulmonary anatomy and insufficient surfactant. Regardless of the history, any foal with a gestational age of less than 320 days, or physical characteristics of prematurity, should be monitored carefully for signs of respiratory disease and dysfunction.

Without surfactant, alveoli tend to collapse at end expiration. The highly compliant chest wall of the equine neonate permits this by failing to prevent lung collapse to below normal functional residual capacity. Weak or hypoglycemic foals are unable to adequately re-expand these alveoli, further exacerbating the problem.

Exposure to the postnatal environment before complete lung maturation has several consequences. Inadequate gas exchange surface area results in decreased oxygen uptake and hypoxemia. Insufficient quantity of surfactant results in increased alveolar surface tension and alveolar collapse at lower lung volumes. Incomplete maturation of type I alveolar cells exacerbates gas exchange difficulties resulting in further hypoxemia. Repetitive collapse and re-expansion of distal airways and alveoli results in shearing trauma, disrupting the gas exchange membrane further and causing protein and edema leakage into the alveolar spaces.

In addition to the direct consequences of being born before complete lung maturation, exposure of the premature lung to the postnatal environment can negatively affect further lung maturation and result in acute lung injury and ultimately chronic lung disease. Both hypoxia and hyperoxia have been shown to disrupt lung maturation (Randell et al 1989, Thibeault et al 1990, Kotecha 2000), probably as a result of the action of pro-inflammatory cytokines and reactive oxygen species (Warner et al 1998). Antioxidant enzyme concentrations are deficient in the developing lung at a time when production of reactive oxygen species is accelerated (Kotecha 2000). Lipid peroxidation appears to be the main oxidative stress on the neonatal lungs. Major causes of increased production of reactive oxygen species include increased inspired oxygen concentrations, ischemia–reperfusion injury, activation of the arachidonic acid cascade and inflammatory cell activation.

Many of the interventions performed to improve pulmonary function can further exacerbate lung injury and inflammation. Mechanical ventilation that is used to improve ventilation and gas exchange also results in further injury as a result of regional overinflation (volutrauma) and positive-pressure ventilation (barotrauma). The premature lung is particularly susceptible to the trauma associated with mechanical ventilation because surfactant may be deficient, the lung matrix is not fully developed, and residual fetal fluids may remain. Excess free radical generation as a result of oxygen toxicity results in

further inflammation in the lung. Consequently, it is easy to see why neonatal respiratory distress syndrome develops and why it can be so difficult to treat.

Clinical signs

At birth, premature foals may show few if any signs of respiratory disease. A slight increase or exaggeration in rib movement may be the first clinical sign of surfactant deficiency. Over time, an increasingly exaggerated respiratory effort, with abdominal breathing and paradoxical chest wall movement, becomes apparent. In an effort to maintain functional residual capacity, the foal may have a prolonged expiratory phase and an end-expiratory grunt may be heard. The foal may be weak and have a poor suckle as hypoxia progresses. The severity of clinical signs in the premature foal depends on many factors, including the extent of prematurity and the severity of the hypoxia, as well as complicating factors including bacterial sepsis, and meconium or milk aspiration. Reversion to fetal circulation and the development of pulmonary hypertension can occur if hypoxia is severe.

Treatment

Premature foals suspected of having surfactant deficiency should be treated with surfactant replacement therapy. Synthetic and animal-derived surfactant products are available. Because of continued degradation of surfactant by inflammatory enzymes and inactivation by surfactant inhibitors, re-treatment may be required to maintain respiratory function (see Surfactant therapy p. 651).

In addition to surfactant replacement therapy, affected foals require oxygen supplementation and mechanical ventilation (see Intensive management of the foal respiratory system p. 649). Broad-spectrum antimicrobial therapy is recommended because of the increased risk of complicating pneumonia and sepsis in these neonates. Foals should be kept in sternal recumbency or standing as much as possible to improve ventilation–perfusion ($\dot{V}/\dot{Q}$) matching.

The prognosis for foals with prematurity and surfactant deficiency depends on the degree of prematurity and potential complications (bacterial pneumonia, systemic inflammatory response syndrome). In general, foals born at a gestational length of ≤ 300 days have a relatively poor prognosis. For foals born after 300 days' gestation, the prognosis depends on additional factors including prepartum illness in the mare, speed of recognition and institution of treatment, and development of secondary complications.

Persistent pulmonary hypertension

Persistent pulmonary hypertension (PPH), also referred to as reversion to fetal circulation, occurs when the cardiopulmonary transition from fetus to foal fails or when disturbance causes reversion back to fetal func-

tion after successful transition. Causes of PPH can be divided into three categories.

Reactive PPH is secondary to underlying pulmonary parenchymal disease such as meconium aspiration or infectious pneumonia. Reactive PPH is triggered by inflammatory and obstructive effects of pulmonary diseases, ventilation–perfusion mismatching, and hypoxemia. The most common cause of reactive PPH in human neonates is meconium aspiration (Findlay et al 1996). Depending on the severity of the underlying pathology, the resulting pulmonary hypertension and hypoxemia may result in reversion to fetal circulation with reopening of the foramen ovale and ductus arteriosus. A similar pathogenesis occurs with bacterial and viral pneumonias, whether the exposure is hematogenous or by inhalation (Hintz et al 2000).

The second category of PPH is the result of structural or functional cardiac or pulmonary abnormalities. Congenital diaphragmatic hernia is associated with underdevelopment of the pulmonary vascular bed, increased pulmonary vascular smooth muscle, and PPH in human infants. Congenital hypoplasia or dysplasia of the lungs can also result in pulmonary hypertension; however, these conditions have not been reported in the equine neonate. Tetralogy of Fallot or pulmonary artery atresia can result in pulmonary hypertension and is associated with a poor outcome (Vitums et al 1973, Reimer et al 1993, Meurs et al 1997). Cardiac-disease-associated PPH will not be discussed in this chapter.

PPH is caused by structural or biochemical derangements of pulmonary circulation and pulmonary vasomotor systems. At birth, increased oxygen concentration stimulates synthesis of vasodilators including nitric oxide, prostacyclin, and prostaglandin E_2. The mechanical effects of lung expansion coupled with the increase in local vasodilators results in pulmonary vascular dilation. Vasodilator effects are mediated by increased intracellular production of cyclic GMP (Raj & Shimoda 2002). Patients with PPH have a reduced production or decreased response to these vasodilators, particularly if hypoxemia persists (Fike & Kaplowitz 1996, Fike et al 1998, Berkenbosch et al 2000). In addition, chronic *in utero* hypoxemia can result in pulmonary vascular remodeling and increased amounts of arteriolar smooth muscle making resolution of PPH after birth difficult (Rabinovitch et al 1979, Steudel et al 1998). In humans, increased pulmonary vascular resistance may develop in fetuses from mothers treated with non-steroidal anti-inflammatory drugs such as indomethacin, but this has not been described in the horse (Tyler et al 1975, Leffler & Cassin 1978).

Clinical signs and diagnosis

Definitive diagnosis of PPH requires documentation of elevated pulmonary artery pressure. This requires passing a catheter via the jugular vein through the right heart into the pulmonary artery to measure pressures. This is

rarely performed in equine neonatology and a tentative diagnosis is generally made using arterial blood gas measurements, response to oxygen supplementation as well as radiographic and ultrasonographic findings. A diagnosis of PPH should be suspected in any foal with severe hypoxemia that is poorly responsive to supplemental oxygen therapy. In cases of PPH, hypoxemia is severe and non-responsive to oxygen therapy as a result of the right to left shunting of a large percentage of the cardiac output in the lungs, and through the foramen ovale and ductus arteriosus. Arterial oxygen content remains low because the blood is never exposed to the oxygen-rich alveolar gases. In contrast, hypoxemia because of ventilation–perfusion mismatching (as may be seen with aspiration pneumonia) without pulmonary hypertension is generally responsive to oxygen supplementation though the response depends on the severity of the underlying disease.

In primary PPH, thoracic radiographs may show evidence of decreased pulmonary vascular markings (Cottrill et al 1987). Physical examination findings of a persistent cardiac murmur, coupled with echocardiographic findings of normal heart structure with right to left shunting through the foramen ovale and ductus arteriosus, are further evidence to support the presence of PPH (Southwood et al 1996).

Treatment

Treatment of PPH should include treatment of any underlying disease process as well as the pulmonary hypertension. Hypoxemia and acidosis trigger pulmonary vasoconstriction; consequently, correcting these abnormalities is vital to the resolution of PPH. Nasal oxygen insufflation should be instituted immediately upon hospitalization. If nasal insufflation fails to significantly improve hypoxemia, mechanical ventilation with supplemental oxygen is indicated. In acute or mild cases, correction of hypoxemia and acidosis may be enough to trigger pulmonary vasodilatation and resolution of pulmonary hypertension.

In refractory cases, inhaled nitric oxide (NO) is recommended to induce relaxation of the pulmonary vasculature. Inhaled NO disperses across the alveolar capillary membrane causing increased cGMP in vascular smooth muscle, so inducing vasodilation. NO is rapidly degraded; consequently the effects of inhaled NO are confined to the local pulmonary circulation without affecting systemic vascular resistance. Inhaled NO has been shown to improve oxygenation, decrease the need for ventilatory support and extracorporeal membrane oxygenation and improve survival in human infants with PPH (Smyth 2000, Finer & Barrington 2001, Goh et al 2001, Sadiq et al 2003). Methemoglobin levels should be monitored during NO treatment, because NO combines with oxyhemoglobin to produce methemoglobin.

Although there are currently no published reports of the efficacy of inhaled NO in the management of PPH in the equine neonate, inhaled NO was effective in an experimental model of PPH in neonatal foals (Lester et al 1999). Inhaled NO attenuated the pulmonary hypertension induced by both hypoxia and the thromboxane mimetic, U46619. The response was dose-dependent between 20 and 160 ppm; however, the equipment used to measure NO flow rates was imprecise. Whether doses of this magnitude are truly needed to effectively resolve PPH in foals remains to be determined as there are anecdotal reports of successful resolution of PPH in equine neonates using concentrations as low as 5 ppm (Wilkins 2003). In human infants, concentrations of > 22 ppm are associated with higher methemoglobin concentrations without any clear benefit on survival or length of hospitalization when compared to lower dosage regimens (Guthrie et al 2004). As many as 30% of human infants fail to respond to inhaled NO therapy and non-responders commonly have underlying pulmonary parenchymal disease (Goldman et al 1996). Rebound PPH has been reported during attempts to wean patients from inhaled NO therapy (Davidson et al 1999).

Magnesium sulfate infusions decrease pulmonary hypertension in human neonates. Because magnesium is relatively inexpensive and easily obtained, it is particularly useful in developing countries (and possibly veterinary clinics) where more expensive therapies may not be readily available or affordable. In a clinical trial in 12 human infants with severe PPH, infusions of magnesium sulfate resulted in improved oxygenation and improved D_{A-aO_2} (Chandran et al 2004). In this report, magnesium sulfate was infused continuously to achieve serum levels of 3.5–5.5 mmol/liter (8.5–13.4 mg/dl). Dopamine infusions were necessary to maintain systemic vascular pressures during treatment. Magnesium sulfate given by bolus injection also causes a significant decrease in pulmonary artery pressure (Haas et al 2002). While magnesium sulfate therapy may be beneficial in PPH, hypermagnesemia can result in central nervous system depression and hypoventilation requiring mechanical ventilation to maintain adequate gas exchange.

Several phosphodiesterase inhibitors effectively resolve pulmonary hypertension. Sildenafil is a specific inhibitor of phosphodiesterase-5, an enzyme that occurs in high concentrations in the smooth muscle cells of the pulmonary vasculature. Inhibition of phosphodiesterase-5 leads to local increase in cGMP that relaxes pulmonary vascular smooth muscle (Rabe et al 1993). In both human and animal experimental models, sildenafil dose-dependently attenuates PPH, in some cases more effectively than inhaled NO (Weimann et al 2000, Ichinose et al 2001, Zhao et al 2001, Lepore et al 2002, Keller et al 2004). It has been used successfully to ameliorate rebound

PPH during weaning from inhaled NO (Lepore et al 2002, Keller et al 2004). While case reports of successful resolution of PPH using sildenafil exist, there are currently no published randomized clinical trials evaluating the efficacy of sildenafil in treatment of neonatal PPH (Atz & Wessel 1999, Michelakis et al 2002, Watanabe et al 2002, Carroll & Dhillon 2003, Mehta 2003). Although used acutely sildenafil has minimal effects on systemic arterial pressures (Weimann et al 2000), but chronic therapy can be associated with systemic hypotension (Ghofrani et al 2003). In a porcine meconium aspiration model of pulmonary hypertension, sildenafil, in combination with inhaled NO, lowered systemic blood pressure and systemic resistance, resulting in profound arterial hypoxemia despite increases in inspired oxygen (Shekerdemian et al 2004). More information is needed regarding the efficacy and potential contraindications of sildenafil use in neonates, particularly those with underlying parenchymal disease or sepsis.

Surfactant supplementation improves hypoxemia and lung function by maintaining alveolar volumes at lower inspiratory pressures and aids in the resolution of PPH (see Surfactant therapy, p. 651, for further discussion).

The prognosis for foals with PPH is fair to good if the condition is recognized early. If recognition and treatment are not instituted quickly, subsequent inflammatory injury and bacterial invasion may result in a poorer prognosis.

Meconium aspiration

Meconium is composed of the remains of glandular secretions, swallowed amniotic fluid, and cellular debris. It is moved along the gastrointestinal tract and stored in the distal colon and rectum. Meconium is a dark brown to black pellet or paste and is usually expelled shortly after birth. *In utero*, hypoxemic fetal stress can result in fetal peristalsis and early expulsion of meconium into the amniotic fluid. Asphyxia triggers fetal gasping, resulting in meconium aspiration into the lungs. The pulmonary effects of meconium aspiration are induced by direct toxic injury to cells, mechanical obstruction as the result of accumulation of debris and secretions, inflammatory cellular influx and activation, and reduced activity of surfactant.

Depending on the severity of aspiration, pathophysiological effects vary from mild ventilation–perfusion mismatching, hypoxemia, and airway obstruction to respiratory failure and pulmonary hypertension. Because meconium is a thick viscous substance it can completely obstruct the airway and cause alveolar collapse or cause intermittent obstruction with air trapping and pulmonary overinflation. The abnormally high lung volumes that develop with intermittent air trapping can result in air

leaks, pneumothorax or interstitial emphysema. Meconium is approximately 75% water with the solid fraction containing lipids (including free fatty acids, triglycerides, and cholesterol) and water-soluble components (including bilirubin, bile acids, and fiber). Bile salts are directly toxic to pneumocytes resulting in epithelial cell injury, apoptosis, and inflammation (Oelberg et al 1990). Both the water-soluble and lipid-soluble fractions of meconium induce inflammatory cell influx, cytokine production and enzyme activation (de Beufort et al 1998, Holopainen et al 1999, Castellheim et al 2004). The inflammatory response and damage to the respiratory membrane results in leakage of proteinaceous edema fluid and alveolar flooding. Alveolar atelectasis, edema accumulation, and airway plugging exacerbate ventilation–perfusion mismatching, hypoxemia, and hypercapnia. In severe cases, profound hypoxemia and acidosis result in pulmonary vascular constriction, pulmonary hypertension, right to left shunting, and further deterioration of ventilation–perfusion mismatching. Surfactant deficiency results from increased degradation of surfactant by enzymes and inflammatory mediators, decreased production as the result of toxic effects on type II pneumocytes, and inhibition of surfactant function by surfactant inhibitors present in meconium (Oelberg et al 1990, Moses et al 1991, Sun 1993b, Holopainen et al 1999, Dargaville & McDougall 2001, Tollofsrud et al 2003, Fuhrman 2004). These inhibitors affect the ability of surfactant to form a monolayer and may inhibit the phospholipid enrichment process that is thought to stabilize the monolayer during compression (Holm & Notter 1999).

Clinical signs

Meconium aspiration syndrome is characterized by evidence of airway obstruction, alveolar atelectasis, pulmonary inflammation, and respiratory failure. Meconium aspiration should be suspected in any difficult or delayed foaling and in foals born with meconium present in the amniotic fluid or with evidence of meconium staining (yellow-brown discoloration) of the coat. However, although meconium-stained amniotic fluid is seen in 10–15% of human births, only approximately 5% of these infants develop meconium aspiration syndrome. The finding of meconium in fluid suctioned from the trachea is further confirmation of aspiration and potential respiratory compromise.

Treatment

Foals suspected of having meconium aspiration should receive supplemental oxygen and have attempts made to remove meconium by suction as soon as possible. Further treatment will be dictated by the severity of clinical signs. Mild cases of meconium aspiration syndrome are treated

with supportive care and oxygen therapy. Ventilatory support is instituted in individuals with evidence of respiratory failure but positive pressure ventilation can result in further air trapping (volutrauma) and baro-trauma. In severe cases of meconium aspiration syndrome the use of extracorporeal membrane oxygenation has improved survival in human infants (Hansell 2003). Surfactant therapy improves respiratory function and decreases the duration of mechanical ventilation, oxygen therapy and hospitalization (Sun 1993a,b, Findlay et al 1996, Cochrane et al 1998, Lotze et al 1998). Depending on the severity of the ongoing inflammatory response, as well as the volume of inhaled meconium, multiple doses of surfactant may be required to maintain respiratory function because of ongoing degradation and continued inhibition. Recent data suggest that the addition of polymers such as dextrans to surfactant solutions may reduce surfactant inhibition in cases of meconium aspiration syndrome (Tashiro & Robertson 2000).

The use of serial, dilute surfactant lavages to remove residual meconium has been reported to improve outcome in human infants with meconium aspiration syndrome; however, a significant portion of infants had to have the procedure halted after developing severe hypoxemia during lavage (Wiswell et al 2002). While the idea of removing residual particulate meconium is attractive, the ensuing inflammatory response appears to be the major factor in the development of acute injury in the lungs. Thus the cost–benefit of these lavages presently is unclear. Smaller volume lavage may offer similar benefits with less potential for complications (Cochrane et al 1998).

Inhaled NO is used in patients with clinical or cardiovascular evidence of pulmonary hypertension. Inhaled NO has been less successful in resolving hypertension in infants with meconium aspiration syndrome than in those with primary PPH (The Neonatal Inhaled Nitric Oxide Study Group 1997). This may be the result of the inability of NO to diffuse down meconium-plugged airways. The use of inhaled NO is discussed in more detail under PPH.

The prognosis for foals with meconium aspiration depends on the severity of aspiration and development of secondary bacterial infection. Uncomplicated cases of mild aspiration may be unrecognized. In more severe cases the prognosis depends on early recognition and aggressive treatment.

Bacterial pneumonia

Bacterial infection is the most common causes of pneumonia in the equine neonate, with bacteremia and aspiration pneumonia the most common routes of exposure. The lungs are the most frequently affected organs in cases of neonatal septicemia. Aspiration can result from congenital anomalies such as a cleft palate, dysphagia secondary to white muscle disease, peripartum asphyxia syndrome, septicemia, or during bottle or syringe feeding.

Foals are at higher risk for bacteremia and sepsis than adults. Foals are born with a functional but naive immune system. The neonatal foal's neutrophils are deficient in phagocytic ability and hydrogen peroxide release making the foals more susceptible to early infection (Demmers et al 2001, McTaggart et al 2001). While functional T cells are present at birth, *in vitro* tests suggest that cell-mediated immunity is still deficient. Foals are capable of producing antibody by 200 days' gestation, but B-cell numbers may not reach adult levels until several weeks of age.

Because of the diffuse epitheliochorial placentation of the mare, foals are born without protective passive immunity and must ingest colostrum during the immediate post-foaling period to receive passive antibody from the mare. Colostrum ingestion not only results in antibody absorption, but also provides local non-specific immunity in the gastrointestinal tract. If a foal does not ingest colostrum during the immediate post-foaling period, the gastrointestinal tract is open to invasion by pathogens. During udder seeking, the foal may suckle dirty environmental surfaces or the contaminated perineal area of the mare resulting in ingestion of potentially pathogenic bacteria. If ingestion of pathogens occurs before ingestion of colostrum, invasion is enhanced and bacteremia is likely. The suckle reflex is one of the first behaviors to deteriorate with illness; consequently the septicemic foal is at greater risk for aspiration of milk when attempting to nurse. Aspiration of milk can also occur when well-intentioned owners attempt to bottle-feed an inappetant foal, resulting in further contamination of the lower respiratory tract. Meconium aspiration during a difficult or delayed parturition can also result in pneumonia and bacterial contamination of the lungs.

