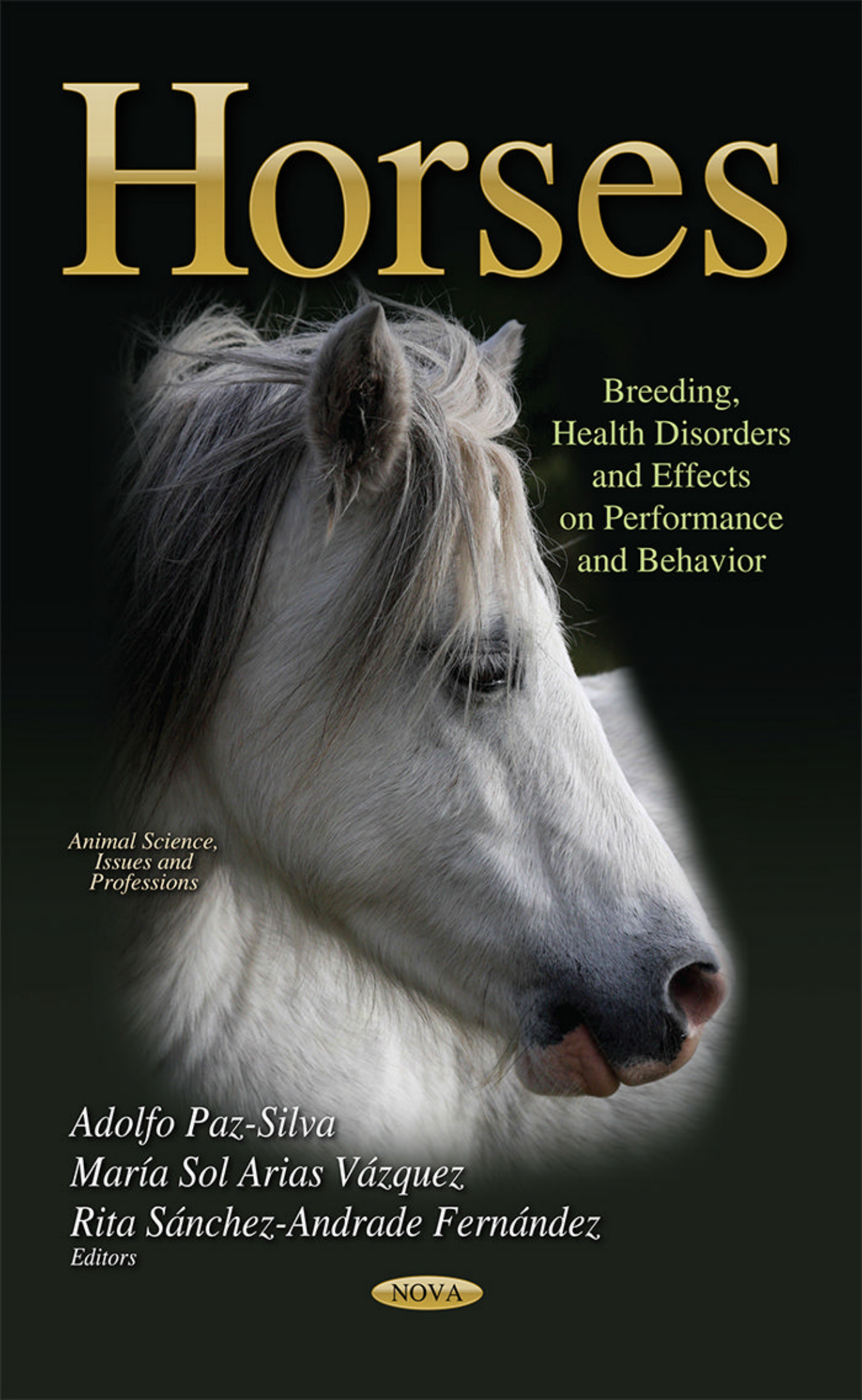
Horses

Breeding, Health Disorders and Effects on Performance and Behavior



Animal Science, Issues and Professions

Adolfo Paz-Silva
María Sol Arias Vázquez
Rita Sánchez-Andrade Fernández
Editors

NOVA

ANIMAL SCIENCE, ISSUES AND PROFESSIONS

HORSES

BREEDING, HEALTH DISORDERS AND EFFECTS ON PERFORMANCE AND BEHAVIOR

ANIMAL SCIENCE, ISSUES AND PROFESSIONS

Additional books in this series can be found on Nova's website under the Series tab.

Additional e-books in this series can be found on Nova's website under the e-book tab.

ANIMAL SCIENCE, ISSUES AND PROFESSIONS

HORSES

BREEDING, HEALTH DISORDERS AND EFFECTS ON PERFORMANCE AND BEHAVIOR

ADOLFO PAZ-SILVA
MARÍA SOL ARIAS VÁZQUEZ
AND
RITA SÁNCHEZ-ANDRADE FERNÁNDEZ
EDITORS

New York

Library of Congress Cataloging-in-Publication Data

ISBN: 978-1-63117-566-4 (eBook)

Published by Nova Science Publishers, Inc. † New York

CONTENTS

PREFACE

There are several questions requiring attention for rearing horses. By providing appropriate care during early ages, important injuries such as lameness or musculoskeletal damage can be avoided, which becomes of capital importance especially among competition horses. In recent years, there has been an increasing body of evidence that the early rearing environment, in particular access to exercise, can have a positive effect on stimulating the musculoskeletal system and priming the tissue for the future demands it will face in the competition arena.

Due to different reasons, such as their participation in competitions or exhibition, sales or pleasure, horses are transported from one location to another. Transportation could be a physical and psychological stressor for horses, contributing to the development of some diseases, thus the effects of transportation on performance and welfare should be appropriately taken into account.

Veterinary clinicians are responsible for ensuring the horses maintain a good health condition. During their training to evaluate and face performance-limiting problems, they provide helpful knowledge and expertise in preventing injuries. By the collection and examination of samples of different origins, the body condition can be estimated. The study of the hemogram provides useful information in diagnostic processes or the formulation of a prognosis. The accurate interpretation of data collected after a blood analysis in horses can reflect the possible presence of anemia or polycythemia and their causes, while the study of white blood cells adds information about immunity, allergies, etc.

For ensuring an adequate environment and appropriate nutrition, horses can be maintained outdoors or indoors. It seems very clear that horse rearing conditions can influence their health status and the risk of exposure to different pathogens. Sometimes certain gastrointestinal parasites are capable of developing inside the horses to cause infection, which is routinely detected by copromicroscopic analyses. Other possibilities consist of collecting these parasites in a *post mortem* exam, but this can be rather difficult in horses on commercial farms. The application of immunoenzymatic assays represents a significant contribution for avoiding these troubles.

Horse rearing conditions have been changing in the last years, mainly due to the economic crisis which can complicate horse feeding. In this way, horses are maintained grazing for long periods, which can also enhance their exposure to unhealthy conditions. Emerging diseases are defined by the WHO as those which appeared in a population for the first time, or that may have existed previously but are rapidly increasing in incidence or

geographic range. Certain emerging diseases affecting horses are very important because of the difficulty of detecting them and/or the absence of successful treatments.

This book offers a global approach on different items to be considered by horse owners, keepers and veterinarians for assuring correct breeding and maintaining an adequate health status. Helpful information has been added concerning care, evaluation of healthiness, risk of exposure to different pathogens, as well as an update on emerging and neglected diseases.

Chapter 1 – The goal of many breeders are to produce a horse that when provided with the opportunity will succeed within their chosen equestrian sport. Lameness and musculoskeletal injury are the primary reasons for wastage within most equestrian sport. Because of this there is considerable focus by competitors and their support team on managing the athletic horse to minimise injury and in rehabilitation of the injured equine athlete. For the breeder a compounding problem is the relatively few foals, estimated to be 20-40%, that are registered for sport. Given these production constraints it is important that the breeder provides the optimal rearing environment so that the foal can achieve its potential.

In recent years there has been an increasing body of evidence that the early rearing environment, in particular access to exercise, can have a positive effect on stimulating the musculoskeletal system and priming the tissue for the future demands it will face in the competition arena. This chapter reviews the current literature on the role early (juvenile) exercise on the equine musculoskeletal system, current management systems and the implications of these for the breeder wanting to maximise the future athletic role of their foal.

Chapter 2 – The effects of transport on individual animals are of paramount concern to those concerned with both animal welfare and performances. Horses are moved from one place to another for different reasons: competitions, breeding, pleasure activities, sales and slaughtering, and could be transported by truck, ferries or airplanes. It is estimated that, in Italy, about 3000 horses were moved daily; therefore, the total number of transported horses all over the world is significantly high. Travel includes handling, loading, transport in self, unloading and often adaptation to a new environment; each of these phases affects horse physiology and behaviour in a different way. Transportation is in fact both a physical and psychological stressor for horses but while transport associated physiological variations are well known, the emotional consequences have only recently started to be investigated. Some studies were conducted for deeply understanding effects of different kind of loading, different journey distance, different travel position and other travel variables on physiological, endocrinal and behavioural parameters in sport horses. For instance, about the distance some authors reported that short journeys are as stressful as long one, causing a higher increase in blood cortisol concentration. Available literature is rich of interesting data, reviewed and presented in this chapter. Physiological responses of transported horses could be not only a welfare concern but also a health problrm, resulting in the development of several medical conditions. During long trips and in hot climatic condition, dehydration of the animals can occur, so the use of electrolytes in the water and resting breaks throughout the journey can help to alleviate physical and emotional suffering of those animals. Another critical point, that may be related to poor air circulation in the trailer, is the development of respiratory illnesses during or after a journey. Race and jumping horses are used to travel before competitions but, after transport, some of them show lower performance than usual. Physiological explanations and proper guidelines about this issue could help horse people to better manage this kind of situation, avoiding related poor performance’s problems. Furthermore, while "Equine sport medicine" has described very accurately the effects of exercise on hematological parameters,

only few studies have been actually published on how training could affect horse behaviour. The results and implication of those studies should be more comprehensible and accessible to horse people. Training, transport and competition are the most important activities that sports horses undergo during their career, probably still the major cause of injuries and health problems and of economic loss for horse breeding and industry. For that reason, a higher competence in this field could be useful for equine technicians.

Chapter 3 – The objective of study of the hemogram is to generate information that would assist the clinician in diagnostic processes, formulation of prognosis, patient management and control. For an accurate interpretation of hematologic data in horses, some characteristics should be considered, such as age, breed, sex, venipunction method, season, reproductive status, feeding, exercise, administration of sedatives, the circadian biological rhythms, etc.). The erythrocyte report data is focused on the possible presence of anemia or polycythemia and their causes, with major relevance in sport horses. The study of white blood cells refers to immunity against infectious microorganisms, through the production of antibodies or by participating in the destruction of microorganisms or allergic responses. Indeed, the leukogram study refers to the inflammatory response in neoplasias. Major alterations that occur in the count of blood platelets are thrombocytosis or thrombocytopenia which may be due to various mechanisms in a large number of inflammatory and ischemic disorders in foals and adult animals. Due to the clinical importance of studying equine hematology, this chapter describes the common quantitative changes affecting erythrocyte, leukocyte and platelet counts, as well as plasma proteins.

Chapter 4 – Horses can be maintained outdoors or indoors. When managed outdoors, they routinely feed on domestic pastures, and are supplemented with grain and roughage when necessary. Other possibilities consist of horses grazing freely on natural pastures in woodland areas, a regime known as silvopasturing. They are never supplemented, and sufficient feeding and veterinary attention is not provided. They are used to reduce unwanted vegetation, and very few economic benefits can be achieved by meat production. In most cases, horses are focused to breeding, farming, silvopasturing or sport (aptitude). With the aim for gaining knowledge on the possible influence of horse rearing conditions on their health status and the risk of sensitization to gastrointestinal parasites also, a survey was conducted in NW Spain. Two blood samples were individually collected from each animal: samples preserved with anticoagulant were examined by means of an automated Coulter-Counter for determining the values of red (erythrocytes, haemoglobin and haematocrite) and white parameters (leukocytes, lymphocytes and granulocytes). Sera were faced to the excretory/secretory antigens of gastrointestinal parasites (cyathostomins and stomach bots). Results were analyzed according to the equine aptitude.

Significantly lower records for erythrocytes, haemoglobin and haematocrit were obtained in silvopasturing horses, while no aptitude-differences in the white blood cells were demonstrated. Horses focused to farming and/or silvopasturing reached the highest percentages of sensitization against cyathostomins, whereas the equines dedicated to sport did it against the stomach bots. The presence of antibodies against strongylids was correlated to erythropenia, anaemia and elevated levels of granulocytes and lymphocytes. Seropositivity to stomach bots was associated with low haematocrite counts and increased numbers of leukocytes. The possible influence of rearing conditions is discussed. Horses feeding on forests reached the highest percentages of strongyle-sensitization among silvopasturing equids, probably due these are generally autochthonous and indigenous breeds difficult to

immobilize for administering any parasiticide. One surprisingly result was the observation of horses dedicated to sport and/or leisure had the greatest counts of exposure to *Gasterophilus*. The possible explanation could be linked to their participation in sporting events held outdoors, where different substances in the sweat, perspiration or smell could serve as attractants for the adult flies.

Chapter 5 – Horse production is a major animal industry in Portugal with horses being bred and trained for for several purposes, including sport (jumping, dressage, horseball), leisure riding, bullfighting, working as draft animals, etc. Strongyles are a very important group of equine parasites, and there are some seventy different species of helminths in this group that are found in horses. The identification of adult strongyles to species is important because it provides information on population of these worms within a horse and suggests the potential risk of the helminths present producing diarrhoea or colic, being associated with anthelmintic resistance, and the effects of animal hunsbandry on their control. Studies comparing worms present within the intestine at *post mortem* with the adult and larval stages shed in faecal samples of horses on the same farms have shown high degrees of corrleation. Thus, for clinical and epidemiological surveys, along with the assessment of deworming efficacy programmes, a great deal can be learned without the need to recover worms from horses at necropsy.