Clinical signs

Because the lungs are the most commonly affected organ system in septicemic foals, routine evaluation including thoracic radiography, arterial blood gas, and thoracic ultrasound should be performed in any foal suspected of being septic. An elevated respiratory rate, presence of abnormal lung sounds on auscultation, and evidence of alveolar disease on radiographic or ultrasonographic examinations are strong evidence of pneumonia. In sick or weak neonates with a poor suckle reflex, the likelihood of aspiration can be detected by auscultating over the trachea as the foal nurses. As discussed previously, the finding of normal lung sounds on auscultation of the thorax should not be overly interpreted because many foals with pneumonia have few if any abnormal adventitial sounds. Radiographs are useful to assess the severity and type of pneumonia. Foals with aspiration pneumonia typically

have ventral consolidation whereas the distribution of lung disease is often more diffuse in septicemic foals. The latter is the result of the hematogenous origin of the bacteria. Ultrasound evaluation is particularly sensitive in detecting abnormalities in acute pneumonia when clinical and radiographic abnormalities may be minimal. Because of the reflection of the majority of ultrasound waves at an air–tissue interface, ultrasound is not accurate in determining the severity of deeper pathology. For this reason, it is recommended that both thoracic radiography and ultrasound be performed to completely evaluate the pulmonary parenchyma.

Bacterial infection is the most common cause of lower respiratory disease and for this reason antimicrobial treatment should be instituted in any foal presented with lower respiratory disease until bacterial involvement is disproved. Definitive diagnosis of bacterial pneumonia is made by culture and cytology of transtracheal aspirates or bronchoalveolar lavage fluid. If respiratory samples are not available for culture, blood culture results (in combination with clinical evidence of pneumonia) can be used as presumptive evidence of bacterial pneumonia. While helpful, blood culture results should be interpreted cautiously. In one study of neonatal septicemia, approximately 20% of foals that were negative on blood culture had bacteria cultured from tissues at the time of postmortem examination (Wilson & Madigan 1989). In addition, blood cultures frequently do not detect multiple bacterial pathogens. The organisms most commonly missed on blood cultures are Gram-negative aerobes. Approximately 50% of bacterial pneumonias have mixed infections including both Gram-negative and Gram-positive pathogens (Wilson & Madigan 1989). Common pathogens in septicemia and bacterial pneumonia include *Escherichia coli*, *Klebsiella pneumoniae*, *Actinobacillus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Enterobacter* spp., *Serratia* spp., *Proteus* spp., and, less commonly, anaerobes including *Clostridium* spp., *Bacteroides* spp., and *Fusobacterium* spp. (Wilson & Madigan 1989, Brewer & Koterba 1990, Marsh & Palmer 2001).

Treatment

The cornerstone for treatment of bacterial pneumonia is broad-spectrum antimicrobial therapy that can be adjusted once culture and sensitivity results are available. In weak foals that are unable or unwilling to nurse, supplemental nutrition should be provided by nasogastric intubation or, if unable to utilize the gastrointestinal tract, via parenteral nutrition. Because of their high metabolic rate and minimal body fat, nutritional support needs to be an integral part of the treatment of foals. Non-steroidal anti-inflammatory drugs are useful in controlling inflammation and minimizing systemic signs (fever, malaise) but should be used cautiously because of the increased susceptibility of foals to their toxic effects, including gastrointestinal ulceration and nephrotoxicity. Because the neonatal chest wall is more compliant than the adult, the dependent lung frequently becomes atelectatic, worsening ventilation–perfusion mismatching. Consequently, foals should be turned frequently or kept in sternal recumbency whenever possible. Oxygen supplementation should be provided in any hypoxemic foal (see Chapter 14) or foal exhibiting respiratory distress or cyanosis.

Because of the potential for false-negative culture results, the response to treatment should be monitored closely and re-evaluation performed if a patient fails to show improvement within 24–48 h. Clinical response should include a resolution of fever, improved attitude and appetite, decrease in respiratory rate or effort, and improved arterial oxygenation. Improvement in lung sounds is not always useful in assessing progress as these frequently worsen during disease resolution. This is because air begins to flow into regions of diseased lung that previously received no airflow. Normalization of white blood cell parameters and serum fibrinogen measurements are also useful in assessing the response to treatment.

The prognosis for foals with bacterial pneumonia varies depending on many factors including the route of infection, underlying disorders, and other sites of infection. In general the prognosis is considered fair to good in uncomplicated cases.

Viral pneumonia

In young foals, viral pneumonia is a relatively rare occurrence but is almost universally fatal. The vast majority of viral respiratory infections in horses result in upper respiratory tract signs rather than pneumonia, particularly in naive weanlings and young adults. Viral pathogens associated with neonatal pneumonia include equine herpes virus-1 (EHV-1), EHV-2, and less commonly EHV-4, equine influenza virus (EIV), equine adenovirus, and equine arteritis virus (EAV).

EHV-1 infection in horses can result in multiple syndromes including late-term abortions, weak or stillborn term foals, upper respiratory infection in weanlings and adult horses and neurological disease (McCartan et al 1995). In neonatal foals, EHV-1 is the most common viral pathogen and almost without exception results in fatal pneumonia and sepsis. Clinical signs in neonates infected with EHV-1 include icterus, petechial hemorrhages, and respiratory distress (Perkins et al 1999). The finding of dilated retinal vessels with retinal hemorrhages has been reported to be a common clinical finding in affected foals. Complete blood counts frequently reveal a toxic, neutropenic, lymphopenic leukopenia; however, these findings are also common in neonatal septicemia making

them unreliable as diagnostic criteria without additional information or a history of a herd outbreak. Post-mortem findings include severe diffuse interstitial pneumonia, hepatitis with viral inclusion bodies and bone marrow depletion. The virus is likely spread from mare and foal reservoirs to other foals and weanlings; consequently the separation of pregnant mares from other horses and foals is an important mechanism for controlling spread and infection of mares in late-term gestation (Gilkerson et al 1999).

EHV-2 also has been reported as a cause of respiratory disease and pneumonia in young foals. EHV-2 is ubiquitous in the equine population and is rarely the cause of significant clinical disease in adult animals (Borchers et al 1997, Kershaw et al 2001). In one abbatoir study, EHV-2 was isolated from tissues in 39 of 40 horses (Edington et al 1994). The majority of infected foals exhibit mild upper respiratory signs but pneumonia, immunosuppression, and death are reported. Epidemiological studies suggest that the majority of foals become exposed to EHV-2 within the first few months of life and subsequently shed the virus for several months (Fu et al 1986, Murray et al 1996).

EHV-4 is primarily associated with respiratory disease outbreaks in young adult horses, particularly those housed in large, transient groups. Exposure to EHV-4 appears to occur early in life with the vast majority of foals becoming seropositive by 2 months of age (Gilkerson et al 1991, Foote et al 2004). Clinical illness is principally an upper respiratory tract disease with high morbidity in naive groups and low mortality.

EAV is a non-arthropod-borne RNA virus in the family Arteritiviridae. Infection of adult individuals with EAV typically results in fever, leukopenia, limb and ventral edema, conjunctivitis, epiphora, and rhinitis (Del Piero et al 1997). Infected foals may present with acute onset of respiratory distress and sudden death (Szeredi et al 2003). Epidemic abortions have also been reported. Affected stallions can become long-term carriers and shed virus in their semen, thus acting as a viral reservoir in the equine population (Gilbert et al 1997). Affected foals present with severe respiratory distress of acute onset. Radiographic findings are consistent with an interstitial pneumonia. Cytological abnormalities of a bronchoalveolar lavage sample in an affected foal included mucus, fibrin, macrophages, epithelial necrosis, and squamous metaplasia (Wilkins et al 1995). The disease is almost uniformly fatal in neonatal foals. Post-mortem findings include interstitial pneumonia with edema and hemorrhage and hyaline membranes in the alveoli. The inflammatory infiltrate is predominantly macrophages with rare neutrophils. Vascular changes include vasculitis, swollen endothelial cells, and fibrinoid necrosis. Viral agents in the endothelial cells and macrophages can be detected using immunohistochemical staining for EAV.

Adenoviral pneumonia has been recognized in immunodeficient foals including Arabian foals with combined immunodeficiency (CID) (Dutta 1975, Whitlock & Shively 1975, Henry 1976, Thompson et al 1976, Perryman et al 1978, Webb & Walker 1981). Equine adenovirus was isolated from 43 of 66 CID-affected foals with evidence of pneumonia (Perryman et al 1978). Only one of these foals had uncomplicated adenoviral pneumonia, the majority of the remainder having concomitant bacterial or fungal infections. Affected foals received hyperimmunized plasma on a weekly basis in an attempt to protect against adenoviral pneumonia. In immunocompetent foals, adenoviral infection rarely results in significant illness.

EIV infection has been reported in association with severe, acute, fatal interstitial pneumonia in two neonatal foals (Britton & Robinson 2002, Peek et al 2004). One case was associated with an upper respiratory tract disease outbreak in horses housed in close proximity (the neonatal intensive care unit) to the affected foal. EIV was isolated from both cases. Post-mortem findings were consistent with a viral pneumonia including a diffuse bronchiolitis, alveolar necrosis, hemorrhage, and fibrin exudation. Immunohistochemical staining for EIV was positive on lung tissue samples.

Acute interstitial pneumonias

Acute interstitial pneumonia is a relatively infrequent cause of acute, severe, respiratory distress and failure in foals from several days to several months of age. The underlying etiology is likely multifactorial with heat stress a common predisposing factor. Prognosis is poor with the majority of foals succumbing to respiratory failure.

Clinical signs include an acute onset of tachypnea, dyspnea, and respiratory distress. Affected foals are frequently cyanotic and the onset of signs is often rapid and severe. In one retrospective study of 23 cases, three foals were found dead on the farm, five died shortly after identification or during transit and two died shortly after arrival at the clinic (Lakritz et al 1993). A 39% (nine of 23) survival rate was reported.

Affected individuals have an elevated rectal temperature, severe respiratory distress, are often cyanotic, depressed, and unwilling to eat. Many individuals have a marked abdominal component to their expiratory effort. Auscultation reveals increased bronchial sounds (hollow tubular characteristics) over the larger airways with diminished sounds in the periphery, supporting decreased ventilation of small airways and terminal alveoli. In individuals that survive, as the lung disease resolves adventitial sounds (crackles and polyphonic wheezes) become increasingly audible in the peripheral lung fields likely as a result of improved ventilation of peripheral airways.

Clinicopathologic findings include hyperfibrinogenemia and a neutrophilic leukocytosis. All but one individual was in respiratory failure at the time of examination; arterial blood gas analysis consistently reveals severe hypoxia and hypercapnia with acidosis less commonly reported. Affected foals are poorly responsive to intranasal oxygen therapy suggesting left to right shunting and/or severe ventilation–perfusion mismatching.

The predominant radiographic lesions in foals with interstitial pneumonia are a diffuse interstitial or broncho-interstitial infiltrate. Lesions range from a diffuse interstitial infiltrate to a heavier, reticulonodular, bronchointerstitial pattern and coalescing alveolar infiltrates. In one retrospective study, multifocal alveolar infiltrates and pulmonary abscessation were also reported in 25% of cases examined radiographically (Lakritz et al 1993).

In the majority of cases the underlying etiology is unknown. Many agents have been proposed including bacterial, viral, fungal, and toxic agents (Buergelt et al 1986, Prescott et al 1991, Ainsworth et al 1993, Lakritz et al 1993, Perron Lepage & Suter 1999, Peek et al 2004). It is unlikely that interstitial pneumonia in foals is the result of one etiological agent; rather it is more likely to be the result of a common response of the lung to injury. Multiple bacterial pathogens have been isolated from transtracheal aspirates and post-mortem tissue specimens including *Rhodococcus equi*, *E. coli*, *Streptococcus* spp., *Actinobacillus equuli*, *Klebsiella pneumoniae*, *Bordetella bronchiseptica*, and *Pseudomonas* spp. (Prescott et al 1991, Ainsworth et al 1993, Lakritz et al 1993, Ewing et al 1994, Perron Lepage & Suter 1999, Nout et al 2002, Peek et al 2004).

Hyperthermia has been proposed to be the common factor triggering the onset of severe respiratory distress. The average environmental temperature was in excess of 32°C (90°F) in one case series and all foals were hyperthermic on admission (Lakritz et al 1993). Pentachlorophenol was found in one farm with two affected foals (Lakritz et al 1993). Pentachlorophenol uncouples oxidative phosphorylation and may have played a role in hyperthermia and heat stress resulting in acute respiratory distress. In other reports, viral and fungal agents have been isolated from affected foals. Therapy with erythromycin has been associated with hyperthermia and was a historical finding in some reported cases (Ainsworth et al 1993, Lakritz et al 1993).

Treatment of acute interstitial pneumonia should include broad-spectrum antibiotics, anti-inflammatory drugs, and oxygen therapy. Bronchodilators and non-steroidal anti-inflammatories have also been utilized with mixed results. In one case series, clinical improvement and survival appeared to be associated with glucocorticoid therapy (Lakritz et al 1993).

Post-mortem examination reveals lungs that are diffusely red, wet, and firm and that fail to collapse when the thorax is opened. Histopathological examination reveals various levels of necrosis of epithelial cells of the distal airways and alveoli. Proteinaceous edema, fibrin, and cellular debris are found within the distal airways and alveoli. Inflammatory cellular infiltrate is generally mild compared to bacterial pneumonia and principally consists of lymphocytes, plasma cells, and alveolar macrophages. Type II pneumocyte proliferation is a common finding.

Pneumocystis pneumonia

Pneumocystis pneumonia is an infrequent cause of respiratory disease in foals but should be suspected in any foal with non-responsive pneumonia, foals that are suspected of being immunocompromised, and foals with radiographic findings of interstitial or reticulonodular patterns in the lung fields. The finding of pneumocystis pneumonia is associated with a poor prognosis for survival. *Pneumocystis carinii* is a fungal pathogen of the respiratory tract of mammalian species. In humans, the vast majority of cases of pneumocystis pneumonia occur in immunocompromised individuals (Al Soub et al 2004). In domestic animals pneumocystis pneumonia has been reported in debilitated or immunocompromised dogs, foals, pigs, and goats (McConnell et al 1971, Farrow et al 1972, Seibold & Munnell 1977, Furuta et al 1994, Cabanes et al 2000). The complete life cycle of *P. carinii* is unknown. There are currently three identified forms of the organism, all of which exist within the mammalian lung: a trophic form that is the primary proliferative stage, a precyst, and a mature cystic form (Cushion 2004). An environmental reservoir or infectious form has yet to be identified.

While the exact mode and source of transmission are unclear, asymptomatic carriers are found in both humans and rats and airborne transmission from asymptomatic carriers to immunosuppressed individuals has been reported (Hong et al 1992, Helweg-Larsen et al 1998, 2002, Dumoulin et al 2000). Subsequent immunosuppression of carrier rats resulted in clinical pneumocystis pneumonia. While transmission between individuals in close contact has been reported, it does not seem to be the primary route of transmission (Helweg-Larsen et al 1998, Beard et al 2000, Manoloff et al 2003). Early neonatal exposure may play a role in infection and transmission (Pifer et al 1978, Miller et al 2002). In a retrospective analysis of respiratory samples from 367 people with bacterial pneumonia, the presence of pneumocystis DNA was associated with old age, glucocorticoid therapy, and concurrent disease (Helweg-Larsen et al 2002). Human and animal models suggest that low numbers of pneumocystis organisms are necessary for transmission and the organism can survive for a period of time in a healthy individual without causing clinical illness

(Dumoulin et al 2000, Gigliotti et al 2003). Whether immunocompromise of a latently infected individual, or a new exposure and infection results in clinical disease is unclear (Hughes 1998).

Pneumocystis pneumonia has been reported in foals with the vast majority of cases occurring in immuno-compromised individuals (Shively et al 1973, Perryman et al 1978, Whitwell 1992, Ainsworth et al 1993, Ewing et al 1994, Tanaka et al 1994, Perron Lepage & Suter 1999, Clark-Price et al 2004). *Pneumocystis carinii* is a common cause of pneumonia and mortality in Arabian foals with CID (Perryman et al 1978). In a three-case series, two foals had a history of receiving glucocorticoids before the onset of respiratory disease. Hypogamma-globulinemia has been reported in association with pneumocystis pneumonia (Whitwell 1992, Flaminio et al 1998). The significance of this finding is difficult to determine given the high prevalence of hypogammaglobu-linemia in 2- to 3-month-old foals. In one case series foals also had *Rhodococcus equi* pneumonia that may have made them more susceptible to subsequent infection with pneumocystis. *Rhodococcus equi* inhibits macrophage phagosome–lysosome fusion and may have affected cellular immunity to *P. carinii*. A transient T-cell deficiency was reported in two foals; both foals responded to treatment for pneumocystis pneumonia and their T-cell function normalized (Flaminio et al 1998, Clark-Price et al 2004). Whether this deficiency in functional T cells was the cause or the result of pneumocystis infection is unclear.

Foals with pneumocystis pneumonia typically present with an acute onset of severe respiratory distress and cyanosis. Many cases have a previous history of milder, chronic bacterial pneumonia that subsequently worsened despite antimicrobial treatment. Radiographic findings vary; the majority of cases have evidence of a diffuse, miliary, reticulonodular or diffuse, bronchointerstitial pattern on thoracic radiographs. Complete blood counts and serum chemistry findings are relatively non-specific. Arterial blood gas results vary with most cases having evidence of hypoxemia. Cough, mucopurulent nasal discharge and lymphadenopathy are reported in approximately half of cases. Many foals with pneumocystis pneumonia have evidence of mixed bacterial infections in their lungs.

Because pneumocystis proliferates in the alveolus, cytological examination of bronchoalveolar lavage samples appears to be a more sensitive diagnostic test than examination of transtracheal wash fluid (Golden 1986, Perron Lepage & Suter 1999, Clark-Price et al 2004). Silver stains are helpful in evaluating histopathological specimens for the presence of pneumocystis organisms. Immunohisto-chemistry and DNA amplification have also been used to diagnose pneumocystosis (Whitwell 1992, Ainsworth et al 1993, Ewing et al 1994, Peters et al 1994, Perron Lepage & Suter 1999, Jensen et al 2001).

Folic acid synthesis inhibitors are the treatment of choice for pneumocystis pneumonia. Trimethoprim–sulfamethoxazole (TMS-SMZ) has been used in all reported cases that have a successful outcome (Ewing et al 1994, Flaminio et al 1998, Clark-Price et al 2004). In one case, after the foal developed salmonellosis, treatment was switched to dapsone, a sulfone antimicrobial used for treatment of pneumocystis pneumonia in humans (Clark-Price et al 2004). Interferon-α and *Propionibacterium acnes* were used in two cases for non-specific immuno-stimulation. The prognosis for affected foals is guarded to poor with the majority of foals dying or being euthanized because of the poor response and severe hypoxemia.

At post-mortem the lungs are often enlarged, fail to collapse and are firm to palpation. A proliferative, interstitial pneumonia with foamy macrophages and occasional multinucleated giant cells with intracellular organisms is present. Type II pneumocyte hyperplasia, edema, and proteinaceous fibrinoid material is frequently found within the alveoli. Depending on the chronicity of illness, interstitial fibrosis may be present.

Idiopathic transient tachypnea

Idiopathic transient tachypnea and hyperthermia has been observed in neonatal foals most commonly in draft, Arabian and thoroughbred breeds. The syndrome is most commonly seen in warm and humid climates. Affected foals develop an elevated respiratory rate and body temperature. Clinical signs typically develop in the first few days of life and may persist for several weeks. Treatment includes body clipping, environmental temperature control, and cool water or alcohol baths. Treatment with broad-spectrum antimicrobials is generally recommended until underlying bacterial pneumonia can be ruled out. The presence of rib fractures should be determined as they can result in an elevated respiratory rate without overt evidence of pulmonary disease.

Neonatal acute respiratory distress syndrome

While the insult leading to neonatal acute respiratory distress syndrome (ARDS) may be different in neonates and adults, the definition remains the same. The syndrome of ARDS is the end result of an inflammatory process that results in destruction of the alveolar–capillary unit. Diagnosis of ARDS includes a history of sudden onset of respiratory distress, radiographic or ultrasonographic findings of diffuse bilateral pulmonary infiltrates, and respiratory failure (severe unresponsive hypoxemia and respiratory acidosis: see Chapter 43). There are many

causes of ARDS in the neonatal foal including prematurity and primary surfactant deficiency, meconium aspiration, asphyxia (as a result of dystocia, peripartum asphyxia syndrome, placentitis or premature placental separation), persistent pulmonary hypertension (cardiogenic or pulmonary), viral and bacterial respiratory infections, systemic inflammatory response syndrome, and septic shock. In human infants the majority of neonatal ARDS develops in premature infants with primary surfactant deficiency. While primary surfactant deficiency in foals is sometimes suspected, the deficiency is rarely confirmed (Rossdale et al 1967). Bacterial and viral infections are the most common cause of ARDS in the equine neonate.