Over several years, 23 horses were selected that had diarrhoea with the natural shedding of adult strongyles in faeces (N=3) or after deworming (N=20). A faecal sample was collected after defecation or 24-48 h after deworming. All 23 horses were found positive for adult stages of strongyles, 30,4% for *Strongylinae and* 100% for *Cyathostominae*, in a total of 2628 worms belonging to 10 genera and 24 species of strongyles (4 *Strongylinae* and 20 *Cyathostominae*). Nematodes of subfamily *Cyathostominae* contributed to 99,7% of the total number of collected strongyles, while *Strongylinae* comprised only 0,34%. An average of 8 species/host (minimum 1 and maximum 17) and 114 worms/sample/host (minimum 1 and maximum 373) were found. The 10 most prevalent species in subfamily *Cyathostominae* (*Cylicocyclus insigne*, *Cylicocyclus nassatus*, *Cyathostomum catinatum*, *Cylicostephanus longibursatus*, *Cylicocyclus asworthi*, *Cyathostomum pateratum*, *Cylicostephanus calicatus*, *Coronocyclus coronatus*, *Cylicocyclus leptostomum*, *Coronocyclus labiatus*), comprised 93,3 % of total studied strongyles, being *C. nassatus* the most abundant one, contributing to 35 % of the total worm count. There was a marked reduction in the *Strongylinae*, namely *S. vulgaris*, when compared with *Cyathostominae*, which comprised 99,7% of the total number of strongyles. This approach to the study of strongyle populations within horses produced very useful data concerning the dominance of cyathostomins when related to the entire strongyle population, provided for the identification of a large number of strongyle species (24), and represents a potentially valid alternative to parasitological studies requiring euthanasia for the purpose of necropsies for worm recovery.

Chapter 6 – In the last decades, loss of productivity has led to the closure of an elevated number of dairy bovine farms in many regions in Europe and the U.S. Accordingly, huge cultivated areas formerly employed for the nutrition of ruminants have been deserted. Cattle have been replaced by other livestock species such as sheep, horses or donkeys. Therefore it is frequent that horses feed on neglected pastures.

Horses can be kept under different management procedures, from extensive grazing on large unfenced plots to housing in box stalls. Fortunately, horses can easily adapt to the adverse atmospheric conditions in nature extensive environments. In the wild, they can spend

most of the day in the meadows and fed on grass, so their diet is rich in fiber and low in starch.One of the greatest benefits created by exercise in grazing horses is in reduction of stress level. Another advantage is the decline in feed costs, although supplementation should be provided. Although grazing ensures the feeding of animals and vegetation control, it could also represent a risk for exposition to some parasitic disorders. There is lack of knowledge regarding the possibility of sensitization against *F. hepatica* in horses grazing in areas where bovines were firstly exploited. Authors carried out a chapter to investigate the presence of antibodies against *F. hepatica* in horses.Data were analyzed regarding several intrinsic (age, gender, breed) and extrinsic factors (housing) of animals. Antibodies against the liver trematode were demonstrated in nearly half of the horses in NW Spain and Uruguay, suggesting that pastures are contaminated by metacercariae (the infective stages). The finding of Pure Breed equines (Spanish Pure Breed and Anglo-Arabs) achieved the highest seroprevalence hints that the authors' initial classification of the horses regarding their housing should be turned into only two categories: mixed and extensive. In the case of horses feeding on pastures previously grazed by ruminants, it is strongly recommended to apply some actions on the environment (grass) to avoid their infection.

Chapter 7 – African horse sickness virus (AHSV) causes a non-contagious but infectious disease of equids and is transmitted by various species of *Culicoides* midges. Horses are the most severely affected by AHSV, whereas mules are less susceptible, and African donkeys and zebra appear to have some natural resistance to the development of acute disease. Although AHSV is endemic in most areas in sub-Saharan Africa, the virus periodically makes excursions beyond its endemic areas and outbreaks have been reported in North Africa, the Middle East and in the Mediterranean Basin. Due to the devastating effect that a widespread outbreak of African horse sickness (AHS) would have on the horse industry of affected countries, great emphasis has been placed on the control of disease incidence. Since there is currently no specific therapy or drug to cure individual horses affected by AHS, vector control and vaccination remains the most practical methods of preventing the disease. This chapter outlines the history and epidemiology of AHS, including information regarding virus structure, transmission, clinical disease, and present and future approaches for controlling the disease.

In: Horses
Editors: A. Paz-Silva, M. S. Arias Vázquez et al.
ISBN: 978-1-63117-560-2

Chapter 1

EARLY EXERCISE IN THE JUVENILE HORSE TO OPTIMISE PERFORMANCE LATER IN LIFE

Chris Rogers, Erica Gee and Charlotte Bolwell
Institute of Veterinary Animal and Biomedical Sciences,
Massey University, Palmerston North, New Zealand

ABSTRACT

The goal of many breeders are to produce a horse that when provided with the opportunity will succeed within their chosen equestrian sport. Lameness and musculoskeletal injury are the primary reasons for wastage within most equestrian sport. Because of this there is considerable focus by competitors and their support team on managing the athletic horse to minimise injury and in rehabilitation of the injured equine athlete. For the breeder a compounding problem is the relatively few foals, estimated to be 20-40%, that are registered for sport. Given these production constraints it is important that the breeder provides the optimal rearing environment so that the foal can achieve its potential.

In recent years there has been an increasing body of evidence that the early rearing environment, in particular access to exercise, can have a positive effect on stimulating the musculoskeletal system and priming the tissue for the future demands it will face in the competition arena. This chapter reviews the current literature on the role early (juvenile) exercise on the equine musculoskeletal system, current management systems and the implications of these for the breeder wanting to maximise the future athletic role of their foal.

Keywords: Horse, epigenetics, early exercise neonate, behaviour

INTRODUCTION

There is an old English saying "*fools breed horses for wise men to ride"*. This, rather than denigrating horse breeders, perhaps reflects the high level of financial and emotional

investment that is required to breed horses and the long and risky period until the new born foal is old enough to first appear in sport.

Irrespective of the production system, the unfortunate reality is that few foals make it to being registered for racing or sport and even fewer are successful. It is estimated that around 30% of Thoroughbred and Standardbred horses bred for racing are never registered with a trainer (Tanner et al., 2011; Tanner et al., 2013) and only 20% of Warmblood foals are registered for sport (Dubois et al., 2008). Of those horses that are in race training between 40-50% fail to start in races (Wilsher et al., 2006; Tanner et al., 2011; Tanner et al., 2013). Within equestrian sport of those horses registered for sport 33% were not re-registered the following year (O'Brien et al., 2005). These data demonstrate that the first limitation for the breeder is successfully rearing the foal to be suitable for the initial stages of training and subsequent entry into sport.

After successfully negotiating the rearing phase and having the resultant foal enter training, after lack of talent, musculoskeletal injury or musculoskeletal reasons for retirement are the major risks to the horse having a successful racing or sport career (Wallin et al., 2000; Perkins et al., 2005a; Perkins et al., 2005b). Irrespective of the sporting use, from racing to dressage, musculoskeletal injury accounts for approximately 2/3rd of all lost training days and 1/3rd of all losses and wastage (Wallin et al., 2000; O'Brien et al., 2005; Perkins et al., 2005b; Murray et al., 2010). This large rate of loss is further compounded by the short registration life of a sporting horse, with a median career of approximately 2 – 3 years for racehorses (Wilsher et al., 2006; Tanner et al., 2011; Tanner et al., 2013) and 3 – 4 years for sport horses (Ricard and Fournethanocq, 1997; Rogers and Firth, 2005; Ducro et al., 2009; Friedrich et al., 2011). For many equestrian sports there are common tissues and predisposing sites susceptible to injury (Murray et al., 2006). This may provide the opportunity for the breeder to proactively prime the foal's musculoskeletal system for the future strains experienced as a mature horse in competition to minimise the risk of injury. Therefore, the challenge to the breeder is to provide a rearing environment that minimises the risk of injury and loss during growth, while balancing the requirement for optimal stimulation of the musculoskeletal system of the future equine athlete.

Nutritional Programming of the Equine Athlete in Utero Participants

Classical genetic theory dictated that once the breeder had selected the sire and dam and the embryo was conceived, the genetic blueprint was hardwired and set for that foal. In recent years we have become increasing aware of the plasticity in which the genotype can be expressed and even the opportunity for the alterations in genotype activation to be transmitted across generations (Jirtle and Skinner, 2007). This recognition of the role of the early intrauterine environment on the genotype of the foal provides the breeder with the opportunity to maximise the phenotype of the foal at two stages of development – gestation and the normal juvenile period. The developmental time periods and the relative windows of opportunity for plasticity and ability to modify the phenotype are schematically presented in Figure 1.

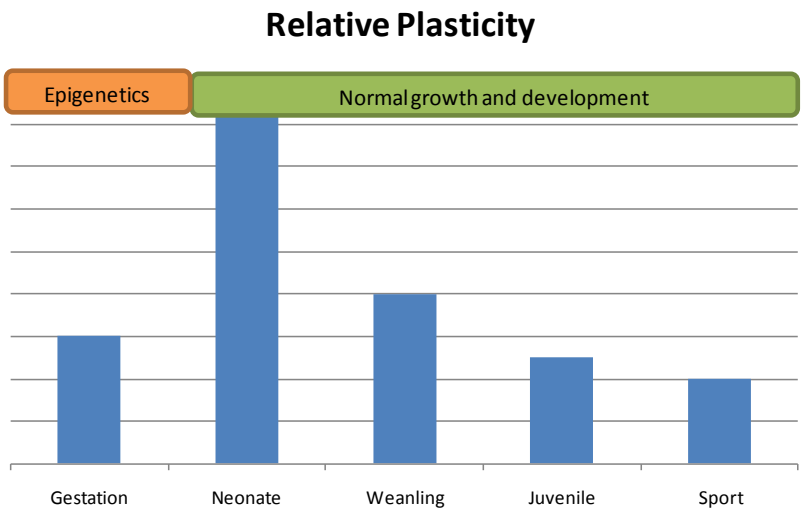


Figure 1.

Nutrition of the dam during pregnancy, and efficiency of placental function are vitally important for normal growth and development of the fetus in utero. The availability of nutrients in utero can be an important determinant of the adult phenotype that develops in the offspring, with poor nutrition in utero being associated with dysfunction of the cardiovascular, metabolic and endocrine systems of the offspring in adult life in several species, including humans (Armitage et al., 2004; Fowden et al., 2013). Fetal tissues can be programmed in-utero by alternations in nutrient supply at specific developmental periods (Ousey et al., 2008). However, in contrast to other species there are relatively few equine data reporting associations between altered maternal nutrition during pregnancy and dysfunction of the offspring (Rossdale and Ousey, 2002; Fowden et al., 2013).

Wilsher and Allen (2006) set out to compare placental and fetal parameters in maiden Thoroughbred fillies fed high or moderate energy diets in early and mid gestation, but the study was confounded by an outbreak of Strangles (*streptococcus equi equi*) between 100 and 140 days gestation, effectively resulting in an acute nutritional insult and resulting in varying degrees of maternal weight loss (approximately 8-11% body weight). In a subset of infected mares, maternal insulin and glucose concentrations declined significantly during, and in the one month following the infection. Male foals from affected mares fed moderate energy diets showed enhanced β cell sensitivity to glucose, suggesting that nutritional programming of these cells had occurred in response to the period of nutritional insult superimposed on a moderate level of nutrition. Feeding high energy diets to pregnant mares resulting in high body condition scores could potentially mitigate the effects of acute nutritional deprivation on the developing fetus (Ousey et al., 2008)

Recent data suggest that relative maternal over-nutrition and specifically the dietary source of energy during pregnancy may also influence intrauterine programming in horses. George et al. (2009) compared the glucose and insulin dynamics in foals from mares fed 2/3rd of their predicted dietary energy requirements as low starch or high diets from around 28 weeks gestation. In foals from mares fed high starch diets glucose concentrations were higher than those of foals from dams fed low starch diets, but all were within normal ranges. The authors also reported a trend for lower insulin sensitivity in foals at 160 days of age from

mares fed high starch diets and concluded the metabolic programming could occur in foals as a result of high starch maternal diets during gestation.

Other research to support the concept of relative over-nutrition and intrauterine nutritional programming in horses includes the study of Allen et al. (2002), where pony embryos were transferred into horse mares, and horse embryos were transferred into pony mares, resulting in differing uterine environments and placental size, therefore influencing fetal nutrition without influencing maternal nutrition. In the resulting large newborn pony foals from Thoroughbred recipient mares higher basal insulin concentrations and increases in β cell sensitivity to glucose were detected compared to other foals (Forhead et al., 2004). Giussani et al. (2003)reported cardiovascular abnormalities in both pony foals from Thoroughbred mares, and Thoroughbred foals from pony mares, indicating that reduced or enhanced fetal nutrition alter post-natal cardiovascular function. Although these studies indicate maternal nutrition can influence the phenotype of the offspring in early life, the longer term implications for the offspring as an athletic are unknown.

Maternal Nutrition and Developmental Orthopaedic Disease in the Equine Athlete

Developmental orthopaedic disease (DOD) is a term to describe a number of conditions affecting articular and metaphyseal cartilage of young foals, including osteochondrosis, which can potentially limit the athletic career of horses. These conditions are likely to have a complex multifactorial aetiopathogenesis with a strong genetic component, and may also be influenced by rapid growth, dietary imbalances, biomechanical stress or trauma and hormonal factors (McIlwraith, 2001). George et al. (2009) suggested metabolic programming could occur in foals as a result of high starch maternal diets during gestation, however, the evidence linking high starch maternal diets and radiographic evidence of osteochondrosis is very weak (Van der Heyden et al., 2013) and requires more rigorous investigation.

Under New Zealand conditions maternal copper supplementation during gestation appears to be protective against some cartilage lesions in foals. Pearce et al. (1998) demonstrated that supplementation of dams with oral copper sulphate in the last 13 to 25 weeks of gestation was associated with improved radiographic distal radial physis scores and reduced frequency of articular lesions in foals at 150 days of age compared to foals from unsupplemented dams. The mechanism by which maternal copper supplementation is protective against DOD in the foal has not been elucidated; Van Weeren et al. (2003) suggested that copper may have a role in repair and resolution of some lesions, thus high liver copper stores at birth may be important for future athletes.

Normal Growth and Development

The horse evolved as a cursorial prey animal and is obligated to be able to walk and even gallop within a relatively short period of time after birth. Even though the mare generally foals at night, possibly as a mechanism to minimise the risk of predation, the foal will be capable of locomotion within a relatively short time post-partum. In the domestic environment a foal will usually be able to stand within 1 hour, suckle within 2 hours and pass

meconium and gallop within 3 hours post-partum (Rossdale, 1989; Dicken et al., 2012). This early locomotor requirement stimulates a unique combination of developmental processes that ensure that very soon after birth the foal's weight relative to height, musculoskeletal and behavioural / neural capabilities are sufficiently developed. As a precocious species the horse has rapid prenatal Central Nervous System development. The neuromuscular capability is relatively advanced and when allometric scaling (relative changes in limb length in relation to growth) are accounted for the locomotor pattern, or kinematic fingerprint, appears consistent and resistant to training (Back et al., 1993; Back et al., 1999).

Normal Growth Weight and Height

The pattern of growth (weight gain and increases in wither height) for the horse is remarkably consistent across breeds. The birth weight of the foal is to a large extent constrained by the size of the dam (Allen et al., 2004). The typical Shetland pony foal weighs ~23 kg at birth, the Thoroughbred foal weighs between 50-60 kg at birth and Warmblood foals are reported to be in the range of 51-76 kg, yet all display similar patterns of weight and height gain (Martin-Rosset, 2004; Hendriks et al., 2009). Weight gain in most domestic livestock species is dependent on birth weight and growth is factorial. Irrespective of the country of origin, and therefore the subtle differences in management system, the rate and pattern of growth is described best with either a sigmoid curve, Gompertz or Richard equation (Morel et al., 2007; Kocher and Staniar, 2013).

In its most simplistic form growth can be described as biphasic with a period of rapid growth pre-weaning and then a period of growth at approximately 2/3rds that of the pre-weaning period (Figure 2) (Rogers et al., 2004; Brown-Douglas et al., 2005). Growth in the pre-weaning period is dependent on age and birth weight, whereas growth in the post weaning period is more susceptible to environmental cues such as season (Morel et al., 2007; Kocher and Staniar, 2013). Across a number of studies seasonal decreases in average daily gain (ADG) have been identified for pasture kept horses during winter, with greater growth rates observed in the summer (Pagan et al., 1996; Brown-Douglas et al., 2005; Kocher and Staniar, 2013). These subtle seasonal variations in growth rate mean that ADG appropriately corrected for season is a more sensitive measure of deviation from desired growth than total body weight at a given age.

In contrast to the sensitivity of bodyweight to seasonal and nutritional challenges, increases in wither height seem relatively resistant to challenge. The relatively high heritability reported for this trait in the literature (~40-50%) clearly demonstrates the limited effect environmental stimuli has on the final wither height of the foal (Hintz et al., 1978). In post weaning New Forest pony foals with a heavily restricted energy intake there was consistent growth in wither height, but presentation of other signs of delayed maturation and delayed closure of the long bone physis permitting some additional compensatory growth once digestible energy restrictions were lifted (Ellis and Lawrence, 1978)

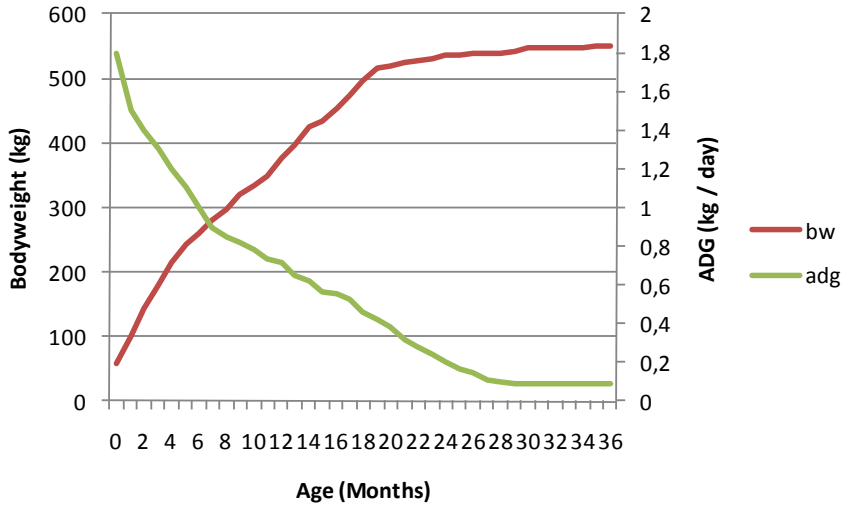


Figure 2. Typical bodyweight and adg for nz tb - gexa data.

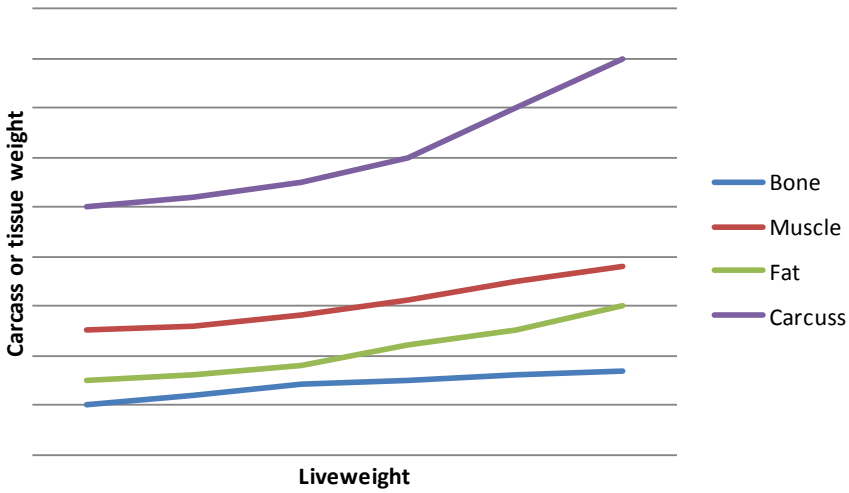


Figure 3. Carcus growth.

Skeletal Muscle

In contrast to other livestock species there is relatively limited literature on the relative composition and rate of growth of the different tissues of the developing foal. As an animal grows the muscle to bone ratio increases, as does the carcass weight as a proportion of live weight. Fat is deposited rapidly and the rate of deposition increases with maturity (Figure 3). Possibly reflecting the rapid growth in the foal, and its requirement for early athletic maturation, the chemical composition of 160 day old Thoroughbred foals was found to be similar to that reported for mature ponies (Gee et al., 2003). In mature horses there appears to be some breed (athletic vs. other breeds) differences in muscle mass and the greater relative growth in some muscles (*femoral* and *longissisimus*) in Thoroughbreds than other breeds

(Gunn, 1979; Gunn, 1987). While there are breed, essentially athletic use, differences in horses there does not appear to be an ability of early exercise early in life to dramatically alter the pre-programmed developmental capability of equine skeletal muscle, but rather what is essentially an exercise only response (Dingboom et al., 1999; Suwannachot et al., 1999). The lack of response observed may be due to what were moderate exercise regimes and may be compounded by the rigorous selection for athletic ability within the Warmblood.

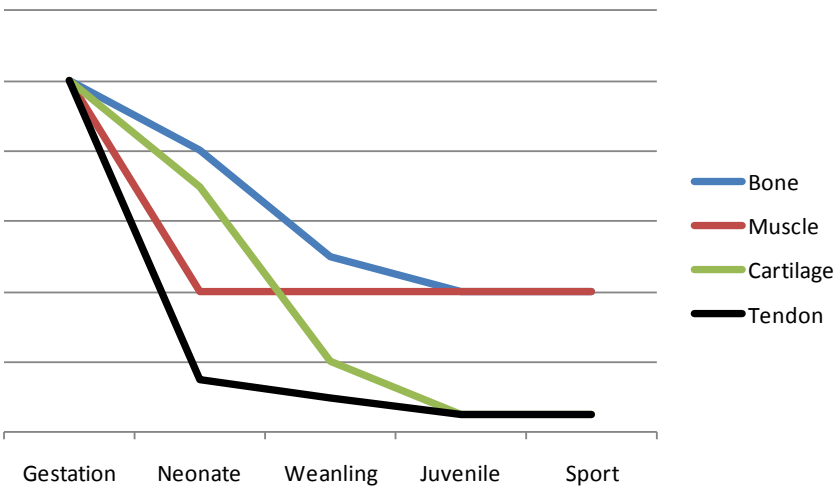


Figure 4.

This pattern of growth of body weight and height reflects the sensitivity and capability of the differing organ systems to respond to environmental stimuli over and above the predetermined rate set by the genotype and the intra uterine environment (Figure 4).

Bone

As previously described the horse is a precocious cursorial animal and to facilitate this the equine skeleton, and limb (long) bones in particular, are capable of load very soon after birth. The third metacarpal (cannon) bone during late gestation is primed for weight bearing and loading with changes in shaft shape from circular to oval. In association with this, within the distal limb maturation or closure of the physis (growth plate) occurs at a relatively young age, providing relative tolerance to loading of the distal limb early in life. This adaptive mechanism is observed as the relatively limited longitudinal growth of the distal limb. The majority of long bone growth in the distal limb is completed by 140 to 210 days old (Fretz et al., 1984; Thompson, 1995), and contributes less than 10% of the increases observed in wither height. While there is some subtle variation between breeds most horses will reach 90% of mature height by 18 months of age.

The limited capability for longitudinal growth in the distal limb bones provides in turn some limitations in the mechanisms available for the bone to respond to the challenges of growth and exercise. In the epiphysis (at the joint) increases in size may be limited and so response is generally an increase in density, which is a consistent pattern across the bones of

the distal limb. In the mid shaft (diaphysis) of the bone there is an opportunity for increases in cross-sectional area and in density, and the nature and rapidity of these changes differ with the bones of the distal limb in relation to the load (primarily longitudinal bending strain) (Firth et al., 2011).

These data imply that bone growth in the horse is pre-programmed and relatively resistant to the influence of environmental stimuli. However, experimental data has demonstrated that given severe nutritional restriction there may be a delay in the closure of the growth plates permitting compensatory growth (Ellis and Lawrence, 1978). In relation to exercise the diaphyseal response is related to the peak bending strains experienced, with relatively few load cycles required to elicit a response. In five month old Dutch Warmblood foals, free exercise at pasture provoked a similar response (increased cross-sectional area of third metacarpus) to the brief sprint exercise superimposed on stall housing / confinement (Cornelissen et al., 1999). Imposition of a 30% increase in workload index for pasture kept horses was associated with greater increase in size and strength in the proximal phalanx and two sites of the third metacarpal bone but not with an increase in density (Firth et al., 2011). In the distal third metacarpal epiphysis (at the site of the fetlock joint) exercised foals had a greater epiphysis diameter, which may provide greater tolerance to the high strains imposed on the joint during training and competition later in life.

These data imply that while bone growth is rapid during the first few months of a foals life there is still some capability to modify the environment to provide positive stimuli to prime the tissue for the strains it will experience in later competitive life.

Cartilage

In many mammalian species, including the horse, the neonate is born with a “blank joint”. This refers to the limited cellular and molecular variation present in the cartilage, with no differentiation in composition obvious between sites of high or low strain. With the imposition of load and strain, due to weight bearing and activity, the cartilage responds rapidly and by five months of age appears to have reached close to maturation. Sites under intermittent high strain have elevated levels of type II collagen and sites under constant low strain are characterised by high quantities of glycosaminoglycan and proteoglycan (Brama et al., 1999; Brama et al., 2000).

Cartilage metabolism is greatest in the neonate and exponentially decreases with age. It is predicted that in mature cartilage collagen has a turnover life of 120 years in dog and 350 years in man (van Weeren et al., 2008). Thus, once maturation has been achieved there is limited opportunity to stimulate the tissue. In Warmblood foals confinement with stall management inhibited the development of biochemical and hence biomechanical variation, which reduced the tolerance for load within the fetlock joint (proximal first phalanx). Rearing foals at pasture during the first five months of life provided the greatest variation and hence capability to tolerate load in cartilage. However, access to pasture after stall confinement for the first five month of life was not sufficient to redress the detrimental effect of stall confinement (Brama et al., 1999). This lack of response may be due to the apparent limited window for differentiation that occurs prior to five months of age.

Additional exercise at pasture from 14 days old in Thoroughbred foals was associated with increased tolerance to high strain loads by stimulating an increased level of post

translational modifications of collagen, and reduced levels of glycosaminoglycans and collagen (van Weeren et al., 2008). This exercise regimen was also associated with stimulation of the chondrocytes, with greater numbers of viable chondrocytes, and hence greater cellular activity, observed in the conditioned foals (Dykgraaf et al., 2008).

These data demonstrate that cartilage in the neonate can respond to environmental stimuli but the window when the tissue is receptive is relatively limited. Heterogeneity of biochemical composition is optimal but the exact loading required to achieve this is still unknown with too much exercise resulting in detrimental effects.

Tendon

In contrast to bone and cartilage, which appear to have some window of sensitivity to stimuli during early maturation and growth, it would appear tendon is resistant to stimuli. In the sport horse the superficial digital flexor tendon (SDFT) is the primary tendon injured during athletic use.

Data from long bone growth in Thoroughbred foals would indicate that longitudinal growth in the SDFT ceases in foals when approximately 10 weeks old. Total longitudinal bone growth in the metacarpus/tarsus is estimated to be 18.5 and 23 mm and ceases abruptly at 10 weeks of age (Fretz et al., 1984). Cross-sectional area (CSA) of the SDFT, which is often used as a crude proxy for strength, appears to increase with age up to 8 months of age and then rapidly plateaus off with limitted increase in CSA. At 12 months of age the CSA of the SDFT is almost the equivalent to the CSA found in mature horses (Perkins et al., 2004; Moffat et al., 2008). It may be that CSA is a better indication of growth and development rather than strength, as there appears to be limited data to support that changes in the SDFT CSA directly translate to greater tensile strength.

At the compositional level most tendon development and maturation occurs when the horse is approximately one year old. Measures of tendon tensile strength include the collagen fibril index (CFI), which measures the percentage area occupied by fibrils, and the mass average diameter (MAD), which measures the relative relationship of fibril diameter with the area of the tendon cross-section containing fibrils. In a cross-sectional study, by 1 year old MAD had peaked at 170 nm and CFI at 70%, close to those observed in mature horses (Patterson-Kane et al., 1997). Exercise may alter the distribution of tendon fibrils, with more small diameter (good) fibrils observed in pasture kept foals than stall raised and control exercise foals. The lowest number of small diameter fibrils were observed in foals just confined to stalls (Cherdchutham et al., 2001). However, access to pasture when the foals were between 6 to 11 months did result in some small fibril development in the foals previously confined to stalls, albeit at lower levels than that seen in the foals that had been pasture kept since birth. In contrast, treadmill exercise, provided in addition to 4 hours of pasture access / free exercise, from birth until 15 months of age did not provide significant changes in SDFT composition compared to a control group (Kasashima et al., 2008). In a different study, additional exercise superimposed over free pasture exercise in foals up to 18 months old also failed to demonstrate a change in either SDFT CSA or in the proportion of active *type 2* tenocytes (Moffat et al., 2008; Stanley et al., 2008). These data imply that in contrast to the receptiveness of other tissues the tendon (SDFT in particular) appear resistant to stimulation from early exercise.