Management of the Foal's Respiratory System

Monitoring

Arterial blood gases and lactate

The primary goal of therapy in the patient with respiratory failure is maintenance of oxygen delivery for aerobic metabolism and removal of carbon dioxide. Oxygen delivery is affected by both the oxygen content of the blood (concentration of hemoglobin and saturation of hemoglobin with oxygen) and the delivery to the tissues (cardiac output and perfusion). The arterial blood gas and blood lactate are the most useful assessments of respiratory function and perfusion. Arterial blood gas samples are most commonly collected from the lateral metatarsal artery. In poorly perfused or hypothermic patients the femoral or brachial artery can be used. In critically ill foals, an arterial catheter can be useful for repetitive sampling; however, in the author's experience, they are difficult to maintain. Arterial and venous hemoglobin saturation, blood lactate, and carbon dioxide are all important in the assessment of oxygen delivery and the response to treatment. Unfortunately, no one measurement alone is enough. For example, an arterial oxygen saturation of 98% is excellent; however, hypoxia may still be present if the foal is hypovolemic and not perfusing its tissues adequately. Consequently, in addition to oxygen content of the blood (assessed by hemoglobin concentration and hemoglobin saturation in the blood), cardiac output and perfusion [evaluated by central venous pressure (CVP), blood lactate, systemic blood pressure, and urine output] need to be maintained. The goal should be to maintain perfusion (normal blood lactate, CVP ≥ 5 mmH$_2$O, normal mean systolic blood pressure), provide adequate arterial oxygen content (oxygen saturation $\geq 90\%$ and adequate hemoglobin) and ensure adequate ventilation (removal of carbon dioxide).

If an arterial blood gas sample cannot be obtained, pulse oximetry can be used to assess oxygen saturation. Pulse oximetry is less accurate than arterial gas analysis and is affected by perfusion deficits, location of the device, pigmented skin, and type of transducer, but it has value as a crude assessment of respiratory function and of response to therapy (Chaffin et al 1996).

Carbon dioxide measurements

The carbon dioxide tension in the blood can be directly measured by arterial blood gas sampling or indirectly via capnography. Capnography uses an infrared sensor to measure end-tidal P_{CO_2}, which correlates with P_aCO_2. Capnography is less accurate in assessing P_aCO_2 in patients with increased physiological dead space (obstructive lung disease, pulmonary embolism, poor cardiac output) or anatomic dead space (shallow breathing).

Cardiac output

Ensuring adequate cardiac output and tissue perfusion is vital in maximizing survival in critically ill neonates. While assessment of a patient's mentation, heart rate, pulse pressure, and the warmth of the distal extremities are all useful techniques to assess cardiac output and perfusion they are not always accurate. In the ideal setting, cardiac output is measured by placement of a pulmonary artery catheter and measured using thermodilution techniques. This is rarely done in the equine neonatal intensive care unit. Instead the clinician must rely on several indirect measurements as well as serial physical examination parameters to assess cardiac output, perfusion and, ultimately, oxygen delivery and uptake by the cells.

Systemic blood pressure

While mean arterial pressure alone does not directly assess perfusion or cardiac output, in combination with other measurements already discussed it is useful in evaluation of these parameters and in assessing response to treatment. Indirect monitoring by use of a tail cuff is most commonly used in our hospital. It is important to utilize the correct size cuff and to maintain consistent technique so that the results are comparable (Marino 1997). Indirect assessment of blood pressure is less accurate than direct measurement but is useful in monitoring patient trends.

Urine output

Urine output is an indirect assessment of cardiac output and renal perfusion. Urine output of $1-2$ ml·kg^{-1}·h^{-1} is adequate although a healthy foal nursing from its dam will

produce significantly greater volumes of urine. Because a healthy foal nurses significantly more milk (to meet nutritional needs) than is needed to meet fluid requirements, urine output is high to excrete the excess fluid. While adequate urine output needs to be determined in relationship to fluid input, urine output values below $1 \, ml \cdot kg^{-1} \cdot day^{-1}$ should alert the clinician to the possibility of a problem.

Central venous pressure

Central venous pressure is an assessment of venous return, blood volume and, indirectly, of cardiac output. Normal CVP is between 0 and 8 cmH_2O (1–6 mmHg). In a hypovolemic foal, CVP is often 0 cmH_2O or less. Evaluating a response to fluid therapy (a progressive increase in CVP) is helpful in determining if fluid replacement is adequate. Care must be taken not to overload the foal with fluids, particularly with septic patients or patients with already compromised lung function, because pulmonary edema will cause further respiratory compromise.

Blood glucose

Frequent monitoring of blood glucose is recommended to try to maintain normoglycemia. Both hypoglycemia and hyperglycemia can have negative effects on respiratory function. The weak, hypoglycemic foal is often unable to generate adequate respiratory muscle activity to maintain tidal volume or functional residual volume. In humans, hyperglycemia and insulin resistance are common complications in patients with sepsis or systemic inflammatory response syndrome, with improved survival seen when glucose is closely regulated (van den Berghe et al 2001). In septicemic neonatal foals insulin therapy may be needed to maintain blood glucose within a normal range.

Treatment

Maximizing respiratory function

The neonatal foal with lower respiratory tract disease requires constant monitoring and care. These foals can be extremely unstable and deteriorate relatively rapidly. Physical care includes keeping the foal in sternal recumbency to minimize further alveolar collapse and improve gas exchange. Foals that cannot be maintained on their sternum should be turned frequently. Many sick foals will have abnormal respiratory patterns and may breath-hold or have long apneic pauses between breaths. These abnormal patterns can result in significant changes in blood gas parameters and may be an indication of central nervous system derangement or muscular weakness. Physical stimulation may help trigger the foal to breathe

more normally. Alternatively, central nervous system stimulants such as caffeine (loading dose of 10 mg/kg then 2.5 mg/kg once a day *per os*) may aid in maintaining a more normal respiratory pattern.

Oxygen supplementation

Supplemental oxygen is provided via a facemask to all foals during their initial work-up at our clinic. If hypoxemia is present, an intranasal cannula is placed and supplemental humidified oxygen is provided via insufflation. If this fails to bring arterial oxygen saturation within an acceptable range, a second cannula is placed in the opposite nostril. The exact inspired oxygen concentration is difficult to determine, and generally flow rates are titrated to deliver enough oxygen to maintain P_AO_2 above 60 mmHg (oxygen saturation ≥ 90%). The use of a percutaneous transtracheal oxygen cannula to deliver higher fractional inspired oxygen (F_IO_2) has been reported (Hoffman 1992). In cases of refractory hypoxemia mechanical ventilation with 100% oxygen is indicated.

Ventilatory support

There are no exact criteria for when to ventilate a foal. Ventilatory support is indicated in foals with moderate to severe hypercapnia, foals with persistent hypoxemia despite oxygen therapy and foals exhibiting a tremendous respiratory effort to achieve "acceptable" oxygen and carbon dioxide levels. Foals requiring ventilation generally fall into one of three categories: (1) foals with CNS depression (potential causes include periparturient asphyxia syndrome and sepsis) that do not increase their ventilation in response to abnormal arterial levels of CO_2 or oxygen; (2) foals that are weak as a result of sepsis, botulism or other disease processes, and while capable of trying to respond to hypercapnia and hypoxia, are unable to ventilate adequately because of respiratory muscle failure; and (3) foals that have primary pulmonary disease (examples include aspiration pneumonia, interstitial pneumonia) leading to gas exchange deficits and decreased lung compliance. Of the three categories, foals with severe underlying pulmonary disease are the most difficult to ventilate and have the worst prognosis for survival.

Ventilation should be considered in any foal with a P_aCO_2 that is consistently > 60 mmHg. In foals with central depression of ventilation (such as foals with perinatal asphyxia syndrome), administration of stimulants such as caffeine may result in correction of hypoventilation and hypercapnia. Foals that fail to respond to stimulants require mechanical ventilation to correct hypercapnia and respiratory acidosis. While foals can tolerate high blood levels of carbon dioxide, there are negative consequences to persistent, severe hypercapnia. Ventilatory support

should also be strongly considered in any foal that cannot maintain a $P_{A}O_2$ of 60 mmHg or better despite sternal positioning and oxygen supplementation. Ventilatory support should also be considered in foals unable to maintain a $P_{A}O_2$ of ≥ 60 mmHg without extreme respiratory efforts. These foals are working at maximum effort and would clearly benefit from ventilatory support.

In general, mechanical ventilation of a foal with significant respiratory compromise is best started earlier than later. Early institution of ventilation is clearly associated with a better outcome in human infants. A detailed discussion of mechanical ventilation is beyond the scope of this chapter. The readers are referred to an excellent, concise review of mechanical ventilation of the foal (Palmer 2005).

Surfactant therapy

Surfactant therapy improves survival and shortens ventilator therapy in human infants (Finer 2004). While primary surfactant deficiency may be rare in equine neonates, secondary deficiency is likely under-recognized (see above).

Surfactant products are very expensive and this limits their use in veterinary medicine. Consequently, there are few published reports of surfactant use in equine medicine (Perry 1993). Both animal-derived and synthetic surfactants are commercially available. Synthetic surfactants containing surfactant proteins SP-B and SP-C are more resistant to inhibitors than those that do not contain these proteins (Mbagwu et al 1999). Doses of 50–200 mg/kg are typically used in human infants. The dose of surfactant is divided and each portion is instilled down the endotracheal tube with the patient in a variety of postures (right lateral, left lateral, dorsal, and ventral recumbency). Manual ventilation should be performed immediately after each bolus dose to maximize distribution to the distal airways and alveoli. Alternatively, surfactant can be given by nebulizer, although the effectiveness of this method is not known. The use of surfactant lavage therapy to remove aspirated debris has been reported (Wiswell et al 2002). Recent data suggest that the addition of polymers such as dextrans to surfactant solutions may also help reduce surfactant inhibition in inflammatory or obstructive conditions (Tashiro & Robertson 2000).

Bronchodilators

The use of bronchodilators is controversial in the foal with acute respiratory distress and respiratory failure. Dilation of airways in consolidated regions of the lung may actually worsen ventilation–perfusion mismatching. In less severely affected foals and those with chronic or resolving pneumonia, the use of bronchodilators may be beneficial. Commonly used bronchodilators include the methylxanthine derivative aminophylline (5 mg/kg *per os* twice daily) and the β_2-adrenergic agonist clenbuterol (0.8 µg/kg *per os* twice daily). Side effects of aminophylline include tachycardia and agitation so it should be avoided in foals receiving caffeine. Oral absorption of aminophylline is variable in the horse. The use of inhaled and nebulized bronchodilator therapy also has been reported (Clarke 1991).

Antioxidants

Many equine clinicians use antioxidant therapy routinely in patients with evidence of systemic inflammation; however, few data exist regarding the benefit of such therapy. A recent report showed a decrease in markers of acute lung injury in preterm infants with respiratory distress syndrome treated with a recombinant human superoxide dismutase (Davis et al 1997). Clinical outcome was the same in both controls and treatment patients. Further research needs to be performed to evaluate the benefit of antioxidant therapy in treatment of neonatal respiratory disease.

Antimicrobial therapy

Treatment of bacterial pneumonia or respiratory disease complicated by bacterial colonization should, whenever possible, be based on the results of culture and sensitivity. However, until these results are available, the clinician must treat empirically rather than withholding therapy and compromising care (Table 45.2). To aid in determining the most appropriate therapy in a particular referral area, the results of bacterial culture and sensitivity from foals treated the previous year are helpful in assessing changes in resistance patterns and determining empirical antimicrobial therapy. Because multiple bacteria are frequently cultured from foals with pneumonia, a combination, broad-spectrum therapy is recommended. Initial broad-spectrum antimicrobial therapy in our hospital consists of a penicillin derivative and an aminoglycoside. It is important to evaluate renal function and hydration status of the foal before using aminoglycosides. Based on the previous year's analysis, this combination is effective in the majority of neonatal foal pneumonia cases seen at our facility. In addition, response to therapy is important in assessing the effectiveness of treatment. Because culture of blood and tracheal aspirate samples does not always identify the offending pathogens, careful evaluation of response is indicated on a daily basis. Favorable response to treatment would include resolution of fever, improved attitude and appetite, improvement in blood gas values, normalization of white blood cell parameters and resolution of hyperfibrinogenemia. Improvement in all values is desirable, while the persistence of abnormalities such as hyperfibrinogenemia or a low-grade fever are signs of inadequate or inappropriate treatment.

Table 45.2. Antibiotic treatment of bacterial pneumonia					
Antimicrobial	**Dosage**	**Frequency**	**Route**	**Comments**	**Spectrum of activity**
Amikacin[1,6,7,9]	22–30 mg/kg	SID	IV	Therapeutic drug monitoring recommended Concentration-dependent activity	Broad Gram –ve activity
Azithromycin[2,3]	10 mg/kg	SID for 5 days then every other day	PO	Concentrates in pulmonary alveolar macrophages	Some Gram +ve and broad Gram –ve aerobes, some anaerobes
Ampicillin[1,3–6,8]	20 mg/kg	QID	IV	Semisynthetic penicillin	Good Gram +ve and some Gram –ve activity
Amoxicillin[1,3–6,8]	30 mg/kg	TID–QID	PO	Poorly absorbed in foals >2 weeks of age	Gram +ve activity
Cephalexin[1,5,8]	25 mg/kg	QID	PO		Good Gram +ve activity, *E.coli*, *Klebsiella* and *Proteus* at high doses
Cephalothin[1,5,8]	25 mg/kg	QID	PO		Good Gram +ve activity, *E.coli*, *Klebsiella* and *Proteus* at high doses
Cefotaxime[1,5,8]	20–40 mg/kg	TID–QID	IV		Antipseudomonal activity, Gram –ve aerobes, streptococci
Ceftazidime[1,5,8]	20–50 mg/kg	BID–QID	IV	Synergism with aminoglycosides	Antipseudomonal activity, Gram –ve aerobes, streptococci
Ceftriaxone[1,5,8]	25 mg/kg	BID	IV		Good Gram +ve and Gram –ve activity, some anaerobes
Ceftiofur[1,5,8]	5 mg/kg	SID–BID	IV/IM		Good Gram +ve and Gram –ve activity, some anaerobes
Chloramphenicol[2,3]				Public health concerns*	Bacteriocidal
Enrofloxacin[1,6,7,9]	2.5 mg/kg	SID	IV/PO	Quinolone-induced arthropathies seen in foals Concentration-dependent activity Post-antibiotic effect	Good Gram –ve spectrum Some Gram +ve activity Poor anaerobic activity
Erythromycin stearate[2,3]	20–30 mg/kg	Q4–6 h	PO	May induce hyperthermia and diarrhea	Some Gram +ve, Gram –ve and anaerobes
Gentamicin sulfate[1,6,7,9]	6.6–8.8 mg/kg	SID	IV	Therapeutic drug monitoring recommended Concentration-dependent activity	Good Gram –ve activity
Imipenem[1,3,4,7,8,10]	5–10 mg/kg	TID	IV	Very irritating to tissues Synthetic penicillin Goal is time over MIC	Good Gram +ve and Gram –ve activity, some anaerobes
Metronidazole[1,3]	15–25 mg/kg	TID–QID	IV/PO		Most anaerobes and some protozoa
Penicillin (K+ or Na+)[1,3–5,8]	20,000–50,000 IU	QID	IV	Goal is time over MIC	Good Gram +ve, some anaerobes
Rifampin[1,3]	5 mg/kg	BID	PO	Used in combination with macrolides	Gram +ve and anaerobes
Ticarcillin[1,3,8]	50–100 mg/kg	TID–QID	IV	Synthetic penicillin Post-antibiotic effect Goal is time over MIC	Good activity against *Klebsiella* and *Pseudomonas*
Ticarcillin/ clavulanic acid[1,3,8]	50–100 mg/kg	TID–QID	IV	Synthetic penicillin Post-antibiotic effect Goal is time over MIC	Addition of clavulanate improves activity against β-lactamase-producing bacteria (both Gram –ve and Gram +ve)
Trimethoprim–sulfamethoxazole[1,3]	30 mg/kg	BID–TID	PO		Gram +ve/Gram –ve spectrum

SID, once a day; BID, twice a day; TID, thrice a day; QID, four times a day; IV, intravenous; PO, *per os*; IM, intramuscular; MIC, minimum inhibitory concentration.
[1]Bacteriocidal; [2]bacteriostatic; [3]good soft tissue penetration; [4]synergism with aminoglycosides; [5]low potential for toxicity; [6]penicillinase resistant; [7]high risk of toxicity; [8]time above MIC; [9]concentration-dependent killing; [10]resistant to β-lactamases.

REFERENCES

Ainsworth DM, Weldon AD, Beck KA 1993 Recognition of *Pneumocystis carinii* in foals with respiratory distress. Equine Veterinary Journal 25: 103–108

Al Soub H, Taha RY, El Deeb Y et al 2004 *Pneumocystis carinii* pneumonia in a patient without a predisposing illness: case report and review. Scandinavian Journal of Infectious Diseases 36: 618–621

Arvidson GAB, Ekelund L, Rossdale PD 1975 Surfactant studies in the fetal and neonatal foal. Journal of Reproduction and Fertility Supplement 23: 663–665

Atz AM, Wessel DL 1999 Sildenafil ameliorates the effects of inhaled nitric oxide withdrawal. Anesthesiology 91: 307–310

Beard CB, Carter JL, Keely SP et al. 2000 Genetic variation in *Pneumocystis carinii* isolates from different geographic regions: implications for transmission. Emerging Infectious Diseases 6: 265–272

Bedenice D, Heuwieser W, Brawer R et al 2003 Clinical and prognostic significance of radiographic pattern, distribution, and severity of thoracic radiographic changes in neonatal foals. Journal of Veterinary Internal Medicine 17: 876–886

Bellezzo F, Hunt RJ, Provost R et al 2004 Surgical repair of rib fractures in 14 neonatal foals: case selection, surgical technique and results. Equine Veterinary Journal 36: 557–562

Berkenbosch J W, Baribeau J, Perreault T 2000 Decreased synthesis and vasodilation to nitric oxide in piglets with hypoxia-induced pulmonary hypertension. American Journal of Physiology: Lung Cellular and Molecular Physiology 278: L276–L283

Borchers K, Wolfinger U, Goltz M et al 1997 Distribution and relevance of equine herpesvirus type 2 (EHV-2) infections. Archives of Virology 142: 917–928

Brewer BD, Koterba, AM 1990 Bacterial isolates and susceptibility patterns in foals in a neonatal intensive care unit. Compendium on Continuing Education for the Practicing Veterinarian 12: 1773–1781

Britton AP, Robinson JH 2002 Isolation of influenza A virus from a 7-day-old foal with bronchointerstitial pneumonia. Canadian Veterinary Journal 43: 55–56

Buergelt CD, Hines SA, Cantor G et al 1986 A retrospective study of proliferative interstitial lung disease of horses in Florida. Veterinary Pathology 23: 750–756

Cabanes FJ, Roura X, Majo N 2000 Pneumocystis carinii pneumonia in a Yorkshire terrier dog. Medical Mycology 38: 451–453

Carroll WD, Dhillon R 2003 Sildenafil as a treatment for pulmonary hypertension. Archives of Diseases of Childhood 88: 827–828

Castellheim A, Lindenskov PH, Pharo A et al 2004 Meconium is a potent activator of complement in human serum and in piglets. Pediatric Research 55: 310–318

Chaffin MK, Matthews NS, Cohen ND et al 1996 Evaluation of pulse oximetry in anaesthetised foals using multiple combinations of transducer type and transducer attachment site. Equine Veterinary Journal 28: 437–445

Chandran S, Haqueb ME, Wickramasinghe HT et al 2004 Use of magnesium sulphate in severe persistent pulmonary hypertension of the newborn. Journal of Tropical Pediatrics 50: 219–223

Clarke A 1991 Practicalities of nebulisation. Equine Veterinary Education 3: 45–49

Clark-Price SC, Cox JH, Bartoe JT et al 2004 Use of dapsone in the treatment of *Pneumocystis carinii* pneumonia in a foal. Journal of the American Veterinary Medical Association 224: 407

Cochrane CG, Revak SD, Merritt TA et al 1998 Bronchoalveolar lavage with KL4-surfactant in models of meconium aspiration syndrome. Pediatric Research 44: 705–715

Cottrill CM, O'Connor WN, Cudd T et al 1987 Persistence of foetal circulatory pathways in a newborn foal. Equine Veterinary Journal 19: 252–255