Summary of Tissue Section

Data on tissue maturation and development in the foal indicates that there may be a number of discrete developmental windows of opportunity. These differ with the tissue of interest and are confounded by the base level of exercise. This complicates the provision of a discrete recipe to provide a programme for optimising musculoskeletal health. Within the sporting literature it is widely recognised that 10,000 repetitions are required to establish a skill as an expert (Ericsson, 2013). However, some tissue such as bone may require as few as 36 cycles at the appropriate strain rate to elicit the desired response (Rubin and Lanyon, 1984). These differing thresholds provide a conflict in the exercise required to achieve the optimal musculoskeletal and neurological development.

Are Feral Horses a Suitable Model – or Just a Guide?

The dramatic changes in the genotype and the phenotype of the domestic horse have resulted in an animal that is physically quite distinct from that of the populations of feral horses, or indeed the last remaining wild horse the Przewalski horse. This, therefore, raises the caveat of whether the feral horse provides a gold standard, or merely a guide, for what typical exercise / daily workload could be.

Data from the tracking of feral horses in Australia indicates that there is significant variation in daily distances covered by horses, which were highly dependent on the quantity and location of water and grazing resources. Across two differing geographical regions feral horses were observed to cover similar mean daily distances 15.9 ± 1.9 km/day (8.1–28.3 km) but the variation in distance was much greater for the central Queensland horses (15 – 55km), that had days of long migrations and then short migrations from the water source (Hampson et al., 2010a). These distances were recorded with feral adult horses, but these distances are typical of the activity seen in family bands of mares and foals.

In a domestic setting paddock size rather than configuration had the greatest influence on daily distance covered. Horses in a 6 m x 6 m yard covered as little as 1.4 km/day. The relationship between paddock size and daily distance was logarithmically associated with daily exercise, with daily exercise starting to plateau out at 4 ha (7.2 (6.7 – 7.6) km/day) (Hampson et al., 2010b). When provided with a 4 ha paddock, foal age did not appear to alter the mean daily distance covered; foals between 4 days old and 3- 5 weeks old travelled 7.3 km/day (range 4.5–9.5 km/day), approximately half that observed for feral horses.

These data provide daily distance travelled but are not able to describe, or quantify, the brief bouts of high speed exercise and play that are so important for priming the musculoskeletal system. Observational data indicates that there may be decreasing bouts of high speed play with increasing age during the first few months of a foal's life. In domestic populations the cumulative workload (a combination of the distance covered and the speed) was approximately twice that in the first month of life than in the fourth month (Kurvers et al., 2006). A similar trend has also been observed in feral Camargue and Przewalski foals (Boy and Duncan, 1979; Boyd, 1988). This indicates that not only is total distance covered of importance but also the dose and the timing (in context of the foals' maturation) of the application of high speed exercise. There also appears to be an interaction of the application of high speed exercise and the base exercise level. One of the interesting findings of the large

exercise trial conducted by the Dutch in the late 1990's was that the imposition of high speed exercise on horses managed conventionally, with partial confinement in loose box stalls, had a deleterious effect on the cartilage quality (Barneveld and van Weeren, 1999; Brama et al., 1999). In contrast, imposition of a 30% increase in workload for pasture kept Thoroughbred foals was not associated with any negative findings and appeared to have a stimulatory effect on a numbers of tissues (Rogers et al., 2008a; Rogers et al., 2008b). These data identify the multidimensional considerations required when quantifying exercise and its impact on the juvenile equine musculoskeletal system.

Current Management of the Juvenile Equine Athlete

Since the mid-20th century there has been a dramatic transition in the role and use of the horses in the western world. This transition from work horse to sport horse has been associated with an increase in the marginal utility and individual economic value of the horse. These changes in role and value (economic and emotional investment) of the horse have been generally associated with a reduction in the per horse workload and more intensive management. In some situations the changes in management reflect what owners consider optimal, but many are not optimal in respect to the health or welfare of the horse (Sillence et al., 2006).

Increasing marginal utility and economic value of the horse are the primary drivers of changes in management. Many horses are kept singularly in loose boxes and it is estimated that between 80-90% of horses in Europe are kept in individual housing systems (Rose-Meierhofer et al., 2010). A primary reason for individual housing, rather than group housing, is believed to be owners' concern of injuries (Mejdell et al., 2010). A retrospective study of injuries of horses admitted to the University of Zurich equine clinic identified that the most common injury was a kick from another horse and that 71% of these injuries occurred while horses were at pasture (Derungs et al., 2004). However, when kept in stable social groups there appears to be few serious injuries and quantification of the magnitude and location of injures indicated that almost 80% of injuries observed were grade 1 (lesion involving hair loss only –alopecia- e.g. superficial bite) and found on the main body area (Mejdell et al., 2010). The authors proposed that these low grade injuries were the result of play and may indicate a positive mental state in the horses.

Pre Weaning

Within Australasia on commercial breeding farms it is typical for the mare to foal outside in a foaling paddock and the mare and foal unit will be kept separate for the first day or two post-partum before joining a larger group of mare and foals (~14 mare and foal pairs) kept within 2.4 ha pasture (Rogers et al., 2007; Dicken et al., 2012). This management of the mare and foal at pasture is possible because of the temperate climate. In the Northern Hemisphere adverse weather early in the breeding season generally restricts foaling within foaling boxes and there is a need to keep the mare and foal unit indoors for the first few weeks of life (Cohen, 1994; Anonymous, 2006). French data indicates that post-partum most mares and foals in Normandy are turned out at pasture after one week but before three weeks of age. The mares and foals are typically managed in large areas of pasture which provides daily free exercise (Lepeule *et al.*, 2009). Published data from other regions of Europe appear limited to

the management of older horses, which because of competition use appear commonly managed in singular loose boxes with limited access to pasture or turn-out (Stock and Distl, 2005).

Weaning

Weaning of foals typically takes place when approximately five months old. Irrespective of the methods of weaning used there is maternal deprivation stress of the foal (Heleski et al., 2002; Rogers et al., 2004). On commercial farms weaning is often associated with a short period of education with foals being individually (or in pairs) housed in loose boxes. This can compound the stress experienced, and confinement during weaning has been reported to alter time budgets. Foals weaned within a paddock / pasture environment while experiencing a maternal stress related weaning check , such as a short term reduction in average daily weight gain, still exhibited time budgets (activity) similar to that reported for feral populations, whereas boxed weanlings had significantly reduced activity (Heleski et al., 2002). Another potential negative for stall confinement of weanlings is the greater risk of developing unwanted stereotypical behaviours. It is reported that stall weaning increases the risk of developing stereotypical behaviours (Relative Risk (RR) 2.19, $P<0.05$) and housing in barns rather than at pasture post weaning was associated with a further increase in risk (RR 2.54, $P<0.01$) (Waters et al., 2002). Stereotypical behaviours while not directly influencing athletic performance can provide a negative perception of the horse and a reduce opportunity for sale.

Post Weaning

Post weaning many foals are kept as groups of same sex weanlings at pasture. On commercial Thoroughbred farms in Australasia foals remain in these cohorts until they are yearlings, with subsequent separation of the sexes and then preparation for yearling sales in the late summer (Rogers et al., 2007; Stowers et al., 2009; Bolwell et al., 2010). In some European countries (e.g. Sweden, Denmark, Switzerland), in recognition of the social needs of the horse, there is legislation in place that requires young horses to be kept in groups. Some larger breeding operations within the Netherlands actively attempt to prolong the period young stock are at pasture. However, adverse weather conditions in the Northern Hemisphere often require housing of the young horses during the winter. Across a number of European countries it has been reported that this can result in a significant reduction in the access to free exercise and social interaction (Lonnell, 2012; Werhahn et al., 2012).

Group housing in open barns can attenuate the restriction in free exercise. Individually housed horses are reported to cover only 0.17km / day whereas within an open barn system horses will cover 1.2 Km / day. Modification of the feeding frequency and provision of different functional areas can increase this to 4.8 Km / day (Frentzen, 1994). However, the primary drivers for activity, either at pasture or within an open barn or activity barns setting, appear to be heavily moderated by the drive for social interaction. Horses do not appear to self-select forced exercise on a treadmill but will select access to pasture if this provides interaction with conspecifics (Lee et al., 2011). Density of horses can also restrict exercise and increase the risk of injury. On North American and New Zealand stud farms mares and foals, and weanlings appear to be kept at a relatively low stocking density of 2 horses / hectare, which is greater than the stocking density of 1 horse / hectare proposed to be optimal to promote pasture activity (Duruttya, 2003).

Foal activity not only provides a mechanism to prime the musculoskeletal tissue but also can modify the expression of a number of diseases. Activity has been implicated in the prevalence of osteochondrosis and developmental orthopaedic disease in a number of studies. Within Belgium a retrospective epidemiological survey indicated that foals kept at pasture up to one year of age were significantly less affected with osteochondrosis than foals boxed or had alternating box and pasture (Van der Heyden et al., 2013). The nature of the pasture and exercise may be a moderating factor on the protective effect observed by Vander Heyden et al (2013). Data from a French prospective study across a number of light horse breeds implicated irregular exercise in large paddocks (Lepeule et al., 2009), which reinforces the negative findings of sprint exercise imposed over stall confinement (Barneveld and van Weeren, 1999).

Foal activity, and play activity in particular, within the first year of life is strongly associated with maternal investment (dams loss in body condition score) in feral horses (Cameron et al., 2008). Further support for a positive association between feeding level (in this case post weaning) and activity / locomotor responses has been observed in Shetland ponies with differing planes of nutrition (Back et al., 2002). These data imply that restrictions in nutrition may not only have a direct effect on decreasing the rate of growth, but may have an interactive effect by inhibiting the level of tissue stimulation provided through bouts of play. This interaction between planes of nutrition and play activity reinforces the difficulty in identifying the single solution or variable that differentiates the injury prone and the injury resistant equine athlete.

Inclusion of a Developmental Plan

Given the volume of data from a number of studies it is apparent that early exercise is essential for the optimal development of the equine athlete. For most tissue the sensitive period for stimuli appears to exist prior to weaning and it is here that the breeder may have the greatest opportunity to modify management practice to stimulate regular exercise. It would appear that access to pasture in groups may provide the simplest and most effective mechanism to stimulate musculoskeletal development, and also the mental health of the future equine athletes. Current industry stocking densities of 2 horse / ha may be within an acceptable range to stimulate free exercise at pasture. However, precise data on the effect of variation of these densities on free exercise is currently lacking and is required before hard recommendations could be made.

The relative size of the pasture may be a significant constraint in some areas and countries. However, it would appear the paddock size and shape may not be the primary drivers or limitations for free exercise. The horse as a social animal appears to seek socialisation over and above free exercise. This drive for socialisation could be managed to ensure the foal is at pasture with other mares and foals. Provided this is a stable cohort, and stocking densities are not too great, there should be relatively limited risk of injury and an opportunity to exploit the foals natural play behaviour to stimulate the musculoskeletal system. Data from foal activity studies indicate that the behaviour patterns of the foal for bouts of high speed play are aligned with what we believe are the most sensitive period for tissue response, with decreases in frequency and volume with increasing age. This overlay of high speed play on a pattern of longer duration low impact exercise, which occurs when at

pasture, appears optimal. Without more precise data on the best mix of the longer duration low impact and high speed exercise it is difficult to predict or recommend how this could be implemented within a management system where pasture turnout / free exercise is not possible. However, common sense would indicate that any changes in management of the foal that permit exercise in a manner that reflects what occurs when at pasture should provide greater optimisation of development than confinement and irregular exercise.

What About Early Introduction to Sport?

If the breeder rears the horse through until it is ready for introduction to sport there may still be opportunities to optimise the duration and success of the athletic career. Epidemiological data from a number of production systems and countries has identified that an early introduction to sport, or even training for sport, is associated with longer and more successful careers.

Due to the robust nature of the recording system within the racing industries the greatest volume of data on benefits of early training relates to racehorses. In both harness racing and flat racing early introduction to training (as a two year old) was associated with a longer and more successful career (Tanner et al., 2011; Tanner et al., 2013). Because of the early focus on sporting career this age of initiating training (generally ~18- 22months) (Bolwell et al., 2012) is earlier than that commonly practised with sport horses. However, even at the relatively later and more mature age of initiating training in sport horses there is an obvious association of early start in sport with longer careers. Data from European Warmblood populations indicates longer career for horses that start in official competitions at a younger age (Ricard and Fournethanocq, 1997; Braam et al., 2011; Ricard and Blouin, 2011).

CONCLUSION

In order to maximise athletic career it appears optimal to provide an early environment that reflects the evolutionary origins of the horse as a cursorial prey species. Free exercise at pasture in groups, which encourages play activity, may provide an environment to stimulate musculoskeletal development. This window for opportunity appears maximal in the pre-weaning period. However, data from epidemiological studies indicate that the opportunity may still exist to prime the horse for its future athletic career with an early introduction to training and / or sport.

REFERENCES

Allen, W. R., Wilsher, S., Tiplady, C., Butterfield, R. M., (2004). The influence of maternal size on pre- and postnatal growth in the horse: III postnatal growth. *Reprod.* 127, 67-77.

Allen, W. R., Wilsher, S., Turnbull, C., Stewart, F., Ousey, J., Rossdale, P. D., Fowden, A. L., (2002). Influence of maternal size on placental, fetal and postnatal growth in the horse. I. Development in utero. *Reprod.* 123, 445-453.

Anonymous, (2006). *Part 1: Baseline Reference of 1998 Equine Health and Management.* Fort Collins, Co, United States Department of Agriculture.pp139.

Armitage, J. A., Khan, I. Y., Taylor, P. D., Nathanielsz, P. W.,Poston, L., (2004). Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? *J. Physiol. (Lond).* 561, 355-377.

Back, W., Schamhardt, H. C., Barneveld, A.,Weeren, P. R. V., (2002). Longitudinal Development of Kinematics in Shetland Ponies and the Influence of Feeding and Training Regimes. *Equine Vet. J.* 34.

Back, W., Smit, L. D., Schamhardt, H. C.,Barneveld, A., (1999). The Influence of Different Exercise Regimens on the Development of Locomotion in the Foal. *Equine Vet. J.*

Back, W., Vandenbogert, A. J., Vanweeren, P. R., Bruin, G.,Barneveld, A., (1993). Quantification of the Locomotion of Dutch Warmblood Foals. *Acta Anat.* 146, 141-147.

Barneveld, A., van Weeren, P. R., (1999). Conclusions regarding the influence of exercise on the development of the equine musculoskeletal system with special reference to osteochondrosis. *Equine Vet. Suppl.* 31, 112-119.

Bolwell, C. F., Rogers, C. W., Firth, E. C.,French, N., (2010). Management and exercise of Thoroughbred yearlings during preparation for yearling sales: a cross-sectional survey. *Proc. N. Z. Soc. Anim. Prod.* 70, 157-161.

Bolwell, C. F., Rogers, C. W., French, N. P., Firth, E. C., (2012). Risk factors for interruptions occurring before the first trial start of 2-year-old Thoroughbred racehorses in training. *N. Z. Vet. J.* 60, 241-246.

Boy, V., Duncan, P., (1979). Time-budgets of Carmargue horses. 1. Developmental changes in the time budgets of foals. *Behav.* 71, 187-202.

Boyd, L. E., (1988). Ontogeny of behavior in Przewalski horses. *Appl. Anim. Behav. Sci.* 21, 41-69.

Braam, A., Nasholm, A., Roepstorff, L., Philipsson, J., (2011). Genetic variation in durability of Swedish Warmblood horses using competition results. *Livest. Sci.* 142, 181-187.

Brama, P. A., Tekoppele, J. M., Bank, R. A., van Weeren, P. R.,Barneveld, A., (1999). Influence of different exercise levels and age on the biochemical characteristics of immature equine articular cartilage. *Equine Vet. J. Supp.* 31, 55-61.

Brama, P. A. J., Tekoppele, J. M., Bank, R. A., Barneveld, A., van Weeren, P.R., (2000). Functional adaptation of equine articular cartilage: the formation of regional biochemical characteristics up to age one year. *Equine Vet. J.* 32, 217-221.

Brown-Douglas, C. G., Parkinson, T. J., Firth, E. C.,Fennessy, P. F., (2005). Bodyweights and growth rates of spring- and autumn-born thoroughbred horses raised on pasture. *N. Z. Vet. J.* 53, 326-331.

Cameron, E. Z., Linklater, W. L., Stafford, K. J., Minot, E. O., (2008). Maternal investment results in better foal condition through increased play behaviour in horses. *Anim. Behav.* 76, 1511-1518.

Cherdchutham, W., Becker, C. K., Spek, E. R., Voorhout, W. F.,van Weeren, P. R., (2001). Effects of exercise on the diameter of collagen fibrils in the central core and periphery of the superficial digital flexor tendon in foals. *Am. J. Vet. Res.* 62, 1563-1570.

Cohen, N. D., (1994). Causes of and Farm-Management Factors Associated with Disease and Death in Foals. *J. Am. Vet. Med. Assoc.* 204, 1644-1651.

Cornelissen, B. P., van Weeren, P. R., Ederveen, A. G., Barneveld, A., (1999). Influence of exercise on bone mineral density of immature cortical and trabecular bone of the equine metacarpus and proximal sesamoid bone. *Equine Vet. J. Suppl.*, 79-85.

Derungs, S. B., Furst, A. E., Hassig, M., Auer, J. A., (2004). Frequency, consequences and clinical outcome of kick injuries in horses: 256 cases (1992-2000). *Wien. Tierarztl. Monatsschr.* 91, 114-119.

Dicken, M., Gee, E. K., Rogers, C. W., Mayhew, I. G., (2012). Gestation length and occurrence of daytime foaling of Standardbred mares on two stud farms in New Zealand *N. Z. Vet. J.* 60, 42-46.

Dingboom, E. G., Dijkstra, G., Enzerink, E., van Oudheusden, H. C.,Weijs, W. A., (1999). Postnatal muscle fibre composition of the gluteus medius muscle of Dutch Warmblood foals; maturation and the influence of exercise. *Equine Vet. J. Suppl.*, 95-100.

Dubois, C., Manfredi, E., Ricard, A., (2008). Optimization of breeding schemes for sport horses. *Livest. Sci.* 118, 99-112.

Ducro, B. J., Gorissen, B., van Eldik, P., Back, W., (2009). Influence of foot conformation on duration of competitive life in a Dutch Warmblood horse population. *Equine Vet. J.* 41, 144-148.

Duruttya, M., (2003). Use of an ethological test to calculate a distance covered by selected categories of Thoroughbred horses. *Czech. J. Anim. Sci.* 48, 46-50.

Dykgraaf, S., Firth, E. C., Rogers, C. W.,Kawcak, C. E., (2008). Effects of exercise on chondrocyte viability and subchondral bone sclerosis in the distal third metacarpal and metatarsal bones of young horses. *Vet. J.* 178, 53-61.

Ellis, R. N. W.,Lawrence, T. L. J., (1978). Energy under-nutrition in weanling filly foal. 2. Effects on body conformation and epiphyseal plate closure in fore-limb. *Br. Vet. J.* 134, 322-332.

Ericsson, K. A., (2013). Training history, deliberate practice and elite sports performance: an analysis in response to Tucker and Collins review-what makes champions? *Br. J. Sports Med.* 47, 533-535.

Firth, E. C., Rogers, C. W., van Weeren, P. R., Barneveld, A., McIlwraith, C. W., Kawcak, C. E., Goodship, A. E., Smith, R. K. W., (2011). Mild exercise early in life produces changes in bone size and strength but not density in proximal phalangeal, third metacarpal and third carpal bones of foals. . *Vet J.* 190, 383-389.

Forhead, A. J., Ousey, J. C., Allen, W. R., Fowden, A. L., (2004). Postnatal insulin secretion and sensitivity after manipulation of fetal growth by embryo transfer in the horse. *J. Endocrinol.* 181, 459-467.

Fowden, A. L., Jellyman, J. K., Valenzuela, O. A., Forhead, A. J., (2013). Nutritional Programming of Intrauterine Development: A Concept Applicable to the Horse? *J. Equine Vet. Sci.* 33, 295-304.

Frentzen, F. (1994). The locomotion activity and behaviour of horses depending on the system of stabling and feeding rhythm, and taking various of loose runs into particular consideration. PhD University of Hannover.

Fretz, P. B., Cymbaluk, N. F., Pharr, J. W., (1984). Quantitative-analysis of long-bone growth in the horse. *Am. J. Vet. Res.* 45, 1602-1609.

Friedrich, C., Konig, S., Rogers, C. W., Borstel, U. K. V., (2011). Examination of Longevity in Dressage Horses - A Comparison between Sport Horses in New Zealand and Hanoverians in Germany. *Zuchtungs.* 83, 68-77.

Gee, E. K., Fennessy, P. F., Morel, P. C. H., Grace, N. D., Firth, E. C., Mogg, T. D., (2003). Chemical body composition of 20 thoroughbred foals at 160 days of age, and preliminary investigation of techniques used to predict body fatness. *N. Z. Vet. J.* 51, 125-131.

George, L. A., Staniar, W. B., Treiber, K. H., Harris, P. A., Geor, R. J., (2009). Insulin sensitivity and glucose dynamics during pre-weaning foal development and in response to maternal diet composition. *Domest. Animal. Endocrinol.* 37, 23-29.

Giussani, D. A., Forhead, A. J., Gardner, D. S., Fletcher, A. J. W., Allen, W. R., Fowden, A. L., (2003). Postnatal cardiovascular function after manipulation of fetal growth by embryo transfer in the horse *J. Physiol. (Lond.).* 547, 67-76.

Gunn, H. M., (1979). The growth of the transverse sectional area of the semitendinosus muscle in the dog and the horse and its relation to the athletic ability in the 2 species. *Anat. Histol. Embryol.* 8, 365-368.

Gunn, H. M., (1987). Muscle, bone and fat proportions and muscle distribution of Thoroughbreds and other horses. *Equine Ex. Phys.* 2, 253-264.

Hampson, B. A., de Laat, M. A., Mills, P. C., Pollitt, C. C., (2010a). Distances travelled by feral horses in 'outback' Australia. *Equine Vet. J.* 42, 582-586.

Hampson, B. A., Morton, J. M., Mills, P. C., Trotter, M. G., Lamb, D. W., Pollitt, C. C., (2010b). Monitoring distances travelled by horses using GPS tracking collars. *Aust. Vet. J.* 88, 176-181.

Heleski, C. R., Shelle, A. C., Nielsen, B. D., Zanella, A. J., (2002). Influence of housing on weanling horse behavior and subsequent welfare. *Appl. Anim. Behav. Sci.* 78, 291-302.

Hendriks, W. K., Colenbrander, B., van der Weijden, G. C., Stout, T. A. E., (2009). Maternal age and parity influence ultrasonographic measurements of fetal growth in Dutch Warmblood mares. *Anim. Reprod. Sci.* 115, 110-123.

Hintz, R. L., Hintz, H. F.,Vanvleck, L. D., (1978). Estimation of heritabilities for weight, height and front cannon bone circumference of Thoroughbreds. *J. Anim. Sci.* 47, 1243-1245.

Jirtle, R. L.,Skinner, M. K., (2007). Environmental epigenomics and disease susceptibility. *Nat. Rev. Genet.* 8, 253-262.

Kasashima, Y., Takahashi, T., Birch, H. L., Smith, R. K. W., Goodship, A. E., (2008). Can exercise modulate the maturation of functionally different immature tendons in the horse? *J. Appl. Physiol.* 104, 416-422.

Kocher, A., Staniar, W. B., (2013). The pattern of thoroughbred growth is affected by a foals birthdate. *Livest. Sci.* in press.

Kurvers, C. M. H. C., van Weeren, P. R., Rogers, C. W., van Dierendonck, M. C., (2006). Quantification of spontaneous locomotion activity in foals kept in pastures under various management conditions. *Am. J. Vet. Res.* 67, 1212-1217.

Lee, J., Floyd, T., Erb, H., Houpt, K., (2011). Preference and demand for exercise in stabled horses. *Appl. Anim. Behav. Sci.* 130, 91-100.

Lepeule, J., Seegers, H., Rondeau, V., Robert, C., Denoix, J. M., Bareille, N., (2009). Risk factors for the presence and extent of Developmental Orthopaedic Disease in the limbs of young horses: Insights from a count model. *Prev. Vet. Med.* 101, 96-106.

Lonnell, C. (2012). *Yard Differences in Training, Management and Orthopedic Injury in Showjumping, Riding School, and Thoroughbred Race Horses.* PhD, Swedish University of Agricultural Sciences.

Martin-Rosset, W. (2004). Growth and development in the equine. *In:* Juliand, V. & Martin-Rosset, W. (eds.) *THe growing horse: nutrition and prevention of growth disorders.* Wageningen, the Netherlands: Wageningen Academic Publishers.

McIlwraith, C. W., (2001). Developmental orthopedic disease (DOD) in horses-a multifactorial process. *Eq. Nutr. Phys. Symp.* 2-23.

Mejdell, C. M., Jorgensen, G. H. M., Rehn, T., Fremstad, K., Keeling, L., Boe, K. E., (2010). Reliability of an injury scoring system for horses. *Acta Vet. Scand.* 52, 1-6.

Moffat, P. A., Firth, E. C., Rogers, C. W., Smith, R. K. W., Barneveld, A., Goodship, A. E., Kawcak, C. E., McIlwraith, C. W.,van Weeren, P. R., (2008). The influence of exercise during growth on ultrasonographic parameters of the superficial digital flexor tendon of young Thoroughbred horses. *Equine Vet. J.* 40, 136-140.

Morel, P. C. H., Bokor, A., Rogers, C. W., Firth, E. C., (2007). Growth curves from birth to weaning for Thoroughbred foals raised on pasture. *N. Z. Vet. J.* 55, 319-325.

Murray, R. C., Dyson, S. J., Tramquille, C., Adams, V., (2006). Association of type of sport and performance level with anatomical site of orthopaedic injury diagnosis. *Equine Vet. J Supp.* 36, 411-416.

Murray, R. C., Walters, J. M., Snart, H., Dyson, S. J., Parkin, T. D. H., (2010). Identification of risk factors for lameness in dressage horses. *Vet. J.* 184, 27-36.

O'Brien, E., Stevens, K. B., Pfeiffer, D. U., Hall, J., Marr, C. M., (2005). Factors associated with the wastage and achievements in competition of event horses registered in the United Kingdom. *Vet. Rec.* 157, 9-13.

Ousey, J. C., Fowden, A. L., Wilsher, S., Allen, W. R., (2008). The effects of maternal health and body condition on the endocrine responses of neonatal foals. *Equine Vet. J.* 40, 673-679.

Pagan, J. D., Jackson, S. G.,Caddel, S., (1996). A summary of growth rates of thoroughbreds in Kentucky. *Pferdeheilkunde.* 12, 285-289.

Patterson-Kane, J. C., Parry, D. A. D., Birch, H. L., Goodship, A. E., Firth, E. C., (1997). An age-related study of morphology and cross-link composition of collagen fibrils in the digital flexor tendons of young thoroughbred horses. *Connect. Tissue Res.* 36, 253-260.

Pearce, S. G., Firth, E. C., Grace, N. D., Fennessy, P. F., (1998). Effect of copper supplementation on the evidence of developmental orthopaedic disease in pasture-fed New Zealand Thoroughbreds. *Equine Vet. J.* 30, 211-218.

Perkins, N. R., Reid, S. W. J., Morris, R. S., (2005a). Profiling the New Zealand Thoroughbred racing industry. 1. Training, racing and general health patterns. *N. Z. Vet. J.* 53, 59-68.

Perkins, N. R., Reid, S. W. J., Morris, R. S., (2005b). Profiling the New Zealand Thoroughbred racing industry. 2. Conditions interfering with training and racing. *N. Z. Vet. J.* 53, 69-76.