Cushion MT 2004 *Pneumocystis*: unraveling the cloak of obscurity. Trends in Microbiology 12: 243–249

Dargaville SM, McDougall PN 2001 Surfactant and surfactant inhibitors in meconium aspiration syndrome. Journal of Pediatrics 138: 113–115

Davidson D, Kattwinkel J, Dudell G et al 1999 Safety of withdrawing inhaled nitric oxide therapy in persistent pulmonary hypertension of the newborn. Pediatrics 104: 231–236

Davis JM, Rosenfeld WN, Richter SE et al 1997 Safety and pharmacokinetics of multiple doses of recombinant human CuZn superoxide dismutase administered intra-tracheally to premature neonates with respiratory distress syndrome. Pediatrics 100: 24–30

de Beufort AJ, Elferink JGR, Berger HM 1998 Effect of interleukin 8 in meconium on in-vitro neutrophil chemotaxis. Lancet 352: 102–105

Del Piero F, Wilkins PA, Lopez JW et al 1997 Equine viral arteritis in newborn foals: clinical, pathological, serological, microbiological and immunohistochemical observations. Equine Veterinary Journal 29: 178–185

Demmers SJA, Grondahl G, Jensen-Waern M 2001 Neutrophil functions and serum IgG in growing foals. Equine Veterinary Journal 33: 676–680

Dumoulin A, Mazars E, Seguy N et al 2000 Transmission of *Pneumocystis carinii* disease from immunocompetent contacts of infected hosts to susceptible hosts. European Journal of Clinical Microbiology and Infectious Diseases 19: 671–678

Dutta S 1975 Isolation and characterization of an adenovirus and isolation of its adenovirus-associated virus in cell culture from foals with respiratory tract disease. American Journal of Veterinary Research 36: 247–250

Edington N, Welch HM, Griffiths L 1994 The prevalence of latent Equid herpesviruses in the tissues of 40 abattoir horses. Equine Veterinary Journal 26: 140–142

Ewing PJ, Cowell RL, Tyler RD et al 1994 *Pneumocystis carinii* pneumonia in foals. Journal of the American Veterinary Medical Association 204: 929–933

Farrow BR, Watson AD, Hartley WJ 1972 Pneumocystis pneumonia in the dog. Journal of Comparative Pathology 82: 447–453

Fike CD, Kaplowitz MR 1996 Chronic hypoxia alters nitric oxide-dependent pulmonary vascular responses in lungs of newborn pigs. Journal of Applied Physiology 81: 2078–2087

Fike CD, Kaplowitz MR, Thomas CJ et al 1998 Chronic hypoxia decreases nitric oxide production and endothelial nitric oxide synthase in newborn pig lungs. American Journal of Physiology 274: L517–L526

Findlay RD, Taeusch HW, Walther FJ 1996 Surfactant replacement therapy for meconium aspiration syndrome. Pediatrics 97: 48–52

Finer NN 2004 Surfactant use for neonatal lung injury: beyond respiratory distress syndrome. Pediatric Respiratory Review 5 (Suppl A): S289–S297

Finer NN, Barrington KJ 2001 Nitric oxide for respiratory failure in infants born at or near term. Cochrane Database Syst Rev 2: CD000399

Flaminio MJ, Rush BR, Cox JH et al 1998 CD4+ and CD8+ T-lymphocytopenia in a filly with *Pneumocystis carinii* pneumonia. Australian Veterinary Journal 76: 399–402

Foote CE, Gilkerson JR, Whalley JM 2004 Detection of EHV-1 and EHV-4 DNA in unweaned Thoroughbred foals from vaccinated mares on a large stud farm. Equine Veterinary Journal 36: 341–345

Fu ZF, Robinson AJ, Horner GW et al 1986 Respiratory disease in foals and the epizootiology of equine herpesvirus type 2 infection. New Zealand Veterinary Journal 34: 152–155

Fuhrman BP 2004 Surface properties and the meconium aspiration syndrome. Pediatric Critical Care Medicine 5: 200–201

Furuta T, Nogami S, Kojima S et al 1994 Spontaneous *Pneumocystis carinii* infection in the dog with naturally acquired generalised demodicosis. Veterinary Record 134: 423–424

Gayle JM, Gayle CN, Chaffin MK 1998 Factors associated with survival in septicemic foals: 65 cases (1988–1995). Journal of Veterinary Internal Medicine 12: 140–146

Ghofrani HA, Rose F, Schermuly RT et al 2003 Oral sildenafil as long-term adjunct therapy to inhaled iloprost in severe pulmonary arterial hypertension. Journal of the American College of Cardiology 42: 158–164

Gigliotti F, Harmsen AG, Wright TW 2003 Characterization of transmission of *Pneumocystis carinii* f. sp. *muris* through immunocompetent BALB/c mice. Infection and Immunity 71: 3852–3856

Gilbert SA, Timoney PJ, McCollum WH et al 1997 Detection of equine arteritis virus in the semen of carrier stallions by using a sensitive nested PCR assay. Journal of Clinical Microbiology 35: 2181–2183

Gilkerson JR, Drummer HE, Studdert MJ et al 1991 Epidemiology of EHV-1 and EHV-4 in the mare and foal populations on a Hunter Valley stud farm: are mares the source of EHV-1 for unweaned foals? Veterinary Microbiology 68: 27–34

Gilkerson JR, Drummer HE, Studdert MJ et al 1999 Epidemiological studies of equine herpesvirus 1 (EHV-1) in Thoroughbred foals: a review of studies conducted in the Hunter Valley of New South Wales between 1995 and 1997. Veterinary Microbiology 68: 15–25

Goh AY, Lum LC, Roziah M 2001 Low-dose inhaled nitric oxide in term and near-term infants with hypoxic respiratory failure: a Malaysian experience. Medical Journal of Malaysia 56: 336–340

Golden J, Hollander H, Stulbarg MS et al 1986 Broncho-alveolar lavage as the exclusive diagnostic modality for *Pneumocystis carinii* pneumonia. Chest 90: 18–22

Goldman AP, Haworth SG, Sigston PE et al 1996 Four patterns of response to inhaled nitric oxide for persistent pulmonary hypertension of the newborn. Pediatrics 98: 706–713

Guthrie SO, Walsh WF, Auten K et al 2004 Initial dosing of inhaled nitric oxide in infants with hypoxic respiratory failure. Journal of Perinatology 24: 290–294

Haas KM, Suzuki S, Yamaguchi N et al 2002 Nitric oxide further attenuates pulmonary hypertension in magnesium-treated piglets. Pediatric International 44: 670–674

Hansell D. 2003 Extracorporeal membrane oxygenation for perinatal and pediatric patients. Respiratory Care 48: 352–362; discussion pp.63–66

Helweg-Larsen J, Tsolaki AG, Miller RF et al 1998 Clusters of *Pneumocystis carinii* pneumonia: analysis of person-to-person transmission by genotyping. Quarterly Journal of Medicine 91: 813–820

Helweg-Larsen J, Jensen JS, Dohn B et al 2002 Detection of Pneumocystis DNA in samples from patients suspected of bacterial pneumonia – a case–control study. BMC Infectious Disease 2: article number 28

Henry JN 1976 Adenovirus pneumonia in an Arabian foal. Canadian Veterinary Journal 17: 220–221

Hintz SR, Halamek LP, Van Meurs KP et al 2000 Secondary infection presenting as recurrent pulmonary hypertension. Journal of Perinatology 20: 262–264

Hoffman AM 1992 A percutaneous transtracheal catheter system for improved oxygenation in foals with respiratory distress. Equine Veterinary Journal 24: 239–241

Holm BA, Notter RH 1999 Multiple mechanisms of lung surfactant inhibition. Pediatric Research 46: 85–93

Holopainen RAH, Laine J, Peuravuori H et al 1999 Human meconium has high phospholipase A2 activity and induces cellular injury and apoptosis in piglet lungs. Pediatric Research 46: 626–632

Hong ST, Ryu JS, Chai JY et al 1992 Transmission modes of *Pneumocystis carinii* among rats observed by karyotype analysis. Kisaengchunghak Chapchi 30: 283–288

Hughes WT 1998 Current issues in the epidemiology, transmission, and reactivation of *Pneumocystis carinii*. Seminars in Respiratory Infections 13: 283–288

Ichinose F, Erana-Garcia J, Hromi J et al 2001 Nebulized sildenafil is a selective pulmonary vasodilator in lambs with acute pulmonary hypertension. Critical Care Medicine 29: 1000–1005

Jean D, Laverty S, Halley J et al 1999 Thoracic trauma in newborn foals. Equine Veterinary Journal 31: 149–152

Jensen TK, Boye M, Bille-Hansen V 2001 Application of fluorescent in situ hybridization for specific diagnosis of *Pneumocystis carinii* pneumonia in foals and pigs. Veterinary Pathology 38: 269–274

Keller RL, Hamrick SE, Kitterman JA et al 2004 Treatment of rebound and chronic pulmonary hypertension with oral sildenafil in an infant with congenital diaphragmatic hernia. Pediatric Critical Care Medicine 5: 184–187

Kershaw O, von Oppen T, Glitz F et al 2001 Detection of equine herpesvirus type 2 (EHV-2) in horses with kerato-conjunctivitis. Virus Research 80: 93–99

Kotecha S 2000 Lung growth: implications for the newborn infant. Archives of Diseases in Childhood 82: F69–F74

Koterba AM 1990 Physical Examination, 1st edn. Lea & Febiger, Philadelphia

Koterba AM, Wozniak JA, Kosch PC 1995a Ventilatory and timing parameters in normal horses at rest up to age one year. Equine Veterinary Journal 27: 257–264

Koterba AM, Wozniak JA, Kosch PC 1995b Changes in breathing pattern in the normal horse at rest up to age one year. Equine Veterinary Journal 27: 265–274

Lakritz J, Wilson WD, Berry CR et al 1993 Bronchointerstitial pneumonia and respiratory distress in young horses: clinical, clinicopathologic, radiographic, and pathological findings in 23 cases (1984–1989). Journal of Veterinary Internal Medicine 7: 277–288

Leffler CW, Cassin S 1978 Effect of indomethacin on pulmonary vascular response to ventilation of fetal goats. American Journal of Physiology 234: H346–H351

Lepore JJ, Maroo A, Pereira NL et al 2002 Effect of sildenafil on the acute pulmonary vasodilator response to inhaled nitric oxide in adults with primary pulmonary hypertension. American Journal of Cardiology 90: 677–680

Lester GD, DeMarco VG, Norman WM 1999 Effect of inhaled nitric oxide on experimentally induced pulmonary hypertension in neonatal foals. American Journal of Veterinary Research 60: 1207–1212

Lotze A, Mitchell BR, Bulas DI et al 1998 Multicenter study of surfactant (beractant) use in the treatment of term infants with severe respiratory failure. Survanta in Term Infants Study Group. Journal of Pediatrics 132: 40–47

Manoloff ES, Francioli P, Taffe P et al 2003 Risk for *Pneumocystis carinii* transmission among patients with pneumonia: a molecular epidemiology study. Emerging Infectious Diseases 9: 132–134

Marino P 1997 Arterial Blood Pressure, 2nd edn. Lippincott, Williams and Wilkins, Philadelphia

Marsh PS, Palmer JE 2001 Bacterial isolates from blood and their susceptibility patterns in critically ill foals: 543 cases (1991–1998). Journal of the American Veterinary Medical Association 218: 1608–1610

Mbagwu N, Bruni R, Hernandez-Juviel JM et al 1999 Sensitivity of synthetic surfactants to albumin inhibition in preterm rabbits. Molecular Genetics and Metabolism 66: 40–48

McCartan CG, Wood JL, Mumford JA 1995 Clinical, serological and virological characteristics of an outbreak of paresis and neonatal foal disease due to equine herpesvirus-1 on a stud farm. Veterinary Record 136: 7–12

McConnell E E, Basson P A, Pienaar J G 1971 Pneumocystosis in a domestic goat. Onderstepoort Journal of Veterinary Research 38: 117–124

McTaggart CYJ, Penhale J, Raidal SL 2001 A comparison of foal and adult horse neutrophil function using flow cytometric techniques. Research in Veterinary Science 71: 73–79

Mehta S 2003 Sildenafil for pulmonary arterial hypertension: exciting, but protection required. Chest 123: 989–992

Meurs KM, Hanson C, Honnas C 1997 Tricuspid valve atresia with main pulmonary artery atresia in an Arabian foal. Equine Veterinary Journal 29: 160–162

Michelakis E, Tymchak W, Lien D et al 2002 Oral sildenafil is an effective and specific pulmonary vasodilator in patients with pulmonary arterial hypertension: comparison with inhaled nitric oxide. Circulation 105: 2398–2403

Miller RF, Ambrose HE, Novelli V et al 2002 Probable mother-to-infant transmission of *Pneumocystis carinii* f. sp. *hominis* infection. Journal of Clinical Microbiology 40: 1555–1557

Moses D, Holm BA, Spitale P et al 1991 Inhibition of pulmonary surfactant function by meconium. American Journal of Obstetrics and Gynecology 164: 477–481

Murray MJ, Eichorn ES, Dubovi EJ et al 1996 Equine herpesvirus type 2: prevalence and seroepidemiology in foals. Equine Veterinary Journal 28: 432–436

Nout YS, Hinchcliff KW, Samii VF et al 2002 Chronic pulmonary disease with radiographic interstitial opacity (interstitial pneumonia) in foals. Equine Veterinary Journal 34: 542–548

Oelberg DG, Downey SA, Flynn MM 1990 Bile salt-induced intracellular Ca^{++} accumulation in type II pneumocytes. Lung 168: 297–308

Palmer J 2005 Ventilatory support of the neonatal foal. Veterinary Clinics of North American; Equine Practice 21: 457–486

Pattle RP, Schock C, Creasey JM 1975 The development of the lung and its surfactant in the foal and in other species. Journal of Reproduction and Fertility Supplement 23: 651–657

Peek SF, Landolt G, Karasin AI et al 2004 Acute respiratory distress syndrome and fatal interstitial pneumonia associated with equine influenza in a neonatal foal. Journal of Veterinary Internal Medicine 18: 132–134

Perkins G, Ainsworth DM, Erb HN et al 1999 Clinical, haematological and biochemical findings in foals with neonatal Equine herpesvirus-1 infection compared with septic and premature foals. Equine Veterinary Journal 31: 422–426

Perron Lepage MF, Suter MM 1999 A case of interstitial pneumonia associated with *Pneumocystis carinii* in a foal. Veterinary Pathology 36: 621–624

Perry B 1993 The use of bovine derived surfactant in foals with respiratory distress. In: 11th Annual Veterinary Medical Forum; 1993 May 20–23; Washington, DC, pp.189–192

Perryman LE, McGuire TC, Crawford TB 1978 Maintenance of foals with combined immunodeficiency: causes and control of secondary infections. American Journal of Veterinary Research 39: 1043–1047

Peters SE, Wakefield AE, Whitwell KE 1994 *Pneumocystis carinii* pneumonia in thoroughbred foals: identification of a genetically distinct organism by DNA amplification. Journal of Clinical Microbiology 32: 213–216

Pifer LL, Stagno S, Woods D 1978 *Pneumocystis carinii* infection: evidence for high prevalence in normal and immunosuppressed children. Pediatrics 61: 35–41

Prescott JF, Wilcock BP, Carman S et al 1991 Sporadic, severe bronchointerstitial pneumonia of foals. Canadian Veterinary Journal 32: 421–425

Rabe KF, Tenor H, Dent G et al 1993 Phosphodiesterase isozymes modulating inherent tone in human airways: identification and characterization. American Journal of Physiology 264: L458–L464

Rabinovitch M, Gamble W, Nadas AS et al 1979 Rat pulmonary circulation after chronic hypoxia: hemodynamic and structural features. American Journal of Physiology 236: H818–H827

Raj U, Shimoda L 2002 Oxygen-dependent signaling in pulmonary vascular smooth muscle. American Journal of Physiology: Lung Cellular and Molecular Physiology 283: L671–L677

Randell SH, Mercer RR, Young SL 1989 Postnatal growth of pulmonary acini and alveoli in normal and oxygen-exposed rats studied by serial section reconstructions. American Journal of Anatomy 186: 55–68

Reimer JM, Reef VB, Saik JE 1993 Aortic origin of the right pulmonary artery and patent ductus arteriosus in a pony foal with pulmonary hypertension and right-sided heart failure. Equine Veterinary Journal 25: 466–468

Rose RJ, Rossdale PD, Leadon DP 1982 Blood gas and acid–base status in spontaneously delivered, term-induced and induced premature foals. Journal of Reproduction and Fertility Supplement 32: 521–528

Rossdale P, Pattle RE, Mahaffey LW 1967 Respiratory distress in a newborn foal with failure to form lung lining film. Nature 215: 1498–1499

Sadiq HF, Mantych G, Benawra RS et al 2003 Inhaled nitric oxide in the treatment of moderate persistent pulmonary

hypertension of the newborn: a randomized controlled, multicenter trial. Journal of Perinatology 23: 98–103

Seibold HR, Munnell JF 1977 *Pneumocystis carinii* in a pig. Veterinary Pathology 14: 89–91

Shekerdemian LS, Ravn HB, Penny DJ 2004 Interaction between inhaled nitric oxide and intravenous sildenafil in a porcine model of meconium aspiration syndrome. Pediatric Research 55: 413–418

Shively JN, Dellers RW, Buergelt CD et al 1973 *Pneumocystis carinii* pneumonia in two foals. Journal of the American Veterinary Medical Association 162: 648–652

Smyth RL 2000 Inhaled nitric oxide treatment for pre-term infants with hypoxic respiratory failure. Thorax 55: S51–S55

Southwood LL, Tobias AH, Schott HC et al 1996 Cyanosis and intense murmur in a neonatal foal. Journal of the American Veterinary Medical Association 208: 835–837; discussion 838–839

Sprayberry K, Bain FT, Seahorn TL et al 2001 56 cases of rib fractures in neonatal foals hospitalized in a referral center intensive care unit from 1997–2001. In: 47th Annual American Association of Equine Practitioners Convention; 2001 Nov 24–28; San Diego, CA, pp.395–399

Steudel W, Scherrer-Crosbie M, Bloch KD et al 1998 Sustained pulmonary hypertension and right ventricular hypertrophy after chronic hypoxia in mice with congenital deficiency of nitric oxide synthase 3. Journal of Clinical Investigation 101: 2468–2477

Stewart JH, Rose RJ, Barko AM 1984 Respiratory studies in foals from birth to seven days old. Equine Veterinary Journal 16: 323–328

Sun B 1993a Exogenous surfactant improves lung compliance and oxygenation in rats with meconium aspiration. Journal of Applied Physiology 77: 1961–1971

Sun B 1993b Surfactant inhibition in experimental meconium aspiration. Acta Paediatrica 82: 182–189

Szeredi L, Denes B, Rusvai M 2003 Equine viral arteritis in a newborn foal: parallel detection of the virus by immunohistochemistry, polymerase chain reaction and virus isolation. Journal of Veterinary Medicine Series B: Infectious Diseases and Veterinary Public Health 50: 270–274

Tanaka S, Kaji Y, Taniyama H et al 1994 *Pneumocystis carinii* pneumonia in a thoroughbred foal. Journal of Veterinary Medical Science 56: 135–137

Tashiro K, Robertson B 2000 Dextran reduces surfactant inhibition by meconium. Acta Paediatrica 89: 1439–1445

The Neonatal Inhaled Nitric Oxide Study Group 1997 Inhaled nitric oxide in full-term and nearly full-term infants with hypoxic respiratory failure. New England Journal of Medicine 336: 597–604

Thibeault DW, Mabry S, Rezaiekhaligh M 1990 Neonatal pulmonary oxygen toxicity in the rat and lung changes with aging. Pediatric Pulmonology 9: 96–108

Thompson DB, Spradborw PB, Studdert M 1976 Isolation of an adenovirus from an Arab foal with a combined immunodeficiency disease. Australian Veterinary Journal 52: 435–437

Tollofsrud PA, Lindenskov PH, Drevon CA et al 2003 Comparison of pulmonary and inflammatory effects of lipid- and water-soluble components in meconium in newborn piglets. Biologia Neonatorum 84: 330–337

Tyler TWR, Leffler C, Cassin S 1975 The effects of indomethacin on the pulmonary vascular response to hypoxia in the premature and mature newborn goat. Proceedings of the Society of Experimental Biology and Medicine 150: 695–698

van den Berghe GW, Weekers F, Verwaest C et al 2001 Intensive insulin therapy in the critically ill patients. New England Journal of Medicine 345: 1359–1367

Vitums AGB, Stone EC, Spencer GR 1973 Transposition of the aorta and atresia of the pulmonary trunk in a horse. Cornell Veterinarian 63: 41–57

Warner BB, Stuart LA, Papes RA et al 1998 Functional and pathological effects of prolonged hyperoxia in neonatal mice. American Journal of Physiology 275: L110–L117

Watanabe H, Ohashi K, Takeuchi K et al 2002 Sildenafil for primary and secondary pulmonary hypertension. Clinical Pharmacology and Therapeutics 71: 398–402

Webb RF, Walker KH 1981 Involvement of adenovirus in pneumonia in a thoroughbred foal. Australian Veterinary Journal 57: 142–143

Weimann J, Ullrich R, Hromi J et al 2000 Sildenafil is a pulmonary vasodilator in awake lambs with acute pulmonary hypertension. Anesthesiology 92: 1702–1712

Whitlock RH, Shively JN 1975 Adenoviral pneumonia in a foal. Cornell Veterinarian 65: 393–401

Whitwell K 1992 *Pneumocystis carinii* infection in foals in the UK. Veterinary Record 131: 19

Wilkins PA 2003 Acute respiratory failure: diagnosis, monitoring techniques, and therapeutics. Clinical Techniques in Equine Practice 2: 56–66

Wilkins PA, Lopez J, Cline M 1995 Immunohistochemical diagnosis of equine arteritis infection in a foal. Equine Veterinary Journal 27: 398

Wilson WD, Madigan JE 1989 Comparison of bacteriologic culture of blood and necropsy specimens for determining the cause of foal septicemia: 47 cases (1978–1987). Journal of the American Veterinary Medical Association 195: 1759–1763

Wiswell TE, Finer NN, Donn SM et al 2002 A multicenter, randomized, controlled trial comparing Surfaxin (Lucinactant) lavage with standard care for treatment of meconium aspiration syndrome. Pediatrics 109: 1081–1087

Zhao L, Mason NA, Morrell NW et al 2001 Sildenafil inhibits hypoxia-induced pulmonary hypertension. Circulation 104: 424–428

Disorders of the Thoracic Wall, Pleura, Mediastinum, and Diaphragm

T Douglas Byars and Bruce C McGorum

Introduction

The thoracic wall and diaphragm function to generate the intrathoracic pressure changes that drive breathing (see Chapter 2) and to protect the vital internal thoracic and abdominal organs. These roles may be compromised by a diverse variety of disorders including thoracic trauma, rib fractures, pneumothorax, pleuropneumonia, neoplasia and botulism, thereby leading to abnormalities in breathing and ultimately even to cardiopulmonary failure.

The anatomy of the thoracic wall, pleura, mediastinum, and diaphragm is detailed in Chapter 1.

Thoracic Wall Trauma

The thoracic wall may be injured by blunt or penetrating trauma. Blunt trauma includes collisions with objects such as automobiles, fences, and other horses, and compression of the neonatal foal's thorax by forceful uterine contractions during parturition. Penetrating injuries include staking injuries and gunshot wounds. As the right pleural sac may extend up the neck, to 2- to 3-cm rostral to the first rib, it is potentially vulnerable to deep puncture wounds in this region. The possibility of trauma should not be eliminated on the basis that a traumatic incident was not observed because the horse has a propensity for developing a variety of traumatic injuries, many of which

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Table 46.1. Sequelae of thoracic trauma

Superficial soft tissue injury
Subcutaneous emphysema
Fractures of the ribs, sternum, and vertebrae
Pneumothorax
Pneumomediastinum
Pleural effusion
Hemothorax
Infections (pleural infection, pleuropneumonia, pulmonary abscess)
Pulmonary contusion or laceration
Diaphragmatic herniation or laceration
Involvement of other systems (pericardial puncture, intestinal puncture, vertebral fractures)

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are not witnessed. Furthermore, the horse's hair coat and dark skin color usually prevent recognition of skin bruising, which is a useful indicator of chest wall trauma in humans.

The sequelae of thoracic trauma are listed in Table 46.1. When assessing horses with thoracic trauma, the clinician should not be blinkered by obvious chest wall injuries, but should perform a thorough evaluation of all body systems. This will ensure early recognition and prompt management of potentially fatal extrathoracic sequelae, such as rupture or perforation of abdominal viscera and vertebral fracture (Laverty et al 1996).

Rib Fractures

Rib fractures are uncommon in adult horses but occur in around 3–5% of neonates, and were considered to be the cause of death in 2.5% of neonatal foals in a postmortem study (Jean et al 1999, Schambourg et al 2003). In neonates, fractures most commonly occur at or near the costochondral junctions of ribs 3 to 8 (Schambourg et al 2003) (Fig. 46.1). They probably result from thoracic compression by forceful uterine contractions during parturition, compounded by focal compression of the cranioventral thorax by the foal's elbow when the forelimb fails to extend fully during delivery. Difficult and primiparous foalings are the only known predisposing factors to thoracic trauma in neonates (Jean et al 1999). While larger birth weights and dystocia are frequently cited as predisposing factors, rib fractures also occur in small-birth-weight foals with uncomplicated deliveries.

Clinical signs

Most foals with uncomplicated rib fractures have no overt clinical signs. Because the chest wall and parietal pleura, but not the visceral pleura, are well endowed with sensory nerve fibers from the intercostal nerves, foals with rib injuries may have variable pleurodynia (thoracic pain), as evidenced by stiffness, expiratory grunting, and reluctance to rise, lie down, circle or nurse. There may be asymmetrical subcutaneous edema or a hematoma over the ipsilateral ventral thorax and olecranon. A flail chest

Fig. 46.1. (A) A foal with pulmonary origin epistaxis caused by laceration of the lung by sharp ends of fractured ribs. (B) Ultrasound image of the rib fragments. (C) Post-mortem view of the inside of the thoracic wall from a foal showing medial displacement of sharp ends of two fractured ribs (arrows) and associated soft tissue damage. (D) Post-mortem view of the lungs from an adult horse that died as a result of pulmonary laceration following thoracic trauma sustained during a fall while racing. Two lacerations are evident on the dorsal surface of the left lung (arrows).
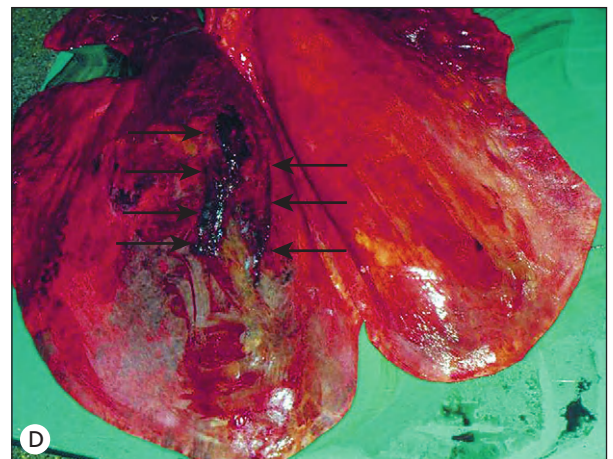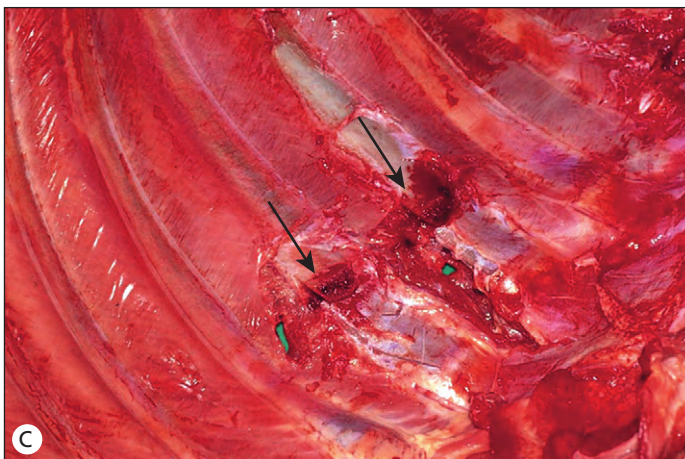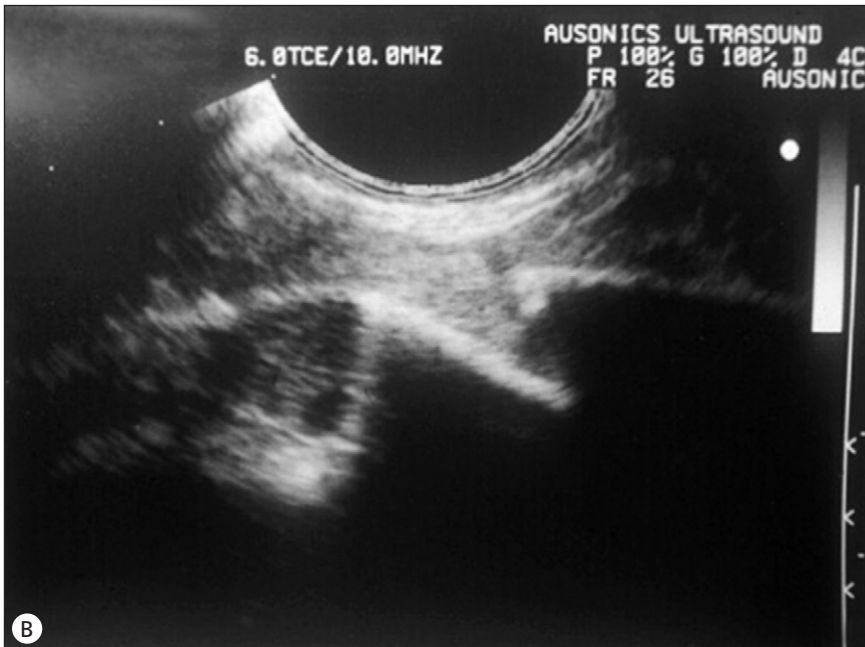
occurs when three or more consecutive ribs are fractured in at least two places, or are separated from the costochondral junction or sternum. The flail segment is unstable and exhibits paradoxical movement, such that it collapses inwards on inspiration, and moves outward on expiration. The inspiratory collapse leads to deviation of the mediastinum towards the contralateral side. The resultant inability to generate adequate intrapleural pressure swings causes generalized hypoventilation and respiratory failure.

Foals with fractured ribs may develop a variety of potentially life-threatening sequelae (Table 46.2), that may lead to additional clinical signs, including:

- dyspnea (pneumothorax, pulmonary contusion, diaphragmatic herniation)
- hypovolemic shock and anemia (hemothorax; laceration of intercostal artery, lung, myocardium, coronary artery, diaphragm, and abdominal viscera)
- bilateral epistaxis (pulmonary laceration; Fig. 46.1A)
- cardiac failure (pericardial and myocardial laceration)
- sudden death.

Diagnosis

Rib fractures may be diagnosed by careful simultaneous palpation of both hemithoraces, especially over the costochondral junctions. This examination is best done with the foal standing, because casting the foal to dorsal recumbency may displace unstable fragments, leading to laceration of internal organs. The clinician should examine for asymmetry of the thoracic wall, pain, swelling, crepitation or the presence of unstable fragments that produce a pathognomonic "click or pop". Ultrasonography and thoracic radiography may aid detection of rib fractures (Fig. 46.1B), with the former technique being more useful for detection of greenstick fractures (Sprayberry et al 2001). Detection and thorough investigation of the sequelae listed in Table 46.2 may require a combination of ultrasonography, radiography, and ultimately exploratory surgery.

Treatment

Uncomplicated rib fractures are treated conservatively, simply by stall rest for 2–3 weeks. Hematoma formation during the course of a few days may provide a cushion over sharp rib fragments, thereby minimizing the risk of further damage. Premature turn-out or exercise of foals with unstable fractures may cause potentially fatal displacement of fragments. Ultrasound evaluation and palpation can be used to assess the stability of callus formation. Foals that spend extended periods of time in recumbency should be placed on a cushion pad with the fracture side down to minimize paradoxical breathing and maximize ventilation. Foals that struggle excessively or that are sufficiently active

Table 46.2. Sequelae to rib fractures
Pneumothorax
Hemothorax and hemopericardium
Pleural effusion
Pleural infection
Pulmonary contusion
Laceration of lung, pericardium, myocardium, diaphragm, abdominal viscera
Diaphragmatic hernia
Sudden death

to risk displacement of unstable fractures may benefit from judicious administration of sedatives (Sprayberry et al 2001). Foals should be assisted to rise and nurse, taking care to avoid direct pressure on the fracture site. Supplemental oxygen is indicated for foals with hypoxemia. Pain control is an important aspect of the management of chest wall injuries to reduce chest splinting and improve regional lung ventilation. This may minimize retention of respiratory secretions and prevent regional atelectasis, which predispose to secondary pneumonia. Intercostal nerve block using a long-acting anesthetic agent such as bupivacaine may provide prolonged analgesia. Where practical, all of the intercostal nerves supplying the region of the thoracic injury, and also the intercostal nerves immediately cranial and caudal to this region, should be blocked. Intercostal nerve block is preferable to narcotic analgesia because it does not result in respiratory depression.

Surgical repair of rib fractures in neonatal foals has been described (Bellazo et al 2004), with internal and external fixation indicated for foals with fractures of the ribs in close proximity to the heart and in foals that develop diaphragmatic laceration secondary to rib fractures. Fixation entails using (1) reconstruction plates with cortical screws and cerclage wires, (2) plates with screws and optional wire, or (3) Steinman pins with stainless steel wire. Closed fixations have also been provided using cerclage wire over external plates or tongue depressors. Surgical complications include hemorrhage, seroma formation, postoperative pneumothorax, and implant failure (R.J. Hunt, personal communication 2004). Stabilization of rib fractures and associated flail chest in an adult horse using a plastic stent as an external fixator secured with orthopedic wire to the ribs under local anesthesia was reported by Laverty et al (1996).

Prognosis

Mortality is significant in patients that have sequelae such as pulmonary contusions, pulmonary lacerations, hemothorax, hemopericardium, and pneumothorax, unless given appropriate prompt medical or surgical treatment

(Sprayberry et al 2001). Foals with significant hemothorax causing pulmonary tamponade and hemorrhagic shock may require pleural drainage and blood replacement.

Diaphragmatic Hernia

Pathogenesis

Diaphragmatic herniation is an uncommon problem that may be congenital or acquired, with the latter resulting from blunt trauma to the thorax or abdomen, such as from falls, kicks or collisions. Herniation may also result from laceration of the diaphragm in animals with caudal rib fractures, as a breeding injury in stallions, and as a complication of parturition in mares. Acquired diaphragmatic hernias have ragged edges with associated hemorrhage and more commonly occur on the left side of the diaphragm. Congenital hernias, occurring in neonates as the result of incomplete closure of the crura, generally involve the dorsal diaphragmatic quadrant and have smooth edges. Horses may have a diaphragmatic hernia for prolonged periods of time without exhibiting clinical signs and then have a sudden onset of clinical signs as a result of further tearing of the diaphragm, sudden passage of viscera into the pleural cavity or incarceration or strangulation of abdominal viscera.

Clinical signs

Diaphragmatic herniation is diagnostically challenging because of its varied presentations. The most common clinical presentation is abdominal pain as the result of intestinal obstruction and strangulation or involvement of other abdominal viscera. Only a minority of horses presents solely with signs of respiratory dysfunction, such as tachypnea, dyspnea, exercise intolerance, and coughing. These horses are likely to have large diaphragmatic defects that allow herniation of considerable amounts of abdominal viscera but without intestinal obstruction or ischemia (Perdrizet et al 1989). The presence of abdominal viscera and secondary effusion in the pleural cavity leads to pulmonary atelectasis and reduced tidal volume. Other signs of diaphragmatic herniation include endotoxemic and hemorrhagic shock and a variety of non-specific complaints. Occasionally, diaphragmatic hernias are identified as apparently incidental findings at post-mortem examinations, suggesting that they may be benign in some horses. Auscultation and percussion of the ventral chest may reveal absence of breath sounds and dullness, respectively. Examination *per rectum* may reveal a relative paucity of viscera within the abdominal cavity.

Diagnosis

Diaphragmatic herniation is frequently confirmed only during exploratory celiotomy or at post-mortem examination. Definitive pre-mortem diagnosis requires identification of abdominal viscera within the pleural cavity using thoracic ultrasonography or radiography. Ultrasonography is more useful because displaced intestine, spleen, or liver are readily identified within the pleural cavity. Ultrasonography may also reveal discontinuity of the diaphragm, and sequelae including hemothorax and pleural effusion. While thoracic radiography may identify abdominal viscera within the pleural cavity and secondary pleural effusions, unlike ultrasonography, radiographic evaluation of the ventral thorax is limited by the cardiac silhouette. Oral barium contrast radiography may aid identification of intestines within the thorax.

Management

Surgical repair is considered by some to be optional for those horses which have relatively asymptomatic chronic hernias, and which have either a small defect in the diaphragm or a large tear that does not incarcerate bowel. Emergency surgical repair is clearly indicated for horses with significant clinical compromise, such as intestinal obstruction and strangulation or hemorrhage, or for horses with concomitant fractured ribs. Diaphragmatic hernias can be treated successfully by primary closure of the defect or insertion of a mesh implant via laparotomy (Steenhaut et al 1992, Santschi et al 1997). Postoperative care includes medical treatment of any associated clinical sequelae, stall confinement, and antibiotic therapy. The long-term prognosis is favorable in horses which are asymptomatic postoperatively and which have a healed surgical site. Horses that are treated successfully can achieve race records similar to their siblings and can produce foals without recurrence of the herniation (Santschi et al 1997).

Pneumothorax

Pathogenesis

The pleural cavity is normally at subatmospheric pressure because of the net effect of the outward elastic recoil of the thoracic wall and the inward elastic recoil of the lungs. The subatmospheric intrapleural pressure prevents lung collapse. Pneumothorax is the accumulation of gas, usually air, within the pleural cavity. The pathophysiology of pneumothorax is described in Chapter 2, Fig. 2.8. Pneumothorax may be classified by etiology (spontaneous, traumatic, iatrogenic), pathophysiology (open or closed), site (unilateral or bilateral) or mechanism (simple or tension). In the horse pneumothorax is commonly bilateral, because the two pleural cavities often communicate through small fenestrations in the caudal mediastinum. Unilateral pneumothorax occurs in horses that do not have fenestrations, and in horses with pleural effusions, because of occlusion of the fenestrations by fluid or inflammatory debris (Boy & Sweeney 2000).

Table 46.3. Causes of pneumothorax and pneumomediastinum
Penetration of the thoracic wall, trachea, esophagus or diaphragm
Fractured ribs
Blunt external trauma causing tearing of the visceral pleura
Bronchopleural fistula
Pleuropneumonia and pneumonia
Wounds of the axilla and ventral neck
Leaking thoracic drains
Complication of tracheostomy, percutaneous transtracheal aspiration, lung biopsy, thoracostomy
Excessive positive-pressure ventilation in foals (barotrauma)
Idiopathic

Open versus closed pneumothorax

Open pneumothorax results from penetrating injuries to the thoracic wall and parietal pleura. Injuries may be accidental (staking injuries, automobile collisions), malicious (gunshots) or iatrogenic (leakage of air through thoracic drains) (Table 46.3). Air is sucked into the pleural cavity through the defect in the thoracic wall during inhalation, as a result of the subatmospheric intrapleural pressure. Accumulation of air within the cavity results in loss of the normal subatmospheric intrapleural pressure that helps maintains lung inflation, leading to collapse of the ipsilateral lung. Thoracic wall defects that are larger than the cross-sectional area of the trachea also result in displacement of the mediastinum towards the contralateral hemithorax.

Closed pneumothorax occurs when air enters the pleural cavity via a route other than a defect in the external thorax. Air may leak through tears in the visceral pleura, caused by laceration of the lung by sharp edges of fractured ribs, blunt thoracic trauma such as occurs during collisions and falls, or barotrauma as the result of excessive mechanical ventilation. Air may also enter the pleural cavity via a bronchopleural fistula, which is an abnormal communication between any part of the bronchial tree and the pleural cavity. Bronchopleural fistulae most commonly result from necrosis and sloughing of subpleural lung tissue in horses with gangrenous pleuropneumonia, but also may occur when lung abscesses or neoplasms erode through the visceral pleura. Fistulae from terminal airways often heal spontaneously by formation of interpleural adhesions, while those involving larger bronchi rarely do so. Closed pneumothorax may also occur as a sequel to pneumomediastinum (see below).