Perkins, N. R., Rogers, C. W., Firth, E. C., Anderson, B. H., (2004). Musculoskeletal responses of 2-year-old Thoroughbred training. 3. In vivo ultrasonographic assessment of the 280 horses to early cross-sectional area and echogenicity of the superficial digital flexor tendon. *N. Z. Vet. J.* 52, 280-284.

Ricard, A., Blouin, C., (2011). Genetic analysis of the longevity of French sport horses in jumping competition. *J. Anim. Sci.* 89, 2988-2994.

Ricard, A., Fournethanocq, F., (1997). Analysis of factors affecting length of competitive life of jumping horses. *Genet. Sel. Evol.* 29, 251-267.

Rogers, C. W., Firth, E. C., (2005). Preliminary examination of the New Zealand event horse production system. *Proc. N. Z. Soc. Anim. Sci.* 65, 372-377.

Rogers, C. W., Firth, E. C., McIlwraith, C. W., Barneveld, A., Goodship, A. E., Kawcak, C. E., Smith, R. K. W., van Weeren, P. R., (2008a). Evaluation of a new strategy to modulate skeletal development in racehorses by imposing track-based exercise during growth: The effects on 2-and 3-year-old racing careers. *Equine Vet. J.* 40, 119-127.

Rogers, C. W., Firth, E. C., McIlwraitht, C. W., Barneveld, A., Goodship, A. E., Kawcak, C. E., Smith, R. K. W., van Weerew, P. R., (2008b). Evaluation of a new strategy to modulate skeletal development in Thoroughbred performance horses by imposing track-based exercise during growth. *Equine Vet. J.* 40, 111-118.

Rogers, C. W., Gee, E. K., Faram, T. L., (2004). The effect of two different weaning procedures on the growth of pasture-reared thoroughbred foals in New Zealand. *N. Z. Vet. J.* 52, 401-403.

Rogers, C. W., Gee, E. K., Firth, E. C., (2007). A cross-sectional survey of Thoroughbred stud farm management in the North Island of New Zealand. *N. Z. Vet. J.* 55, 302-307.

Rose-Meierhofer, S., Klaer, S., Ammon, C., Brunsch, R., Hoffmann, G., (2010). Activity Behavior of Horses Housed in Different Open Barn Systems. *J. Equine Vet. Sci.* 30, 624-634.

Rossdale, P. D., (1989). *The horse from conception to maturity.* London, J. A. Allan.pp174.

Rossdale, P. D.,Ousey, J. C., (2002). Fetal programming for athletic performance in the horse: potential effects of IUGR. *Equine Vet. Edu.* 14, 98-111.

Rubin, C. T.,Lanyon, L. E., (1984). Regulation of bone-formation by applied dynamic loads. *J. Bone Jt. Surg. (Am.).* 66A, 397-402.

Sillence, M., Noble, G.,McGowan, C., (2006). Fast food and fat fillies: The ills of western civilisation. *Vet. J.* 172, 396-397.

Stanley, R. L., Edwards, L. J., Goodship, A. E., Firth, E. C., Patterson-Kane, J. C., (2008). Effects of exercise on tenocyte cellularity and tenocyte nuclear morphology in immature and mature equine digital tendons. *Equine Vet. J.* 40, 141-146.

Stock, K. F., Distl, O., (2005). Survey on the development of Hanoverian Warmblood horses selected for sale at auction in 1991 to 1998. *J. Equine Vet. Sci.* 25, 210-223.

Stowers, N. L., Rogers, C. W.,Hoskin, S. O., (2009). Management of weanlings on commercial Thoroughbred studs farms in the North Island of New Zealand. *Proc. N. Z. Soc. Anim. Prod. 69*, 4-9.

Suwannachot, P., Verkleij, C. B., Weijs, W. A., van Weeren, P. R., Everts, M. E., (1999). Effects of training on the concentration of Na+, K+-ATPase in foal muscle. *Equine Vet. J. Suppl.*, 101-5.

Tanner, J. C., Rogers, C. W., Firth, E. C., (2011). The relationship of training milestones with racing success in a population of Standardbred horses in New Zealand. . *N. Z. Vet. J.* 59, 323-327.

Tanner, J. C., Rogers, C. W., Firth, E. C., (2013). The association of 2-year-old training milestones with career length and racing success in a sample of Thoroughbred horses in New Zealand. *Equine Vet. J.* 45, 20-24.

Thompson, K. N., (1995). Skeletal growth rates of weanling and yearling thoroughbred horses. *J. Anim. Sci.* 73, 2513-2517.

Van der Heyden, L., Lejeune, J. P., Caudron, I., Detilleux, J., Sandersen, C., Chavatte, P., Paris, J., Deliege, B., Serteyn, D., (2013). Association of breeding conditions with prevalence of osteochondrosis in foals. *Vet. Rec.* 172, 68-71.

van Weeren, P. R., Firth, E. C., Brommer, H., Hyttinen, M. M., Helminen, H. J., Rogers, C. W., DeGroot, J., Brama, P. A. J., (2008). Early exercise advances the maturation of glycosaminoglycans and collagen in the extracellular matrix of articular cartilage in the horse *Equine Vet. J.* 40, 128-135.

Van Weeren, P. R., Knaap, J., Firth, E. C., (2003). Influence of liver copper status of mare and newborn foal on the development of osteochondrotic lesions. *Equine Vet. J.* 35, 67-71.

Wallin, L., Strandberg, E., Philipsson, J., Dalin, G., (2000). Estimates of Longevity and Causes of Culling and Death in Swedish Warmblood and Coldblood Horses. *Livest. Sci.* 63, 275-289.

Waters, A. J., Nicol, C. J., French, N. P., (2002). Factors influencing the development of stereotypic and redirected behaviours in young horses: findings of a four year prospective epidemiological study. *Equine Vet. J.* 34, 572-579.

Werhahn, H., Hessel, E. F., Van den Weghe, H. F. A., (2012). Competition Horses Housed in Single Stalls (II): Effects of Free Exercise on the Behavior in the Stable, the Behavior during Training, and the Degree of Stress. *J. Equine Vet. Sci.* 32, 22-31.

Wilsher, S., Allen, W. R., (2006). Effects of a Streptococcus equi infection-mediated nutritional insult during mid-gestation in primiparous Thoroughbred fillies. Part 1: Placental and fetal development. *Equine Vet. J.* 38, 549-557.

Wilsher, S., Allen, W. R.,Wood, J. L. N., (2006). Factors associated with failure of Thoroughbred horses to train and race. *Equine Vet. J.* 38, 113-118.

In: Horses
Editors: A. Paz-Silva, M. S. Arias Vázquez et al.
ISBN: 978-1-63117-560-2

Chapter 2

EFFECT OF TRANSPORT AND EXERCISE ON BEHAVIOUR OF SPORT HORSES

Barbara Padalino*
Department of Veterinary Medicine, University "Aldo Moro" of Bari, Italy, Valenzano (Bari) - Italy

ABSTRACT

The effects of transport on individual animals are of paramount concern to those concerned with both animal welfare and performances. Horses are moved from one place to another for different reasons: competitions, breeding, pleasure activities, sales and slaughtering, and could be transported by truck, ferries or airplanes. It is estimated that, in Italy, about 3000 horses were moved daily; therefore, the total number of transported horses all over the world is significantly high. Travel includes handling, loading, transport in self, unloading and often adaptation to a new environment; each of these phases affects horse physiology and behaviour in a different way. Transportation is in fact both a physical and psychological stressor for horses but while transport associated physiological variations are well known, the emotional consequences have only recently started to be investigated. Some studies were conducted for deeply understanding effects of different kind of loading, different journey distance, different travel position and other travel variables on physiological, endocrinal and behavioural parameters in sport horses. For instance, about the distance some authors reported that short journeys are as stressful as long one, causing a higher increase in blood cortisol concentration. Available literature is rich of interesting data, reviewed and presented in this chapter. Physiological responses of transported horses could be not only a welfare concern but also a health problrm, resulting in the development of several medical conditions. During long trips and in hot climatic condition, dehydration of the animals can occur, so the use of electrolytes in the water and resting breaks throughout the journey can help to alleviate physical and emotional suffering of those animals. Another critical point, that may be related to poor air circulation in the trailer, is the development of respiratory illnesses during or after a journey. Race and jumping horses are used to travel before competitions but, after

* Department of Veterinary Medicine, University "Aldo Moro" of Bari, Italy, Str. Pro. Per Casamassima Km 3, 70010 Valenzano (Bari) - Italy, Email: barbara.padalino@uniba.it.

transport, some of them show lower performance than usual. Physiological explanations and proper guidelines about this issue could help horse people to better manage this kind of situation, avoiding related poor performance's problems. Furthermore, while "Equine sport medicine" has described very accurately the effects of exercise on hematological parameters, only few studies have been actually published on how training could affect horse behaviour. The results and implication of those studies should be more comprehensible and accessible to horse people. Training, transport and competition are the most important activities that sports horses undergo during their career, probably still the major cause of injuries and health problems and of economic loss for horse breeding and industry. For that reason, a higher competence in this field could be useful for equine technicians.

Keywords: Transport, exercise, behaviour, performance, horses

INTRODUCTION

Horses are transported more frequently than any other type of livestock (Friend, 2001); they have been moved for many different reasons: in the past they travelled almost for war, while, nowadays, horse owners transport them for competitions, breeding, pleasure activities, sale, or slaughter (Fazio et al., 2008a). In Italy about 3000 horses were moved daily in 2004 (Giovagnoli, 2010); so the total number of transports for horses is very high all over the world.

In the Unites States, there are an estimated 6.9 million horses and their owners spend an estimated $2.17 billion per year transporting those horses (American Horse Council, 1996). Even though transport is expensive and considered stressful and dangerous for the horses, the owners prefer transport their horses from one country to another instead to buy a new one, because it is not easy to find a new horse with the same temperament and performance quality of the one's own. Gibbs et al. (1997, 1998) reported that the typical Texas horse owner transports 2.5 horses on 24 trips per year, averaging 380 km per trip.

Horse transport has had a long history, that started many years before Christ and will continue until the horse will be considered essential for humans.

Figure 1. First method to horse transport.

In the past, one of the most famous method of transportation was horse-drawn wagons (Figure 1) and there are many later accounts of them in many parts of the world; in this case the better horses were transported by the worse ones. Later, horses started to be transported by ship and this method has been quite popular since the last 3500 years (Cregier, 1982). It is claimed that as early as 480 B.C. Xerxes moved his entire cavalry by ship across the Hellpont against the Acient Greeks and Hannibal is recorded as having used rafts to get his horses across the Rhone in 218 B.C. Sea transport was the only means of transport for horses until late Nineteenth Century; nowadays, it is still in use because is the cheapest; but the disadvantages of sea travel are principally of time and risk of injury (Judge, 1969). Horses carried by modern cargo ships travel in boxes from 4.5 x 4.5 metres to 6.0 x 4.5 metres either as deck cargo o between decks and have access to a sand yard for exercise (Waran et al., 2007). Transport by sea has been very popular to transport horses from South America to Europe almost for slaughtering, but also for training and pleasure. Cavallone et al. (2002) conducted a trial to study the variations of cortisol on horses imported in Italy from Argentina and concluded that the adaptation to the journey was very good; whereas, stress conditions appeared only in the first period after unloading, mainly concerned to the change of management, some hierarchical conflicts and variation of circadian circle.

Transport by rail was very common during the middle to the late 1800s, horses have been carried in cattle wagon, where more than one animal occupied a wagon and there was some risks due to treads and kicks from fellow travelers. Two types of wagon were used, the single four-wheel type carries eight horses, and the bogie carries 16-20 horses depending upon their size. Valuable or race horses were transported in special "race horse vans" which were added to passenger trains and where horses were kept isolated from the others. The disadvantages of this mode of transport lie mainly in rough uncomfortable journey and often prolonged waiting at rail-heads for collection or delivery (Judge, 1969). Nowadays, transport by rail is still in use, because it is reasonably cheap, in particular in developing countries.

Transport by rail gave way to transport by trucks after World War II following the building of the interstate highways system and the development of more reliable trucks and trailers (Friend, 2001). The 1960s and 1970s became known as the "Trailer age" for horse transport (Cregier, 1982) because many different kinds of trailers and trucks were designed and produced. Consequently, nowadays, there are many different types of road vehicles used to transport horses; the major differences is between lorry and trailer. The "motorized horse box" or "horse lorry van" varies in capacity from 1 to 10, 12 or even 16 or more individually or grouped horses. The trailer has a rear and sometime a side ramp, although in some countries (e.g. the US), there is no ramp and the horse are trained to "step up". The container is attached to the towing vehicle at one point via a tow hitch, which makes them particularly susceptible to the vectoral changes acting across the hitch when on minor roads with many bends/corners. For this reason, some owner prefer another type of trailers, that are designed where the trailer sits over the rear axle of the towing vehicle and are called "goose-neck" or "tractor-type" trailers. However, trailers are less stable than lorries or trucks.

Horses were first air-freighted in late 1946 (Judge, 1969) and air transport is actually became very common for breeding (today stallions from the world's leading studs may be mated with mares in both the Northern and Southern Hemispheres in the same year) and competition reasons, even though flying is the most specialized and expensive of all means of transporting horses. Quarantine regulations are applied to air travel, to prevent the spread of disease, even though Ohmura et al. (2012) demonstrated that the staying in the quarantine

boxes caused an increase in the heart rate correlated to new environmental stress. On arrival to air transport, horses are transferred to air stables or jet stalls, which have been designed to accommodate a maximum of three horses, side by side, separated by partitions (Waran et al., 2007).

Stewart et al. (2003) conducted a study about the effect of air transport on heart rate and behaviour of 16 horses; they reported that during short haul flights (3–4 h) heart rates were significantly higher during transitional events (i.e. while loading and unloading the truck and aircraft, and during ascent and descent) than when horses were resting or when the aircraft was in flight. In level flight, horses' heart rates were close to resting levels and they would regularly doze and rest. In comparison, during ascent and descent, social behaviour, including aggression and submission, increased, and horses were seen to regularly change body postures to maintain balance. The authors tested that there was no difference between short and long haul flights (10–15 h) and concluded that, although some sharp increases in heart rate and activities suggested agitation during transitional stages of air transport, these events did not appear to be frequent or long enough to be a significant welfare concern and consequently that horses appear to adapt well to air travel. A recent study (Munster et al., 2013) confirmed those data, and concluded that the loading into a jet stall, causing the highest increase in Heart Rate (HR), was considered the most stressful event for horses during air transport; but HRs were still less increased than HRs during loading for road transport. During transit, HR, HR Variability and Behavioural Score were comparable with resting levels, and were lower than in horses undergoing road transport. On the basis of these studies, it could be supposed that air transport, causing a less increase in HR in horses, may be less stressful than road transport.