Simple versus tension pneumothorax

Simple pneumothorax is self-limiting and non-progressive. *Tension pneumothorax*, in contrast, leads to progressive, rapid accumulation of large volumes of air within the pleural cavity, with resultant rapid life-threatening dete-

rioration in cardiopulmonary function. Tension pneumothorax results from a defect in the visceral pleura (closed tension pneumothorax) or parietal pleura (open tension pneumothorax) that acts as a one-way valve. This permits increasing volumes of air to enter the pleural cavity from the lungs during inspiration, but prevents an equal egress of air from the pleural cavity during exhalation. The intrapleural pressure increases progressively with successive attempts to inhale, until it exceeds that of the atmospheric pressure, and the thorax becomes fixed in maximal expansion and effective breathing ceases. Ventilation–perfusion mismatching and intrapulmonary (right to left) shunting of blood through the rapidly collapsing lung cause the $P_{A}O_2$ to decline rapidly. In severely affected horses, alveolar hypoventilation may lead to hypercapnia and exacerbate hypoxemia. The increasing intrapleural pressure displaces the mediastinum towards the contralateral side, compromising the function of the contralateral lung and decreasing venous return to the heart by compressing the large intrathoracic veins. Rapid cardiopulmonary collapse may ensue.

Clinical signs and diagnosis

Pneumothorax causes variable degrees of respiratory distress, anxiety, sweating, pleurodynia, and thoracic expansion. Horses with open pneumothorax usually have an obvious external wound, especially if a "sucking" sound is audible as air enters the thorax during inspiration. Thoracic auscultation and percussion may reveal absence of breath sounds and hyperresonance, respectively, over the dorsal thorax. Thoracic radiography and ultrasonography may help confirm a clinical suspicion of pneumothorax. Thoracic radiographs are best taken during expiration. The dorsal margin of the collapsed lung is seen as a horizontal linear opacity lying ventral to the thoracic vertebrae (see Chapter 10, Fig. 10.22). Radiographs may also reveal underlying causes of pneumothorax including rib fractures or penetrating radiodense foreign bodies. Ultrasonography reveals a static gas reverberation artifact that does not move with breathing (see Chapter 11, Fig. 11.16). Ancillary diagnostic procedures may not be possible in cases that present as acute onset, life-threatening emergencies. In such cases, aspiration of air from the pleural cavity, and the consequent rapid clinical improvement, confirms the diagnosis. Once the patient is stabilized, further diagnostic procedures can then be performed.

Management

Simple uncomplicated closed pneumothorax without dyspnea may be treated conservatively, with stall rest and close observation. Air is absorbed from the pleural space, albeit slowly, because the venous blood within the pleural vessels contains a lower total pressure of dissolved gases

than does the alveolar air or atmospheric air. In humans with pneumothorax, approximately 1.25% of the air volume is absorbed daily when patients breathe atmospheric air (Chadha & Cohn 1983).

Open external wounds should be closed, ideally by primary closure following local intercostal nerve blocks and aseptic preparation. In emergency situations, further ingress of air may be prevented by manual compression with a gloved hand or application of a non-porous pressure pad. Large and complex thoracic defects may require surgical repair under general anesthesia. In these circumstances, care must be taken to stabilize patients and remove air from the pleural cavity before inducing anesthesia. Positive-pressure ventilation of horses with tension pneumothorax must be performed with care because exacerbation of the pneumothorax may not be apparent until cardiac arrest occurs.

Air is aspirated from the pleural cavity via a 10-cm blunt teat cannula or 8–10 French catheter inserted in the dorsal lung field at the level of 13th intercostal space, using a suction apparatus or a large syringe with a three-way stopcock. Drainage may be performed intermittently, or employing a continuous aspiration system with a Heimlich valve (Fig. 46.2) or an underwater seal device, with or without suction. Bilateral pneumothorax is usually resolved by aspiration from only one hemithorax. However, in severe bilateral pneumothorax, and tension pneumothorax, bilateral tube thoracostomy should be performed. If pneumothorax is the result of leaking thoracostomy tubes or valves, the apparatus should be replaced and suction applied to the new indwelling chest tube and to a drain inserted into the dorsal pleural cavity. In humans, when a large volume pneumothorax has persisted for over 24 h, rapid evacuation of air from the pleural cavity may occasionally induce severe, life-threatening interstitial edema, termed "re-expansion pulmonary edema" (Rozenman et al 1996). Consequently slow evacuation using not more than 20 cm water suction pressure is recommended. Ultrasonography and radiography can be used to assess restoration of lung inflation. Failure of thoracostomy to alleviate the respiratory distress should prompt examination for concomitant injuries such as fractured ribs or diaphragmatic hernia, or continued leakage of air into the pleural cavity. The latter may result from (1) a defect in the visceral pleura, (2) iatrogenic lung puncture during insertion of chest drain, which is rare, (3) leakage around the thoracostomy tube or one-way valve, or (4) failure to adequately close external thoracic wounds.

Ancillary treatment is important. Supplemental intranasal oxygen (10–20 ml·kg^{-1}·min^{-1} or 5–10 liters/min) should be provided if available as it helps reduce hypoxemia and aids absorption of air from the pleural cavity, by washing out nitrogen from the blood (Chadha & Cohn 1983). Horses with pleurodynia should receive analgesia to minimize chest splinting and its detrimental sequelae.

Antibiotic therapy is indicated for horses with thoracic wounds. Pleural lavage (see below) may be indicated in horse with penetrating thoracic wounds to minimize secondary bacterial pleuritis.

Pneumomediastinum

Pathogenesis

Pneumomediastinum is the accumulation of gas, usually air, within the mediastinum. It often occurs in conjunction with pneumothorax because of movement of gas between the two spaces. Pneumomediastinum can occur in horses which have tension subcutaneous emphysema as a result of wounds to the trachea or axilla, or as a rare complication of transtracheal puncture and tracheostomy (Farrow 1976, Hance & Robertson 1992, Kelly et al 2003). Tension subcutaneous emphysema occurs when air enters through a wound that acts as a one-way valve, preventing egress of an equal volume of air. Air then dissects along the subcutaneous fascial planes of the neck and into the mediastinum; thereafter air may pass through the mediastinal pleura into the pleural cavity to cause concomitant pneumothorax. Pneumomediastinum may also result from blunt thoracic trauma. Pneumomediastinum, and concomitant acute bacterial mediastinitis, bacterial pleuropneumonia, and pneumothorax, may result from perforation of the esophagus. Pneumomediastinum may also result from rupture of alveoli in horses with pulmonary disorders that lead to air trapping or emphysema. Air tracks from the ruptured alveoli through the bronchovascular sheaths to the lung hilus and mediastinum and thereafter into the subcutaneous tissues of the dorsum and neck. If the rate of egress of air from the mediastinum into the subcutaneous tissues is insufficient, the mediastinal pleura may rupture, resulting in concomitant pneumothorax.

Diagnosis

Pneumomediastinum *per se* is rarely life-threatening, but it may occasionally result in severe respiratory distress and death as the result of acute cardiopulmonary decompensation. On thoracic radiographs, air in the mediastinum outlines structures that are not normally visible, including the mediastinal blood vessels and esophagus. In contrast, free air surrounding the trachea makes it more difficult to identify on radiographs. Localized or generalized subcutaneous emphysema is usually readily palpable in horses with tension subcutaneous emphysema.

Management

The treatment of pneumomediastinum is largely determined by the primary cause. External wounds, such as tracheal perforations, should be closed. Concomitant pneumothorax should be managed with a tube thoracostomy. Stall rest should be provided.

Fig. 46.2. (A) Various indwelling chest tubes; (B) placement of a 24 French blunt catheter with a condom one-way valve; (C) an indwelling chest catheter with a Heimlich valve.
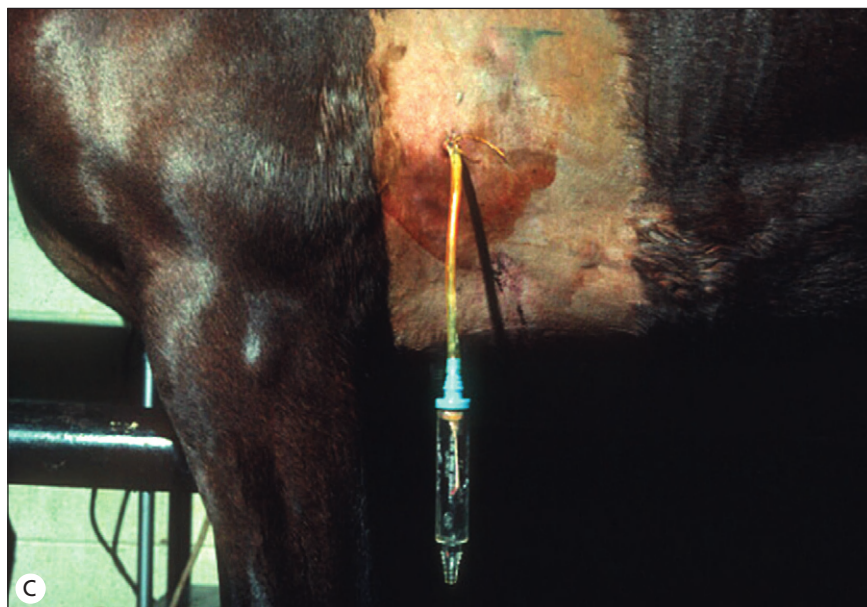
Table 46.4. Causes of hemothorax
Trauma (blunt and penetrating)
Complication of lung biopsy
Coagulopathy
Rupture of a major vessel or aneurysm
Bleeding tumors, especially hemangiosarcoma
Abscess
Pulmonary infarction

Hemothorax

Hemothorax is rare in horses, but may be caused by various disorders (Table 46.4). Horses with acute intra-pleural hemorrhage are likely to present with hemorrhagic shock rather than with respiratory failure. This is because the volume of blood loss that causes death as a result of acute hemorrhagic shock (approximately 15 liters for a 500-kg horse) is insufficient to cause life-threatening pulmonary tamponade. Thus the prime therapeutic consideration is replacement of blood volume. The underlying cause of the hemothorax should also be sought, because this is an important determinant of subsequent case management. Whether it is beneficial to drain free blood from the pleural cavity is unclear, as there are insufficient data in the literature to answer this question. Some case reports indicate that hemothorax may resolve successfully without thoracostomy (Perkins et al 1999). In these cases, rapid autotransfusion of blood from the pleural cavity via the pleural lymphatics may have led to rapid resolution of hemothorax. However, other authors state that free blood should be drained (Laverty et al 1996). Drainage of blood may be beneficial because it facilitates pulmonary re-expansion, which may arrest hemorrhage arising from the low-pressure pulmonary parenchymal vessels. Furthermore, free blood left within the pleural cavity represents an ideal culture medium for bacteria, and, in humans, may lead to fibrous pleurisy and permanent impairment of lung expansion. To date, there are no reports of fibrous pleurisy as a sequel to hemothorax in the horse. Disadvantages of tube thoracotomy include iatrogenic introduction of micro-organisms into the pleural cavity, injury to the lung or heart, and disruption of blood clots causing recurrence of bleeding from a pleural defect (Perkins et al 1999).

Pulmonary Contusion

Lung contusion results from blunt trauma to the thorax when the glottis is closed (e.g. automobile collisions and horses falling at jumps). It may occur in conjunction with other traumatic thoracic injuries. If severe, it carries a poor prognosis because it causes a rapid ventilation–perfusion mismatch and has life-threatening sequelae including hemorrhagic, infarctive, and gangrenous pneu-

monia. There is no specific treatment. Horses should be rested in a quiet environment. Antibiotics may be administered to counteract secondary bacterial infection, which is a common sequel. Analgesics should be administered to reduce pleurodynia. Bronchodilators may aid clearance of blood and exudate from the airways. As contused lung is susceptible to developing edema and exacerbation of ventilation–perfusion mismatching following crystalloid infusions, fluid replacement should be performed cautiously.

Pleural Effusion

Pathogenesis

The pleural cavity is a potential space that normally contains only a small volume of clear to slightly turbid yellowish, non-clotting pleural fluid. Tight regulation of the volume and composition of pleural fluid is essential for efficient mechanical coupling between the lung and chest wall. This mechanical coupling facilitates instantaneous transmission of forces of breathing, which are applied perpendicularly to the pleural surface, while simultaneously providing lubrication to enable frictionless movement between the two pleural surfaces in response to shear stresses. In the normal horse, pleural fluid is produced by a net filtration pressure gradient through the parietal pleura, while it is absorbed by a net absorptive pressure gradient through the visceral pleura. These pressure gradients are, in part defined by the Starling–Landis equation (see Chapter 43). Pleural fluid is also resorbed by electrolyte-coupled liquid absorption through mesothelial cells in both pleurae, and by vesicular transport of liquid associated with protein transocytosis (Zocchi 2002). Pleural fluid, protein, and cells are also absorbed by lymphatic drainage through numerous stomata present in the parietal pleura. Pleural effusions occur when fluid is produced at a rate faster than it is removed; there are five general mechanisms by which this may occur:

- increased transpleural hydrostatic pressure gradient (e.g. congestive cardiac failure)
- decreased colloid osmotic pressure (e.g. hypoproteinemia)
- impaired lymphatic drainage (e.g. obstruction by neoplasm)
- increased vascular permeability (e.g. inflammation)
- excessive volumes of peritoneal fluid may pass via diaphragmatic lymphatics or diaphragmatic defects into the pleural cavity.

The first two mechanisms produce a bilateral pleural transudate or modified transudate. If congestive heart failure is present, there may also be tachycardia, abnormal jugular distension, and ventral edema. The third and fourth mechanisms will produce unilateral or bilateral pleural exudates. The last mechanism will produce either a

Table 46.5. Causes of pleural effusion
Pleural infections (pleuropneumonia; bacterial, mycoplasma, viral or fungal) Thoracic neoplasia Penetration of chest wall, esophagus, or diaphragm Thoracic trauma Extension of peritoneal effusion Liver disease Congestive heart failure Hypoproteinemia Diaphragmatic hernia Pulmonary hydatidosis Pulmonary granulomas Idiopathic

Table 46.6. Normal constituents of equine pleural fluid	
Appearance	clear to slightly turbid, yellowish, non-clotting fluid
Cells	$< 10 \times 10^9$/liter, with approximately 32–90% neutrophils, 5–66% mononuclear/meso-thelial type cells, 0–16% lymphocytes and 0–0.5% eosinophils
Total protein	< 35 g/l
pH	similar to blood pH
Glucose	> 2 mmol/l
Bacteriologically sterile	

Adapted from Brobst & Parry 1987, with permission.

transudate or exudate, depending on the nature of the peritoneal fluid. Some disorders may produce effusion via several of the aforementioned mechanisms. For example, thoracic neoplasia may cause pleural effusion by (1) obstruction of lymphatics or mediastinal lymph nodes, (2) increased hydrostatic pressure as a result of venous obstruction, and (3) increased permeability because of inflammation around neoplasms.

Some of the numerous causes of pleural effusion are listed in Table 46.5. Approximately two-thirds of all pleural effusions in North American horses are the result of pleuropneumonia, pneumonia or lung abscesses (Raphel & Beech 1981), while in the UK approximately 60% of pleural effusions are associated with neoplasia (Mair 1987).

Clinical signs

Horses with small volumes of pleural effusion may be asymptomatic, while large volumes may cause respiratory distress. Exudates are associated with pleurodynia. Coughing is rarely the result of pleural effusion, and is more likely to be attributable to underlying pulmonary disease.

Investigation

The cause of the pleural effusion should be determined, where possible, by signalment and case history, clinical examination, pleural fluid analysis, radiography, ultrasonography, and pleuroscopy. Thoracocentesis should be performed in all horses with suspected pleural effusion, to confirm the presence of pleural effusion, to improve ventilation, to remove inflammatory debris, toxins and bacteria, and to obtain samples for laboratory analysis. Thoracocentesis is a relatively simple and safe technique. Normal horses have small volumes of clear to slightly turbid, yellowish, non-clotting pleural fluid (Table 46.6). Detailed information on the collection, processing, and analysis of pleural fluid is presented in Chapter 9.

Treatment

Treatment of pleural effusions is directed at the underlying cause, and at drainage of pleural fluid to improve ventilation (see below). In human medicine, persistent or recurrent pleural effusions that are caused by primary lesions for which there is no effective treatment may be managed using chemical pleurodesis. This involves instillation of irritants such as tetracycline or talc to produce adhesions between the visceral and parietal pleurae to reduce the dead space. While this procedure may be considered for horses (Ainsworth & Hackett 2004), welfare implications mean that selection of cases is critical. For example, it is not an appropriate option for horses in the terminal stages of thoracic neoplasia. Furthermore, in humans it causes significant pleurodynia and may initially exacerbate pleural effusion. Clinicians considering performing pleurodesis should consult a detailed medical text (Lee & Light 2004).

Chylothorax

Chylothorax, the accumulation of chyle within the pleural cavity, is rarely reported in horses (Mair et al 1988, Schumacher et al 1989, Brink et al 1996, Scarratt et al 1997). Chylothorax results from a leak in the thoracic duct, which drains lymph from the gastrointestinal tract, liver, abdominal wall, and hind limbs. Chyle has a high protein content and contains predominantly lymphocytes. Chyle from suckling foals has a high chylomicron content, which imparts a characteristic milky opacity, while chyle from adults has a lower chylomicron content and may consequently lack a milky appearance (Brink et al 1996). The underlying disorders associated with chylothorax include congenital diaphragmatic hernia, meconium impaction, neoplasia, and idiopathic causes. Differentiation of

chylothorax from pseudochylous effusion that results from severe chronic inflammatory processes is discussed in Chapter 9. Management of chylothorax is reported infrequently in horses, but may include thoracic drainage, restricted exercise, low-fat diets, and ligation of the thoracic duct. Prognosis is guarded.

Pleuropneumonia

Etiopathogenesis

Pleuropneumonia most commonly occurs in conjunction with bacterial pneumonia and lung abscesses (Table 46.7). Thus the risk factors for pleuropneumonia are similar to those for pneumonia, and include factors that:

- cause aspiration of oropharyngeal bacteria
- inhibit clearance of secretions from the lower airways
- compromise pulmonary defense mechanisms.

Risk factors include long-distance transportation (>800 km), strenuous exercise, general anesthesia and surgical procedures, respiratory viral infections, corticosteroid therapy, disorders of the upper airway, exercise-induced pulmonary hemorrhage, systemic diseases, and dysphagia (Chaffin & Carter 1993, Austin et al 1995, Raidal 1995, Raidal et al 1995, Racklyeft et al 2000). Long-distance transportation is the single most important risk factor because prolonged head elevation prevents postural drainage of respiratory secretions, and facilitates aspiration of upper respiratory tract bacteria into the lower airways. This leads to accumulation of purulent respiratory secretions within the distal airways (Raidal et al 1997a). In addition, long-distance transportation is immunosuppressive. It may also expose horses to environments that have poor air hygiene, resulting in inhalation of bacteria and pro-inflammatory agents. Strenuous exercise is also a risk factor because it is immunosuppressive and because it results in inhalation of particulates from the racetrack and aspiration of oropharyngeal secretions deep into the lung. Consequently, even a single episode of strenuous exercise significantly increases the numbers of aerobic and anaerobic bacteria within the lower airway (Raidal et al 1997b). Pleuropneumonia most commonly affects younger horses, 1–5 years old, associated with active competition and frequent long-distance transport. A wide

range of aerobic and anaerobic bacteria are involved, most of which are commensals of the oropharynx (Sweeney et al 1985, 1991, Mair & Lane 1989, Byars & Becht 1991, Chaffin & Carter 1993). Common aerobic isolates include β-hemolytic streptococci, *Pasteurella* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., and *Actinobacillus* spp. Common anaerobic isolates include *Clostridium* spp., *Fusobacterium* spp., *Peptostreptococcus* spp., and *Bacteroides* spp. Anaerobic bacteria are most commonly associated with disease of more than 5–7 days' duration (Sweeney et al 1991), and their presence is associated with a poor prognosis (Sweeney et al 1985), in part because it suggests that substantial tissue damage has occurred to convert the normal aerobic environment of the lung to an anaerobic environment. Polymicrobial infections, and mixed aerobic and anaerobic infections, are more common than single isolates. Viruses (African horse sickness, equine infectious anemia), mycoplasmas (*Mycoplasma felis*), fungi (*Coccidioides immitis*, *Cryptococcus neoformans*) and hydatid cysts are less common causes of pleuropneumonia. Carr et al (1997) reported 21 horses which had suppurative hemorrhagic pleural effusion associated with hemorrhagic pulmonary infarction, necrotizing pneumonia, and, in some cases, pulmonary thromboembolism. This etiology of this syndrome was not determined, but many cases followed strenuous exercise and the pathology resembled that of porcine *Actinobacillus pleuropneumoniae* infection. *Actinobacillus* spp. and *Streptococcus equi* var. *zooepidemicus* were cultured from some affected horses. This syndrome had a poorer prognosis than conventional pleuropneumonia.

Three phases are recognized in the pathogenesis of equine pleuropneumonia (Chaffin & Carter 1993):

- *Exudative phase* – initially bacteria colonize the peripheral lung, causing localized pneumonia and/or pulmonary micro-abscessation. There is a predilection for involvement of the ventral aspects of the cranial lung lobes and the cranial aspects of the caudal lung lobes, especially on the right side, consistent with aspiration being the major route of infection. Extension of the inflammatory response to the contiguous visceral pleura increases the permeability of the capillaries within the visceral pleura, leading to production of a sterile, protein-rich inflammatory parapneumonic effusion. Parenchymal damage predisposes to secondary colonization with anaerobes. Systemic antibiotic therapy alone may be effective at this early stage.
- *Fibrinopurulent phase* – direct extension of bacterial infection into the pleural cavity causes production of large volumes (up to 80 liters) of septic fibrinopurulent exudate. Large quantities of fibrin cover the pleural surfaces, producing a complex network of fibrin strands and loculi.
- *Organization phase* – organization of fibrin into fibrous tissue results in a thick "pleural peel" that limits thoracic

Table 46.7. Causes of pleuropneumonia
Extension of pneumonia or lung abscess
Penetrating and blunt thoracic injury
Penetrating injuries of the esophagus or diaphragm
Systemic spread (bacteremia or septicemia)
Pulmonary hydatidosis
Neoplasia

expansion and may lead to interpleural adhesions. The residual pleural exudate, containing inflammatory and necrotic debris, may become more viscous to produce a pleural empyema. There may be significant lung necrosis and bronchopleural fistulae may develop.

Clinical signs

The clinical signs of pleuropneumonia are variable. Affected horses are commonly "sick" (i.e. depressed, anorexic, febrile), in contrast to horses with recurrent airway obstruction. Additional signs include lethargy, anorexia, pyrexia, tachycardia, tachypnea, various degrees of respiratory distress, elbow abduction, and ventral edema. Nasal discharge may be absent, or may be mucopurulent, purulent or serosanguineous. Malodorous breath and fetid nasal discharge may suggest anaerobic infection or tissue necrosis, and is consequently a poor prognostic sign. Mucous membranes may appear normal, cyanotic or toxic. Pleurodynia (thoracic pain) is common and often severe, resulting in an anxious facial expression, a fast shallow breathing pattern, stilted gait, reluctance to turn in tight circles and to lie down, a characteristic soft suppressed cough, expiratory grunting, especially during palpation of the chest, and forelimb pointing. Forced rebreathing is contraindicated in horses with pleurodynia. Clinicians must be careful not to mistake pleurodynia for colic because this is likely to lead to inappropriate diagnostic evaluation and treatment. The occurrence of secondary constipation and ileus in horses with pleurodynia may confound this error. Pleuropneumonia may also be mistaken for laminitis, rhabdomyolysis (Chaffin et al 1994a) or hypocalcemia.

Auscultation reveals absence of lung sounds over the ventral thorax, and increased lung sounds, with or without adventitious sounds, in the dorsal lung fields. Horses with exudates within the large airways commonly have coarse crackles audible over the distal cervical trachea. Cardiac sounds may be transmitted more dorsally than normal. Pleural friction rubs are rarely present in acute cases but may be audible in chronic patients. Percussion will reveal dullness over the ventral thorax and may elicit coughing and evidence of pleurodynia. Patients with cardiovascular compromise may have tachycardia, jugular distension, and toxic mucous membranes.

Diagnosis and investigation of pleuropneumonia

Ultrasonographic examination

Ultrasonography is the best diagnostic modality for detecting and evaluating suspected pleural disease (Chapter 11). Ultrasonography can be used to assess the nature of pleural fluid (echodense, echolucent, gas echoes), volume of fluid, determine whether it is unilateral or bilateral, identify

sequelae such as fibrin deposition, loculi formation, pleural adhesions, and pneumothorax, and select the optimal site for thoracocentesis (Rantanen et al 1981, Reimer et al 1989, Chaffin et al 1994b). Gas echoes in the pleural effusion have been described with anaerobic infections, gangrenous pneumonia, bronchopleural fistulae, and iatrogenic leakage or air during thoracocentesis. Ultrasound can also detect concurrent cardiopulmonary lesions, such as pulmonary consolidation (hepatization), compression atelectasis, abscesses, infarction and pericardial effusions (Fig. 46.3).

Thoracic radiography

Radiography is diagnostic of pleural effusion whenever a fluid line is noted that obliterates the normally distinct heart shadow and caudal vena cava (Chapter 10, Fig. 10.20). However, radiography does not readily permit identification of the affected hemithorax, assessment of the character of the fluid, or evaluation of the ventral lung field deep to the fluid. Radiography, ideally performed after thoracocentesis, may allow evaluation of the mid and dorsal lung fields for the presence of underlying disorders such as abscesses and neoplasms. Radiography may identify lesions within the axial lung fields, which cannot be identified by ultrasonography. Thoracic radiography is fully discussed in Chapter 10.

Thoracocentesis and transtracheal aspirate

Thoracocentesis is essential to confirm a diagnosis of pleuropneumonia, and to provide samples for cytological, biochemical, and microbiological testing (laboratory evaluation is discussed in Chapter 9). Diagnostic microbiology is often pivotal to the case outcome, by providing data to guide appropriate antimicrobial selection. Ideally, antibiotics should be withheld for a minimum of 24 h before microbiological sampling. Bacterial culture of both pleural fluid and transtracheal aspirates will optimize detection of organisms associated with pleuropneumonia.

Pleuroscopy

Pleuroscopy is rarely warranted in cases of acute pleuropneumonia. However, it may provide additional information regarding sequelae such as fibrin deposition, loculi formation, pleural adhesions, bronchopleural fistulae, and lung abscesses. Pleuroscopy is discussed in Chapter 20.

Hematology and biochemistry

Horses with acute pleuropneumonia commonly have an inflammatory hematological profile, i.e. leukocytosis, neutrophilia, possibly with a left shift and toxic neutrophils, hyperfibrinogenemia, hyperglobulinemia, and hypoalbuminemia. Patients with advanced disease may have a toxic neutropenia, hypoproteinemia, and hemoconcentration.
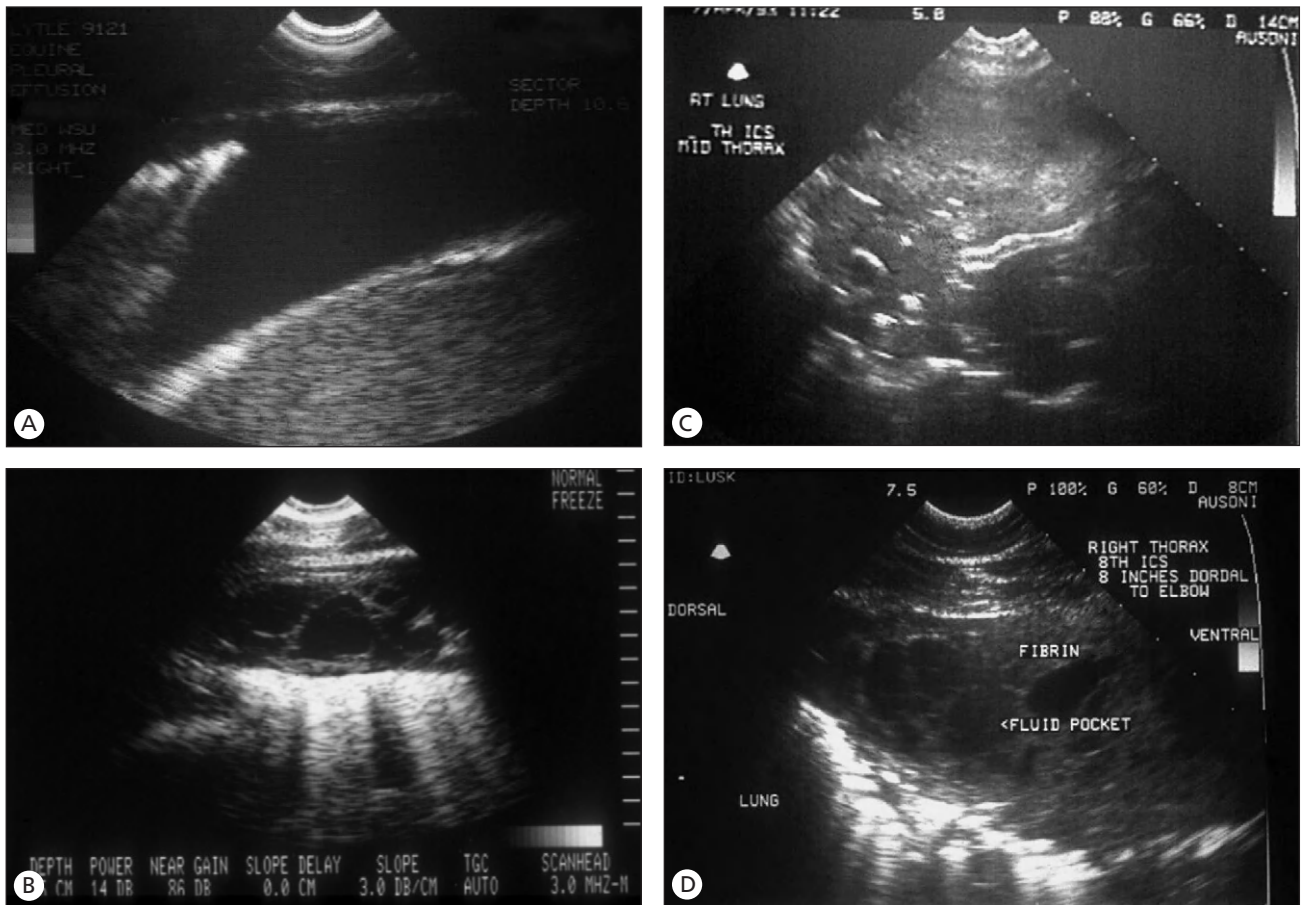
Fig. 46.3. Ultrasound images from horses with pleuropneumonia: (A) pleural effusion outlining the ventral border of the free-floating lung with diaphragm and spleen to the right of the image; (B) loculated fibrinous pleuropneumonia; (C) hepatized lung – note intrapulmonary air trapping and outline of an airway; (D) pleural abscess formation secondary to pleuropneumonia.

Treatment of pleuropneumonia

A successful case outcome is dependent on early recognition and prompt initiation of appropriate treatment. Ideally treatment should be commenced within 48 h of a predisposing event to prevent significant bacterial invasion of pulmonary parenchyma (Raidal 1995). Treatment is usually prolonged and is determined by the degree of cardiopulmonary compromise evident at the time of diagnosis and by the development of sequelae during the course of treatment. Treatment involves:

- prompt administration of systemic, broad-spectrum, bactericidal antibiotics
- drainage of pleural effusion
- ancillary treatments
- prompt recognition and appropriate treatment of sequelae.

Antibiotic therapy

Administration of antibiotics to which the causal organism(s) are sensitive is essential for patient survival. Transtracheal aspirates and pleural fluid should be cultured to provide retrospective antimicrobial sensitivity data. Empirical therapy should be commenced immediately, while awaiting culture and sensitivity data. Analysis of Gram-stained smears of these samples may provide data to guide selection of an empirical antibiotic regimen. Suitable empirical combinations should be effective against many of the bacteria that cause pleuropneumonia and include penicillin, gentamicin, and metronidazole, or ceftiofur and metronidazole. Selection of antimicrobials for long-term therapy, such as metronidazole or potentiated sulfonamides, should be based on the sensitivity of the most recent isolates. It is important to recognize that most antibiotics are ineffective in the presence of exudates because of inhibitory effects of components of purulent secretions,

changes in pH, or volume dilution of the drug to non-therapeutic levels. It is therefore essential to drain thoracic fluid in patients with significant volumes of pleural fluid.

Pleural drainage

Pleural drainage should be performed to improve ventilation and remove microorganisms, toxins, inflammatory mediators, and inflammatory debris. It may be performed intermittently or continuously, depending upon the degree of patient compromise, and the volume and nature of the fluid. Continuous drainage, via an indwelling chest tube, is indicated in most patients with pleuropneumonia, especially those with suspected anaerobic infections and those in which intermittent drainage is relatively ineffective. Intermittent drainage is reserved for horses with small volumes (< 5 liters) of fluid. A 24 French blunt thoracostomy tube is recommended for most patients including foals, as smaller diameter tubes such as teat cannulae often become occluded by inflammatory debris (Fig. 46.2). The optimal site is ideally defined by ultrasound evaluation, but the sixth or seventh intercostal space is usually suitable. It is not essential to select the most ventral site for tube insertion because lung expansion usually moves the fluid towards the internal tube opening.

The site is surgically prepared and local anesthetic (6–10 ml) is infiltrated from the skin to the sensitive parietal pleura. A stab incision is made with a no.10 scalpel through the skin, and enlarged slightly to accommodate the chest tube and trocar. The tube is inserted directly between the ribs. Subcutaneous tunneling is not recommended as this may lead to kinking and occlusion of the drain. As the insertion of large-bore chest drains through the intercostal muscles requires considerable force, care must be taken to prevent advancing the drain too deeply when the resistance suddenly decreases as the tube enters the pleural cavity. The tube is inserted to a depth of approximately 8 cm, and anchored to the skin with a suture attached through "dog-eared" adhesive tape or by a pursestring suture to prevent slippage. The external opening of the tube is connected to a one-way valve to prevent air aspiration when fluid drainage is complete (Fig. 46.2). A Heimlich valve, or a condom or a finger of a surgical glove with the closed end cut off, will suffice (Fig. 46.2). For horses with bilateral effusions, bilateral indwelling tubes are placed only if drainage via a unilateral drain does not effectively drain both hemithoraces. Rapid removal of large volumes of pleural fluid should be avoided to prevent hypovolemia. Indwelling tubes should be monitored regularly and can be kept in place as long as they are productive. Tubes should be removed when non-productive or if a painful local cellulitis ensues. Complications of chest drainage are uncommon but include:

- hypovolemia and collapse if fluid is removed too rapidly
- local cellulitis
- pneumothorax
- cardiac dysrhythmia
- cardiac, pulmonary or diaphragmatic laceration.

Pleural lavage

This may be of benefit in selected cases to aid removal of tenacious exudate, bacteria, toxins, fibrin, and inflammatory debris. It should be performed early in the disease course, before the development of fibrin loculi and adhesions, which would otherwise limit its value. Between 5 and 20 liters of warmed sterile saline are instilled via an ingress catheter into the dorsal pleural cavity, and the fluid is removed via an egress catheter inserted in the ventral pleural cavity. It is rarely possible to recover the full volume of the instilled fluid. This technique is probably contraindicated in horses with patent bronchopleural fistulae as the lavage fluid will enter the airways, further compromising lung function and also inducing coughing.

Thoracotomy

Standing thoracotomy is indicated for horses with large quantities of thickened, organized fibrinopurulent debris or empyema, especially those with fetid contents that cannot be removed by conventional pleural drainage and lavage (Grant 1997). Patient selection is critical for a successful outcome (Chaffin et al 1994b). It is of most benefit for horses with large, persistent, unilateral localized pockets of thick, inspissated debris following resolution of the disease in the contralateral hemithorax. The disease should be confined to one hemithorax or walled off from the remainder of the ipsilateral hemithorax. The mediastinum must be complete, or a visceral to parietal adhesion must seal off the abscess cavity from the surrounding ipsilateral pleural cavity. Prior to thoracotomy the area is fully investigated using ultrasonography. A large-bore open thoracic drain is inserted into the lesion and the patient is monitored for 2 h; if this is well tolerated without development of unilateral or bilateral pneumothorax, it indicates that the lesion is walled off from the remainder of the pleural cavity. The onset of respiratory distress indicates development of pneumothorax and contraindicates the procedure. The surgical site is prepared and infiltrated with local anesthetic. A vertical incision, capable of allowing either finger or hand insertion, is made into the thorax, usually at the seventh or eighth intercostal space, from the level of the lateral thoracic vein to the ventral border of the latissimus dorsi. This allows removal of all accessible necrotic tissue and fibrin by both lavage and manual extraction using

appropriate forceps. It is not necessary to remove all fibrin at this stage, as residues will be removed by flushing over the following days (Grant 1997). The pleural cavity is then lavaged with warmed 1% povidone iodine solution. The contralateral pleural cavity is monitored for pneumothorax, which is managed by tube thoracostomy if required. The incision is left open and debris is removed daily both manually and by flushing until granulation tissue closes the thoracic wound. Alternatively a rib resection or large thoracotomy may be performed to provide even greater access to the pleural cavity. These last procedures, however, have increased postoperative morbidity and usually preclude return of the horse to athletic function (Shearer et al 1986, Chaffin et al 1994b, Grant 1997). A loss of thoracic compliance compromises future athletic performance in all cases.

Ancillary treatments

Supplemental oxygen may be administered to horses with significant hypoxemia, provided the procedure is well tolerated. Analgesia is indicated to alleviate pleurodynia. Fluid and plasma therapy may be required in horses with acute pleuropneumonia, to counteract significant losses of fluid and protein into the pleural space. The effects of toxemia may be minimized by administration of flunixin, hyperimmune plasma, colloids, and pentoxifylline.

Complications of pleuropneumonia

Byars et al (1991) and Byars & Becht (1991) described a variety of serious sequelae in horses with infectious pleuropneumonia, including cranial thoracic masses (7.2% of horses), bronchopleural fistulae (6.5%), pericarditis (2.6%), and laminitis (1.3%). Resolution of many of these complications necessitates invasive intervention. Other sequelae include pleural adhesions (Chapter 11, Fig. 11.8), pleural empyema, pneumothorax, catheter-associated jugular thrombophlebitis, disseminated intravascular coagulation, colitis, and colic.

Cranial thoracic masses (abscesses)

The heart may act as a ball valve to trap effusion and inflammatory debris in the cranial thorax. Organization of this material may result in cranial thoracic masses including encapsulated abscesses and pockets of empyema (Byars et al 1991). Cranial thoracic masses can produce subtle clinical signs or cause tachycardia, jugular pulsation and ventral edema as a result of heart failure because of compression of the great vessels. Forelimb "pointing" may occur in the absence of laminitis. The diagnosis is confirmed by ultrasonographic imaging of the mass in the cranial thorax. It may be necessary to pull the right forelimb forward to image this region.

Medical treatment of cranial thoracic masses, including antibiotics, diuretics, digoxin, and anti-inflammatory drugs, is effective for some horses. Surgical drainage may be required for horses that are unresponsive to medical therapy. Drainage is usually performed on the standing sedated horse, or less commonly under short-duration general anesthesia. The horse's right foreleg is held forward and a large-bore trocar and catheter are inserted under ultrasound guidance into the mass to permit suction and lavage. Alternatively a laparoscope may be used to penetrate the abscess; this facilitates visualization of each tissue layer as it is penetrated, thereby allowing safe introduction of the trocar (Fischer 2002). The decrease in volume of the mass postoperatively should result in rapid resolution of the clinical signs of heart failure. Drainage is best performed as a single procedure; indwelling tubes are not recommended because the triceps musculature causes crimping and occlusion of the catheter and stimulation of the heart by the catheter may cause dysrhythmias (Byars et al 1991). Repeated drainage may be required if the mass recurs.

Extrapulmonary abscesses

Extrapulmonary (pleural) abscesses may require surgical drainage when antibiotic therapy alone is ineffective because of the thick capsule and large volume of exudate and necrotic debris. They are usually amenable to percutaneous drainage via a large-bore (no. 20F to 40F) chest tube, inserted under ultrasonographic guidance. The sharp tip is thrust through the capsule without pushing the lesion away from the chest wall and causing leakage of exudate into the thoracic cavity. Both suction and lavage are employed while moving the tube in and out to break up the debris. As the abscess is walled off from the rest of the pleural cavity, a one-way valve is usually not required. Antibiotics are administered, based on culture of the exudate. Anaerobic infections are often present and the necrotic debris is often fetid.

Pleural empyema

Accumulation of tenacious pus within the pleural cavity commonly necessitates thoracotomy.

Bronchopleural fistula

These develop when gangrenous pneumonia, abscesses or neoplasms cause necrosis of peripheral lung tissue and lead to direct communication between the pleural cavity and the airways. Diagnosis is confirmed by pleuroscopy or by instillation of fluorescein dye (from sterile ophthalmic strips) into the pleural cavity, followed by observation of dye at the external nares or within the trachea. During pleural lavage patients with bronchopleural fistulae will often cough vigorously because the lavage fluid can pass

from the pleural cavity into the airways. Fistulae commonly spontaneously seal because of adhesion development between the adjacent visceral and parietal pleurae, following resolution of the underlying lesion, although this process may take several months. Partial pneumonectomy has been used successfully to treat a bronchopleural fistula and pulmonary abscess in a filly (Sanchez et al 2002).