Since the major part of the studies concerning the effects of transport on the stress of horses has been focused on road transport and this is the most popular and common way of travelling, the rest of the dissertation will be concerned to it.

DESTINATION OF TRANSPORT: TOWARD A SLAUGHTERHOUSE, A NEW STALL OR A HORSE TRACK?

Horses travel for different reasons, but the primary difference, that can influence the kind of travel is the destination: a slaughterhouse or a different stall.

Equine Slaughter Transport

Horsemeat consumption became popular after World War II with lower-income people in Europe, where beef was scarce, and old or lame draft horses were processed for affordable meat. Horsemeat have been sought for its high iron content and leanness (Stull et al., 2001) and in Europe is recently considered a delicacy and associated with costly prices. Canada reported 64,519 horses slaughtered in 1997, while Europe processed in 1998 approximately 140,000 horses for human consumption (USDA, 1999). However, countries such as the United Kingdom and Canada never accepted horsemeat in their diets (Reece et al., 2000).

In South America, Argentina is the largest equine meat exporter and in Chile nearly 50 thousand equines are slaughtered yearly (Werner and Gallo, 2008). The number of horses in the United

States that are slaughtered for human consumption at USDA-inspected facilities has gradually declined over the last decade: in 1992, approximately 243,000 horses were processed, whereas only 62,813 horses in 1999 (USDA, 1999). In Northern America there are few equine slaughterhouses and so animals must travel for long time, horses are slaughtered and exported for human consumption, primarily to Europe. 25.000 tons of horsemeat were produced in 2009. Actually most Americans oppose the slaughtering of horses for meat consumption and in 2013, the Obama administration promoted an initiatove to abolish funding for US Department of Agriculture inspections of horse slaughter plans in the 2014 financial year.

The effects of the travel on the meat quality are well studied in cattle and pigs (Ritter, 2008), but also for horses the stress correlated with transport, lairage and stunning should be minimal to avoid poor meat quality.

Werner and Gallo (2008) reported that transport induced an increase in blood lactate, cortisol and glucose concentration and hematocrit values, the latter related to the catecholamine release and not due to dehydration. The rise in blood lactate and glucose could affect the meat quality, so a correct lairage period is recommended.

Generally, meat horses travel loose in the truck toward the slaughterhouse and the density of transport is still on discussion, because in high-density compartment, severe injuries from kicking may be avoided, because a direct, aggressive kick is not always possible (Collins, 2000); in contrast in a lower density horses can escape biting situation and the possibility to escape aggression is a less stressful mental situation for horses. Whiting (1999) found that most of 296 loads of loose transported horses were at or below the maximum Canadian recommended density of 400 Kg/m^2. Iacono et al. (2007a) concluded that density and water access did not substantially affect aggression, dehydration, physiological indicators of stress, or weight loss in slaughter horses, but in high density condition horses could fall during shipments, which could result in injury or death. Aggressive behaviour during transport seems not to be correlated more to individual horses (dominance) than density or other travelling conditions. Further studies are needed in this field.

Transport of Companion Horses

The number of horses is approximately 58 million all over the world (FAOSTAT, 2013[1]). They are still reared for working in developing countries (Pritchard et al, 2009), but in industrialized countries horses are mostly reared for pleasure or racing. Most horse owners or trainer transporting horses for pleasure or recreation travel their animals in single stalls within the vehicle, whereas the horses transported in group tend to be those unhandled. Confinement is usually achieved by means of partitions made of wood or metal, attached to the sides of the vehicle so that they can be moved or opened to enable the animal to be loaded and then shut in on both sides. Depending on the vehicle (trailer or lorry) and the internal layout, there may also be straps, bars or gates that enclose the horse's front and rear. The design of these internal fittings is crucial for safe and comfortable carriage of the horse (Weeks et al., 2012). Many horses travel for breeding reason and in that case particularly attention is given to foals that

must travel with the mare and at least a week after foaling. Another reason of horse transport is sale, in that case in addition to the transport stress, there is a new stall adaptation period. The horse must adapt not only to the new owner, but also to cope any change in management and training; in this case the diet change and sometimes any contact with new bugs are of particular importance in relation to the health status.

LEGISLATION OF ANIMAL TRANSPORT

There are many commercial carriers within each country but since they are mainly unregulated, it is impossible to determine the size of the industry (Waran et al., 2007).

Horse civil registry is not the same all over the world and recording the total number of trips and movements of horses within and across countries is complicated. In Europe, for instance, horse exports and importss have not been recorded until recently, and it has been done to and from countries outside the European Union (EU) only. Therefore it is impossible to quantify the number of transported horses and the number of times each horse was carried from one place to another. However, horse movements are now recorded trough the new EU Trade Control and Expert System (Traces system) (Herholz et al., 2008). TRACES is an electronic system which replaced the animal movement (ANIMO) control system in 2004. It is used to exchange information on intra-Community trade, import or transit of live animals, semen or embryos and some animals product. These data have became a very valuable information resource (Leadon et al., 2008).

Anyway different legislations are updated and amended constantly at least in developed countries during the last decades, but since The World Organization for Animal Health (OIE) formulated and approved the guidelines on animal welfare in relation to transport during the 73rd OIE General Session in May 2005, all countries have developed rules according with them.

Countries within the European Union are expected to enforce national regulation based on the appropriate European Directive as the minimum standard and currently the EC Regulation 1/2005 is applied to the transportation of all domestic animals including solipeds; recommendation N°R(87)17 sets forth standards on the transport of horses. For example, within the European Union (EC1/2005) unhandled equines must be transported in groups of no more than 4 animals per section, no equines may be carried in the upper decks of multi-tier vehicles and the height above the withers of the tallest animal must be at least 75 cm. A particular welfare license is, actually, needed by the drivers, but not if the transported animals have no commercial status, or if the journey is shorter than 65 km. A journey log is needed when the journey is longer than 8 hours or a border is crossed. Records of the compulsory onboard navigation system are used to check journey limits and rest times. Furthermore, an assessment of the condition of the animals at the end of the journey is required (Weeks et al., 2012).

The Australian Animal Welfare Standard and Guidelines for livestock transport (AWS[2]) have been endorsed by the Primary Industries Ministerial Committee for legislation in 2012, but the part concerning horse transport is still in an early stage.

Official guidelines for horse air-transport are issued by the International Air Transport Association (IATA[3]). Requirements for jet stalls and the loading and unloading of horses are

prescribed in the Live Animal Regulations (LAR[4]). Flying grooms must be highly experienced professionals, who in addition to their horse management skills should be certified on air safety matters.

All movements of horses carry some risk of disease transmission; safeguarding the horse industry against the dangers of disease spread is one of the major responsibility of the equine clinician. The occurrence in August 2002 in Sydney of West Nile fever in a stallion in post arrival quarantine or the catastrophic outbreak of equine influenza in August 2007 in Australia are some examples of the above menzioned risk. Clinicians also need to be aware of the problems relating to horse transport and the way in which these problems arise (Herzolz et al., 2008).

PHASES OF TRANSPORT AND THEIR EFFECTS ON HORSE BEHAVIOUR AND HEALTH STATUS

The transport of animals is a complex procedure involving several potential stressors, including handling, loading, unloading, separation from familiar physical and social environments, confinement, vibration, changes in temperature and humidity, inadequate ventilation, and often deprivation of food and water (Waran, 1993). Factors that induce stress during transport are mostly psychological (White et al., 1991), but physical factors also contribute, as the motion of the trailer, noise, driver's ability and road conditions (Jones, 2003). Confinement is stressful for horses (Mal et al., 1991), but a stationary vehicle than a moving one is generally considered to be less stressful for farm animals (Tarrant, 1990). Indeed, during transport, horses are subjected to changing forces due to, for example, acceleration, deceleration, and turning movements of the vehicle (Waran and Cuddeford, 1995).

Lee et al. (2001) conducted a survey to determine what types of problems with trailering the horses exhibited, as well as the techniques the owners had used to mitigate the problem. Horses had problems with loading (53.4%) and traveling (51.5%). Of the horses who exhibited problems during travel, most had problems when the vehicle first began to move (53%) or when it went around a curve (47%). Less than half the horses (28.2%) had been cured by the methods the owners used. They concluded that there is no breed differences in type of problems, in incidence of multiple problems, and in improvement were compared, whereas orientation in the trailer and association of the trailer with aversive experiences may be important components of the etiology of trailering problems.

It should be underlined that horses used for sporting and recreational purposes, for which a number of relatively positive experience of loading and transport is reported, are adversely affected by the bare transport in minor way than horses with no experience and horses living through previous negative experiences, such as fall, over-crowding etc (Leadon et al., 2008).

Handling

The handling methods can have a great effect on animal welfare, in particular for slaughter horse handling animals without the use of sticks or electric goads results in better

welfare and lower risk of poor meat quality (Broom, 2005). During the handling, isolation is another important factor of stress, because during restraint for routine husbandry procedures, animals are often separated from their co-specifics. In addition, both handling and transport involve the interaction of animals with humans and it is important to know how animals react to human behaviour in order to effectively move and restrain them (Fazio and Ferlazzo, 2003); in that case also the taming method (ethological or traditional), influencing the horse-man relationship, could affect the horse behaviour during handling procedure (Casamassima et al., 2008).

Age, sex and physiological conditions also affect the behaviour of horses during handling and transport; indeed handling young animals, such as foals and yearlings, which are usually not tamed yet, can be more difficult and although it is generally assumed that intact males are more difficult to be handled than castrates, this difference may be age dependent. Rearing environment and previous experience can also be expected to be of considerable importance. Animals respond to challenges in their immediate environment through several interacting mechanisms including behavioural, hemato-chemical, physiological and neuro-hormonal patterns. Identifying of stressful situations would promote greater well-being, health and reproductive efficiency of domestic animals as well as protection of their performance and economic potential. Behavioural indicators of discomfort are vocalization, attempts to escape, kicking, or struggling. Finally, the response of animals to handling and transport depends on their sensory capabilities and the visual field and flight zone of animals are important behavioural concepts.

Loading (Injuries and Fear)

Loading is considered to be one of the most stressful components of transport (Waran, 1993); fear of entering into a closed space as well as the height of the step up onto the ramp and the instability and incline of the ramp, all combine to a fear of loading (Houpt and Leib, 1993). During loading inexperienced horses exhibit considerable evasive behaviour and are reluctant to step up onto the rump. Houpt (1982) suggested that accustoming the foal to loading as foal prevents behavioural problems associated with loading and transport later in life. Anyway, heart rate during loading is usually higher than average heart rate during transport recorded in experienced and inexperienced horse. In fact, climbing a ramp is probably a frightening experience for a naive horse, and although horses may become accustomed to the situation, they are still stimulated in some way. This elevation in heart rate could be ascribed partly to the energy expended in climbing the ramp and partly to the emotional fear (Waran, 1993). It seems that evasive behaviour during loading was typical of very young horses, and that the time of loading is influenced by the age. In fact, Waran and Cuddeford (1995) reported that the yearlings took more time to load (368 s) than that of 2-year-olds (29.5 s), 3-year-olds (21.5 s) and those over 3 years old (5 s).

Due to the fear loading the horse onto a trailer or horsebox can be a source of stress and injury for both the horse and handler, because many horses fight during loading. They exhibit behaviours such as rearing, pulling back, head tossing, pawing, and turning sideways. These behaviours are likely to be negatively reinforced when the owner fails to load the horse (Baron, 1991). The combination of a horse that fights loading and an owner who uses physical force can produce a very dangerous situation. Injuries to the trainer can include rope

burns, lost fingers, broken bones, or bruises and bleeding. Injuries to the animal can include lacerations to the head from banging into the trailer, scrapes and cuts on the legs, broken legs from falling, or even a broken back if the animal falls backwards while rearing (Ferguson and Rosales Ruiz, 2001).

Consequently, some studies have been conducted to understand in which way this fear could be decreased to avoid these accidents.

To reduce the likelihood of injury, horses that have difficulty loading may be trained to load more willingly. According to Scoggins (1996), successful training involves increasing the horse's confidence by breaking loading into simpler, separate tasks that can be accomplished in a relaxed mental and physical state. These tasks include moving forward on command, stepping onto—and backing off—an unstable floor, and moving into a confined space.

The Tellington-Touch Equine Awareness Method (TTEAM), developed by Linda Tellington-Jones and described by Curcio-Wolfe (1996), takes this concept of relaxed, progressive training one step further. This method uses nonaversive touch and commands in novel situations as a means for inducing behavioural changes in horses. Horses are generally neophobic (Houpt, 1982), and TTEAM is specifically designed to teach horses to relax and function in the presence of novel and potentially frightening stimuli. Shanahan in 2003 conducted a study and concluded that non-aversive retraining method (based on TTEAM) was effective in reducing loading time for horses with a history of reluctance to load onto a trailer. There also was a significant decrease in heart rate and saliva cortisol during the loading assessment after training. As indicated by a decrease in heart rate and saliva cortisol, this suggests that the training improves willingness to load and also may decrease stress at loading.

Cross et al. (2008) investigated whether lighting conditions either inside or outside the trailer influence the welfare of horses during loading into an enclosed trailer either from a dark or lit arena and into a dark or lit trailer. They recorded that heart rate increased from the start to the end of each test in all treatments, suggesting that the horses experienced some fear of the loading process. However, there was no effect of lighting treatment on the increase in heart rate or the maximum or mean heart rates. Neither was there any effect of treatment on the speed of loading or number of refusals, which when examined with the heart rate data, suggests that there was no effect of lighting treatment on the horses' fear of loading. However, horses loading from a lit arena were more likely to turn away from the trailer or lower their head than horses loading from a dark arena. In addition, those loading from a lit arena to a dark trailer sniffed the ground more, showing increased exploration of their environment. It is concluded that the amount of fear shown by horses was not affected by lighting conditions inside or outside the trailer, but there was some evidence of negative emotions when they loaded from a lit arena, particularly when they entered a dark trailer (Ferguson and Rosales Ruiz, 2001).