Pneumothorax

Pneumothorax develops in horses with pleuropneumonia as a result of a bronchopleural fistula or air leaking around thoracic drains, or via the site of thoracocentesis, thoracotomy or transtracheal puncture.

Laminitis

Laminitis is a relatively infrequent complication of pleuropneumonia. The onset may be subtle and the forelimb involvement is frequently asymmetrical; consequently it may be mistaken for pleurodynia. The "sinker syndrome" is often encountered and hoof separation at the coronary band may eventually result in the patient being subjected to humane euthanasia.

Colic

Colic is a common complication of pleuropneumonia. It is most often associated with ileus, and production of scant dry feces. It may result from anorexia, dehydration, pleurodynia, and the lack of effective thoracic compression for defecation.

Prognosis of pleuropneumonia

The prognosis is generally favorable, with survival rates ranging from 48 to 98% in horses receiving prompt and aggressive treatment (Raphel & Beech 1982, Byars & Becht 1991, Seltzer & Byars 1996). Complicated sequelae such as cranial thoracic masses, multiple abscesses, and bronchopleural fistula are associated with decreased survival (Byars & Becht 1991). The prognosis for future athletic performance can also be considered fairly good, with the exception of those that have complicated sequelae (Reef 1990). Indeed Seltzer & Byars (1996) reported that 61% of thoroughbreds raced after recovery from uncomplicated infectious pleuropneumonia, with 56% of these horses winning at least once.

Pleural Fibrosis and Neoplasia

Pleural fibrosis and neoplasia are described in Chapter 43. Pulmonary neoplasia may occasionally cause hypertrophic osteopathy (Fig. 46.4).

Mediastinal Masses

These relatively uncommon lesions include abscesses, neoplasia, and lymphadenopathy. Mediastinal abscesses are relatively uncommon but can result from esophageal rupture or chronic lymph node infection, particularly with *Streptococcus equi* subsp. *equi* (bastard strangles). Thoracic neoplasia is reviewed in Chapter 43. Clinical signs of mediastinal masses reflect compression of the trachea or larger bronchi (respiratory distress, wheezing), esophagus (regurgitation), airways (cough) or large thoracic veins and lymphatics (head and neck edema, jugular vein distension). Horner syndrome may reflect dysfunction of the sympathetic innervation to the head. Diagnosis is made principally by thoracic radiography, ultrasonography, and endoscopy. Ultrasonographic examination is restricted to the cranial mediastinum, because air-filled lung reflects ultrasound and precludes examination of the central and caudal mediastinum. Endoscopy of the esophagus or trachea may reveal a compressive mass. Treatment will be dependent on the nature of the mass. Antibiotic therapy and drainage of accessible abscesses may be indicated.

Neuromuscular Disorders Which Compromise Breathing

Neuromuscular disorders which cause paresis, paralysis or spasm of the intercostal muscles and diaphragm can compromise the horse's ability to breathe (Tables 46.8 and 46.9). Horses with botulism in particular may die from respiratory failure as a result of flaccid paralysis of the respiratory muscles. The clinician should therefore be aware that horses with botulism are unable to maintain an increased depth and rate of breathing because of flaccid paralysis of respiratory muscles and diaphragm. Consequently, increased respiratory effort will not herald the

Table 46.8. Neuromuscular disorders which compromise breathing
Disorders of the neuromuscular junctions Botulism
Disorders which cause myodegeneration of respiratory muscles Atypical myoglobinuria Nutritional myodegeneration Ionophore toxicity
Disorders which cause tetany of breathing muscles Tetanus
Miscellaneous disorders Diaphragmatic muscle paralysis
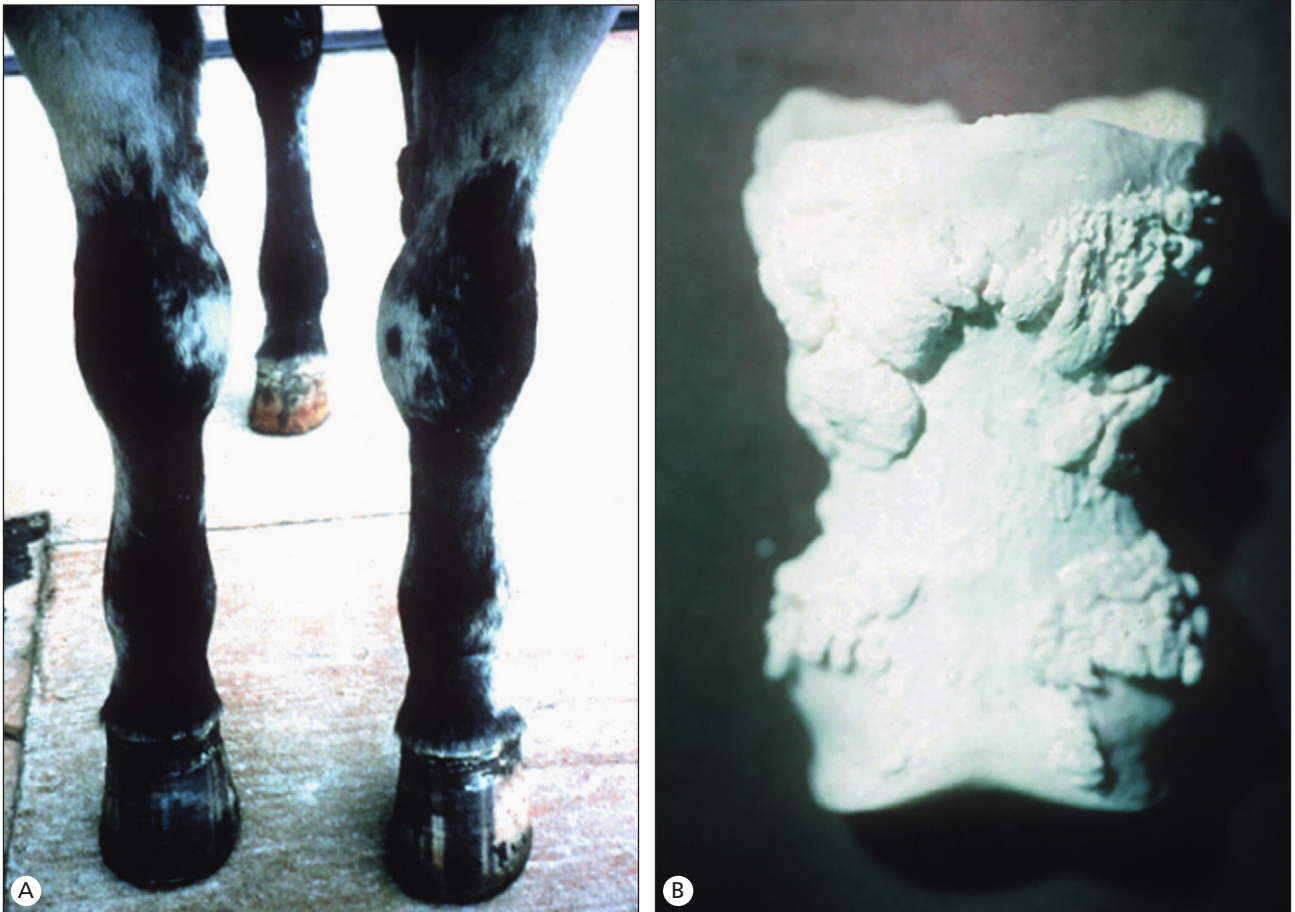
Fig. 46.4. (A) A 14-year-old Appaloosa gelding with hypertrophic osteopathy; (B) post-mortem specimen of proximal phalanx from the same horse.

onset of respiratory failure, as it does in disorders such as recurrent airway obstruction. Instead, the first sign of respiratory failure may be cyanosis. Mechanical ventilation and oxygen supplementation are indicated in horses with respiratory paralysis caused by botulism. Myodegeneration of intercostal and diaphragmatic muscles occurring in atypical myoglobinuria, nutritional myodegeneration, and ionophore toxicity may lead to respiratory dysfunction. Bilateral idiopathic diaphragmatic paralysis can result in significant hypoventilation, paradoxical breathing and respiratory failure (Amory et al 1994).

Synchronous Diaphragmatic Flutter ("Thumps")

"Thumps" produced by unilateral or bilateral contraction of the diaphragm may be heard or palpated over the thorax and flanks in horses with synchronous diaphragmatic flutter. It is probable that acid–base and electrolyte disturbances, and in particular disturbances of calcium,

chloride, magnesium, and bicarbonate, make the phrenic nerve sensitive to the depolarizing electrical activity of the adjacent myocardium. The involuntary diaphragmatic contraction and resultant "thump" are thus synchronous with the heartbeat and not with the breathing cycle. Synchronous diaphragmatic flutter is most commonly associated with exhaustive exercise including racing and endurance rides, and with hypocalcemic syndromes such as lactational tetany, transport tetany, cantharidin (blister beetle) toxicity, and idiopathic hypocalcemia. Diagnosis is straightforward because of the pathognomonic clinical signs, with laboratory confirmation being made by electrolyte analysis, including assessment of total and ionized calcium and magnesium levels.

Treatment consists of parenteral administration of calcium and occasionally chloride solutions. The response to treatment is usually rapid, thus supporting the diagnosis. The prognosis is considered favorable, although a rest period of 7–10 days is often indicated. Supplementation of electrolytes either in the feed or in the water may prevent this condition in endurance horses.

	Table 46.9. Summary of features that may aid differentiation of neuromuscular disorders that can compromise breathing		
	Signalment	**Clinical signs**	**Diagnostic aids**
Botulism	Ingestion of contaminated food including silage. Foals grazing pastures which harbor spores. Rarely toxicoinfection from wounds. Outbreaks possible	Diffuse myasthenia. Variable autonomic signs including ileus, constipation and megaesophagus	Definitive diagnosis rarely achieved. Diagnosis supported by detection of toxin in fresh food, serum and feces, or culture of organism from feces or food. False positives and negatives occur. EMG findings are supportive
Atypical myoglobinuria	Typically affects horses and ponies that are grazing poor quality pasture, with no supplementary feeding and no exercise. Outbreaks may occur	Stiff, stilted gait. Variable muscle pain, distress, sweating, tachycardia, tachypnea. Myoglobinuria. Many horses are alert, responsive and have normal appetite. Variable ileus, dysphagia and urinary retention	Markedly elevated muscle enzymes. Myoglobinuria. Histopathology of muscle biopsies
Nutritional myodegeneration (subacute skeletal form)	Regions with selenium-deficient soils. Mainly foals <1 year old. Group outbreaks may occur	Stiff, stilted gait. Firm, swollen, painful muscles. Myoglobinuria. Variable distress, sweating, dysphagia, tachycardia and tachypnea	Markedly elevated muscle enzymes. Myoglobinuria. Low erythrocyte glutathione peroxidase activity. Low blood and tissue selenium and possibly low blood and tissue vitamin E
Acute ionophore toxicity	Ingestion of ionophores such as monensin. Outbreaks may occur	Acute toxicity causes muscular weakness, hypovolemic shock and hemolysis. Possible generalized sweating, ataxia, myoglobinuria, cardiac dysrhythmias, ileus, colic, diarrhea, dyspnea and sudden death	Detection of monensin in feed or stomach contents. Markedly elevated muscle enzymes. Myoglobinuria
Tetanus	Unvaccinated horses, usually with puncture wound	Tetany of skeletal muscles, stiff gait, "rocking horse stance", raised tail head, anxious facial expression, ileus, third eyelid flicks across during menace response. Progresses to recumbency and opisthotonus	None

Adapted from McGorum 2003, with permission.
EMG, electromyography.

REFERENCES

Ainsworth DM, Hackett RP 2004 Disorders of the respiratory system. In: Reed SM, Bayly WM, Sellon DC (editors) Equine Internal Medicine, 2nd edn. WB Saunders, Philadelphia, pp.289–353

Amory H, Lomba F, Lekeux PM et al 1994 Bilateral diaphragmatic paralysis in a pony. Journal of the American Veterinary Medical Association 205: 587–591

Austin SM, Foreman JH, Hungerford LL 1995 Case–control study of risk factors for development of pleuropneumonia in horses. Journal of the American Veterinary Medical Association 207: 325–328

Bellazo F, Hunt RJ, Provost P et al 2004 Surgical repair of rib fractures in 14 neonatal foals: case selection, surgical techniques and results. Equine Veterinary Journal 36: 557–562

Boy MG, Sweeney CR 2000 Pneumothorax in horses: 40 cases (1980–1997). Journal of the American Veterinary Medical Association 216: 1955–1959

Brink P, Wilkins LP, Spano JS 1996 Chylothorax due to primary thoracic haemangiosarcoma in a horse. Equine Veterinary Journal 28: 241–244

Brobst DF, Parry BW 1987 Normal clinical pathology data. In: Robinson NE (editor) Current Therapy in Equine Medicine 2. WB Saunders, London, p.729

Byars TD, Becht JL 1991 Pleuropneumonia. Veterinary Clinics of North America; Equine Practice 7: 63–78

Byars TD, Dainis CM, Seltzer KL et al 1991 Cranial thoracic masses in the horse: a sequel to pleuropneumonia. Equine Veterinary Journal 23: 22–24

Carr EA, Carlson GP, Wilson WD et al 1997 Acute haemorrhagic pulmonary infarction and necrotizing pneumonia in thoroughbred racehorses: 21 cases (1967–1993). Journal of the American Veterinary Medical Association 210: 1774–1778

Chadha TS, Cohn MA 1983 Non-invasive treatment of pneumothorax with oxygen inhalation. Respiration 44: 147–152

Chaffin MK, Carter GK 1993 Equine bacterial pleuropneumonia. Part I. Epidemiology, pathophysiology, and

bacterial isolates. Compendium on Continuing Education for the Practicing Veterinarian 15: 1642–1650

Chaffin MK, Carter GK, Relford RI 1994a Equine bacterial pleuropneumonia. Part II. Clinical signs and diagnostic evaluation. Compendium on Continuing Education for the Practicing Veterinarian 16: 362–379

Chaffin MK, Carter GK, Byars TD 1994b Equine bacterial pleuropneumonia. Part III. Treatment, sequelae, and prognosis. Compendium on Continuing Education for the Practicing Veterinarian 16: 1585–1596

Farrow CS 1976 Pneumomediastinum in horse – complication of transtracheal aspiration. Journal of the American Veterinary Radiology Society 17: 192–195

Fischer AT 2002 Management of pleuritis. Equine Veterinary Education 14: 293–294

Grant BD 1997 Thoracotomy. In: Rantanen NW, Hauser ML (editors) The Diagnosis and Treatment of Respiratory Disease. Proceedings of the Dubai International Equine Symposium. Rantanen Design, USA pp.419–424

Hance SR, Robertson JT 1992 Subcutaneous emphysema from an axillary wound that resulted in pneumomediastinum and bilateral pneumothorax in a horse. Journal of the American Veterinary Medical Association 200: 1107–1110

Jean D, Laverty S, Halley J et al 1999 Thoracic trauma in newborn foals. Equine Veterinary Journal 31: 149–152

Kelly G, Prendergast M, Skelly C et al 2003 Pneumothorax in a horse as a complication of tracheotomy. Irish Veterinary Journal 56: 153–156

Laverty S, Lavoie JP, Pascoe JR et al 1996 Penetrating wounds of the thorax in 15 horses. Equine Veterinary Journal 28: 220–224

Lee YCG, Light RW 2004 Management of malignant pleural effusions. Respirology 9: 148–156

Mair TS 1987 Pleural effusions in the horse. In: Grunsell CSG, Hill FWG, Raw ME (editors) The Veterinary Annual, 27th edn. Scientechnica, Bristol, pp.139–146

Mair TS, Lane JG 1989 Pneumonia, lung abscesses and pleuritis in adult horses: a review of 51 cases. Equine Veterinary Journal 21: 175–180

Mair TS, Pearson H, Waterman AE et al 1988 Chylothorax associated with a congenital diaphragmatic defect in a foal. Equine Veterinary Journal 20: 304–306

McGorum BC 2003 Diffuse skeletal muscle weakness. In: Robinson NE (editor) Current Therapy in Equine Medicine 5. WB Saunders, London, pp.740–745

Perdrizet JA, Dill SG, Hackett RP 1989 Diaphragmatic-hernia as a cause of dyspnea in a draft horse. Equine Veterinary Journal 21: 302–304

Perkins G, Ainsworth DM, Yeager A 1999 Hemothorax in 2 horses. Journal of Veterinary Internal Medicine 13: 375–378

Racklyeft DJ, Raidal S, Love DN 2000 Towards an understanding of equine pleuropneumonia: factors relevant for control. Australian Veterinary Journal 78: 334–338

Raidal SL 1995 Equine pleuropneumonia. British Veterinary Journal 151: 233–262

Raidal SL, Love DN, Bailey GD 1995 Inflammation and increased numbers of bacteria in the lower respiratory tract of horses within 6 to 12 h of confinement with the head elevated. Australian Veterinary Journal 72: 45–50

Raidal SL, Taplin RH, Bailey GD et al 1997a Antibiotic prophylaxis of lower respiratory tract contamination in horses confined with head elevation for 24 or 48 hours. Australian Veterinary Journal 75: 126–131

Raidal SL, Love DN, Bailey GD 1997b Effect of a single bout of high intensity exercise on lower respiratory tract contamination in the horse. Australian Veterinary Journal 75: 293–295

Rantanen NW, Gage L, Paradis MR 1981 Ultrasound as a diagnostic aid in pleural effusion in horses. Veterinary Radiology 22: 211–216

Raphel CF, Beech J 1982 Pleuritis secondary to pneumonia or lung abscessation in 90 horses. Journal of the American Veterinary Medical Association 181: 808–810

Reef VB 1990 Outcome and return to performance in horses with pleuropneumonia evaluated ultrasonographically. Proceedings of the 8th American College of Veterinary Internal Medicine, pp.573–575

Reimer JM, Reef VB, Spencer PA 1989 Ultrasonography as a diagnostic aid in horses with anaerobic bacterial pleuropneumonia and/or pulmonary abscessation: 27 cases (1984–1986). Journal of the American Veterinary Medical Association 194: 278–282

Rozenman J, Yellin A, Simansky DA et al 1996 Re-expansion pulmonary oedema following spontaneous pneumothorax. Respiratory Medicine 90: 235–238

Sanchez LC, Murphy DJ, Bryant JE et al 2002 Use of diagnostic thoracoscopy and partial pneumonectomy for the treatment of a pulmonary abscess and bronchopleural fistula in a Thoroughbred filly. Equine Veterinary Education 14: 290–293

Santschi EM, Juzwiak JS, Moll HD et al 1997 Diaphragmatic hernia repair in three young horses. Veterinary Surgery 26: 242–245

Scarratt WK, Wallace MA, Pleasant RS et al 1997 Chylothorax and meconium impaction in a neonatal colt. Equine Veterinary Journal 29: 77–79

Schambourg MA, Laverty S, Mullim S et al 2003 Thoracic trauma in foals: post mortem findings. Equine Veterinary Journal 35: 78–81

Schumacher J, Brusie R, Spano J 1989 Chylothorax in an Arabian filly. Equine Veterinary Journal 21: 132–134

Seltzer KL, Byars TD 1996 Prognosis for return to racing after recovery from infectious pleuropneumonia in thoroughbred racehorses: 70 cases (1984–1989). Journal of the American Veterinary Medical Association 208: 1300–1301

Shearer DC, Slone DE, Moll DE et al 1986 Rib resection and thoracotomy as a treatment for chronic pleuritis. Proceedings of the American Association of Equine Practitioners 31: 265–268

Sprayberry KA, Bain FT, Seahorn TL 2001 56 cases of rib fractures in neonatal foals in a referral center intensive care unit from 1997–2001. Proceedings of the American Association of Equine Practitioners 47: 395–399

Steenhaut M, Verschooten F, Demoor A et al 1992 Surgical correction of diaphragmatic-hernia in the horse – a report of 6 cases. Vlaams Diergeneeskundig Tijdschrift 61: 181–186

Sweeney CR, Divers TJ, Benson CE 1985 Anaerobic bacteria in 21 horses with pleuropneumonia. Journal of the American Veterinary Medical Association 187: 721–724

Sweeney CR, Holcombe SJ, Barningham SC et al 1991 Aerobic and anaerobic bacterial isolates from horses with pneumonia or pleuropneumonia and antimicrobial susceptibility patterns of the aerobes. Journal of the American Veterinary Medical Association 198: 839–842

Zocchi L 2002 Physiology and pathophysiology of pleural fluid turnover. European Respiratory Journal 20: 1545–1558

Index