People have used winches, whips, war bridles, chains, cattle prods, and a variety of other punitive methods to get horses to load. Although professional horse trainers do not openly advocate extremely aversive methods, most of their methods of loading horses include some form of negative reinforcement and the use of punishment for inappropriate behaviours.

In horses which refused to load, Hendriksen et al. (2011) compared two different training methods, that is the negative reinforcement (NR) versus the positive reinforcement (PN) training, and concluded that the PR group provided the fastest training solution and expressed

less stress response. Thus, the PR procedure could provide a more advisable training solution when training horses in potentially stressing situations. Slater and Dymond (2011) also conducted some trials about horses with loading problem and their results support the use of applied equine training systems based on positive reinforcement to increase appropriate behaviour during common handling procedures.

Transport in Self

During the journeys onto the truck horses can experience large-scale changes in environmental temperature, relative humidity and exposure to environmental contaminants (Leadon et al, 1990). They also may have to adapt to different management strategies including mixing with unfamiliar traveling companions, confinement in unfamiliar spaces, unfamiliar movement beneath their feet, climbing and descending, unfamiliar drinking water and so on.

Confinement and Isolation

Once loaded into the vehicle, the horse is placed in a restricted space either due to being confined in an individual stall using portions, or due to pressure on space by the rest of the group of loose horses with which it may be travelling. Mal et al. (1991) conducting some studies demonstrated that both confinement and isolation are stressful for horses.

Since often trailers require the animal to travel alone, Kay and Hall (2009) conducted a study to investigate the effects of transporting horses alone, in company or with a mirror that provided surrogate companionship. The behavioral and physiological responses of 12 mature horses during a 30-min journey by trailer under the three treatments were compared. Behaviors (vocalizing, eating, head-tossing, pawing, and head-turning) and physiological parameters were recorded. When traveling with a live companion significantly less time was spent vocalizing, head-turning, head-tossing and pawing, whereas eating behavior increased. Heart rate and temperature also significantly decreased when travelling with a live companion. Travelling with the mirror did not significantly affect physiological responses compared yo travelling alone, but when travelling with a mirror significantly less time was spent turning the head, vocalising and head-tossing; instead eating behavior increased. The only significant difference between travelling with a live companion and a mirror was that when travelling with a live companion the time spent turning the head round was lower. In general, the provision of surrogate companionship in the form of a mirror was found to be preferable to travelling alone, but where possible a live companion is recommended. Finally, isolation during transportation was found to suppress feeding behavior.

Effect of Density and Food and Water Intake

During transport with loosing horses moderate stocking density would likely reduce injury and bruising during transportation, but would also increase transport costs. High stocking densities create a situation of constant struggle for the horses. Decreasing density

would reduce the overall stressfulness of long distance transport by allowing the horses some maneuvering room to avoid aggressive horses, to stand in a more comfortable position, to adopt their preferred orientation, and perhaps allow them to rest during periods when the truck is stopped (Collins et al., 2000).

For unloosing horses, the individual stall size is important and there is legislation about the provided minimum space allowances during transport according with ages/types of horses by road. Aniway, minimum space allowances differ from country to country (Waran et al., 2007).

Depending on the reason for transportation, horses may or may not have the opportunity to feed and drink en route. Sport horses are allowed to feed some hay, usually offered in a net, because it will not impair performance (Waran and Cudderford, 1995). Other studies suggest avoiding the presence of the hay into the lorries for the air quality or to put the hay on the floor and allow the horses to eat with a low head position to reduce the development of respiratory disease. With or without feeding opportunity during travel, weight losses are reported after a journey (Waran, 1993).

During long travel water should be offered to the horses whilst the vehicle is stationary at least every 2 to 4 hours, especially when external temperature are high (Houpt and Leibs, 1993). It is important to underline that however horses tend to reduce feed and water intake during the journey, because they are less willing to feed and drink in unfamiliar and stressful surroundings and from an unaccustomed source (Mars et al., 1992).

Environmental Challenges

The space inside the moving vehicle is not usually conducive to a healthy environment. Good ventilation is vital to ensure acceptable air temperature, relative humidity and levels of contaminants such as gases and dust. Studies of air flow around and within lorries for horses were conducted by Leadon et al. (2008): air usually enter the horse lorry through the windows or vents along the side of the lorry, but this air tends to drop toward the floor and becomes contaminated. The air quality consequently in a lorry become very poor and this field should be more studied.

Traub-Dargatz et al. (1988) demonstrated that following exposure to ammonia, nitric oxide and carbon monoxide, horse respiratory clearance was reduced. This is due to damage to the pulmonary epithelial barrier, which then becomes more permeable to bacteria. Although it seems that no upper limit for exposure to ammoniac and other gases have been recommended for equids, Cregier (1982) suggested that this level should be up to 20 ppm.

Another problem is the control of temperature and humidity, which is difficult inside the lorry, luckily healthy horses have a wide temperature tolerance and they have a good thermoregulation mainly via evaporation from the skin and the respiratory tract. Consequently, in heat and humid condition inside the lorry, horses profusely sweat, that can lead to dehydration, but potential heat stress could be occur when the vehicle is stationary and in overcrowding condition (Waran et al., 2007).

Effect of Body Orientation during Travelling

The anatomical conformation of the horse is such that it carries 60% of its body weight over its forelegs, and the hindquarters are poorly designed for continual shifting of weight and direction (Cregier, 1982). It is probably for this reason that the most commonly observed body posture of horses during transit is that of standing with the front limb and hind limb apart and the forelegs stretched forward. This exaggerated limb position during transit perhaps helps the horse to retain its balance by exerting inclined thrusts with one leg or the other, as occasion demands (Robert, 1990).

Inappropriate orientation, and consequent loss of balance, may be the cause of injuries during horse transport (Collins et al., 2000). Among experts, there are different opinions about travel position for minimizing transport stress and optimizing horses' post-transport performance (Gibbs and Friend, 1999). Several studies have been carried out to determine the effects of orientation on a horse's ability to maintain balance during transport, but results have often been conflicting due to differences in trailer design and lack of simultaneous comparisons. Clark et al. (1993) found that when transported in a 2-horse trailer, backward-facing horses had fewer side and total impacts and losses of balance as compared with forward-facing horse. Waran et al. (1996) found that horses transported in a 2-horse lorry without a saddle compartment and facing backward had a significantly lower heart rate, moved less frequently, and showed a greater tendency to rest their rumps on a partition. Toscano and Friend (2001) concluded that some horses demonstrated a superior ability to maintain their balance in a particular orientation; therefore, individual characteristics and other factors, rather than travel orientation alone, may be responsible for the ability of horses to maintain their balance during transport.

Padalino et al. (2012) compared three different positions during a three hours journey: backward, forward and sideward and concluded that although facing backward was the travel position that provoked the greatest number of horses' movements, it did not have a negative effect on physiological and behavioural parameters during and after the journey. These authors suggested that for Standardbred trotters accustomed to travel, the facing backward may be the less stressful position during a 200 Km transport.

Effect of Distance on Physiological, Endocrinological and Behavioural Parameters: Short versus Long Trip

Many studies have be conducted to deeply understand the effects of road transport on equine physiological and behavioural parameters and these changes are usually in accordance with the journey duration.

Baucus et al. (1990) studied the effects of 9 hours of transportation in mares and, even though the early-gestation mares showed increased concentrations of cortisol and progesterone, no early embryonic death rate was reported.

Long transport might strongly affect equine physiological and endocrinal parameters. Stull and Rodiek (2000) studied the effect of 24 hours transport and reported that body weight immediately after unloading showed a 6% loss whereas at 24 h following transit, a 3% deficiency in body weight loss remained. The White Body Cell (WBC) counts showed a progressive increase with duration of travel and peaked at the termination of transport.

Dehydration measures of hematocrit and total protein increased during transport and returned to baseline during the post-transport period. Serum concentrations of lactate, creatine kinase and asparate aminotransferase increased during transport and in the early post-transit period, but returned to baseline values after 24-h from unloading. Glucose concentration increased with the initiation of transport and did not decrease to baseline concentration at the end of the 24-h post-transport period. Plasma cortisol and neutrophil:lymphocyte ratio increased with duration of transit and returned to baseline during the post-transport period. These data clearly showed physiological responses of horses undergoing 24 h of transport including changes in muscle metabolism, stress indices, dehydration, immune parameters, and body weight. These responses may increase disease susceptibility and influence energy availability for athletic performance following long-term transport of horses.

Due to the long journey, stress respiratory disease and even death have been reported in horses (Anderson et al., 1985; Oikawa et al., 1994; Austin et al., 1995). Laegreid et al. (1988) demonstrated that in healthy horses traveling 36 h (1,100 Km) in a trailer, the number of alveolar macrophages and their bactericidal function were decreased and cortisol concentration was elevated for one week after transport; that situation could favor the development of low airways disease.

In agreement with the European law a journey is considered to be long if it lasts more than 8 hours and some rest must be planned to avoid the mentioned healthy problems, safeguarding equine welfare.

Anyway, horses were transported mainly on shorter distances in particular before competitions, so the effects also of short journey must be take in account.

Codazza et al. (1974) compared the effect of exercise and training and concluded that in racehorses, a short-term (300 Km) transportation or an exercise bout of cantering 1,500 m affected similarly serum enzyme and metabolic changes.

Tateo et al. (2012) compared a one hour-journey with a three hours-one: the number of movements recorded per kilometer was greater during the short journey than the long one; in fact, more forward and backward oscillations were recorded, as a result of the greater agitation shown by the horses at the start of the journey. Many authors have argued that horses need around 5 hours to adapt to the journey and to the vehicle; so the first phase of a journey was defined as "the most critical" (Schmidt et al., 2010; Baucus et al., 1990). In Tateo et al. paper, movements (forward and backward) were also interpreted as a part of an adaptation to the new situation. In addition, horses on the short journey not only moved more in the truck but also showed a higher serum cortisol concentration at unloading, suggesting that they could not adapt to the new situation in 1 hour. Anyway, the three hours journey had a greater effect on muscles than did the short journey, causing some minimal muscle damage and slight dehydration.

Other studies confirmed that the period of the adaptation to the journey is longer of one hours, so particular attention must be put on short journey, because the horse is under an acute stress situation and needs to be properly handled (Fazio et al., 2008a).

Unloading

Unloading is part of the journey and it is another physical stress, because for some horses could be difficult to unload if the ramp is very steep or if the animals present some lameness

problems. It seems that it is easier unload from the truck than load into it, indeed, Padalino (not published data) concluded that for horses is easier unload from a lorry ramp that from a step.

Tateo et al. (2012) tested that the heart rate (HR) remained high at the moment of unloading after a three hours journey. This is in agreement with Waran and Cuddeford (1995) who reported that HR remained higher up to 30 minutes after unloading, but it is hard to understand if this increase is due to the unloading in self or to the total transport stress.

Adaptation Period in a New Environment

Little is known of the behaviour of horses after a journey, and very often, behavioural alterations noted may be a result of the changed environment (Waran, 1993). Tateo et al. (2012) studied the behaviours in horses after both short and long journey, but to avoid any confounding effect of changed environment included a control group that did not experience a journey, but changed also box. Comparing the 3 groups at the arrival in the new stalls, it was clear that horses that had made the journey sniffed less and snorted later than the control group. In the first 2 hours after travelling, horses were attracted immediately by concentrated feed and then, they spent more time in hay feeding. After the long journey, the horses performed more drinking bouts and drank earlier than they did after the short journey and when they had not travelled, likely due to the slight dehydration caused by the long journey, which can have positive feedback on drinking behaviour (Iacono et al., 2007b). Two hours after unloading, the horses tended to spend more time standing or playing and yawned more frequently. This is in agreement with Waran (1993) who found that after a 6-hour journey, horses also rested only after drinking and eating.

Overall, after a journey, horses are most interested in feeding than in other behaviours, including exploration, rest, and play activities, that are usually concentrated in the post-feeding hours. In addition, after travelling and during the adaptation period to a new stall travelled horses rested less than control group likely due to their need to feed for recovering energy spent to maintain balance in the truck (Padalino et al., 2012). Consequently, to guarantee favorable adaptation to a new stall, it would be recommend that food and fresh water are offered to the horses onto the boxes.

MAJOR PATHOLOGY CONNECTED WITH TRAVEL

Cregier (1982) reported that long transport could be associated with the incidence of acute colitis, laminitis, transit tetany, trailer choke and mild azoturia. However, horses that have become accustomed to travel by road may also suffer from problems associated with frequent journeys. The pressure placed upon the frequent traveller are likely to be associated with physical demands associated with fatigue, disrupted feeding patterns, loss of weight and restricted movements.

Traumatic Injuries

The simplest traumas are the minor abrasions acquired from horses being placed in an unusual environment. A specific type of abrasion would be from the halter rubbing at the poll or over the nasal area. These injuries occur from braking against a short tie rope and can be prevented by a more experienced driver, protection such as a head bumper or soft wrapping around the halter, and a longer tie rope. Tail rubbing can cause significant abrasions at the base of the tail; careful protective bandaging and eliminating contact of the horse's tail with any fixed object can resolve this problem. Biting can occur if appropriate head ties or protective screens are not available. Wither wounds can present serious healing difficulties because of gravity drainage resulting in infection dissecting into the shoulder fascial planes. These wounds occur from vehicle ceilings which are inhumanely low or from accidents. Leg wounds can occur from loss of balance as a result of braking and cornering episodes. One leg will often strike another, most commonly in the pastern and coronet areas. Another serious injury is vertebral injury caused by quick braking, when a horse has his head firmly tied and the vehicle rapidly decelerates. With this injury the vertebral column is whiplashed from a rapid stop causing serious muscle and ligamentous pulling as the hind legs whip under the horse's body. Vertebral fracture and dislocation can also occur. Head injuries from lipping over when loading or trauma during travel can be serious. Besides direct obvious trauma to the head and eye area, the optic nerve can become stretched in some accidents causing non repairable blindness (Mansmann and Woodie, 1995).

Respiratory Illness Post Transport

In horses, a history of recent transport is frequently associated with the development of pleuropneumonia or *shipping fever*, which is a mixed bacterial pneumonia with varying involvement of the pleural space. Although its aetiological role is uncertain, *Streptococcus zooepidemicus* has been frequently isolated from pneumonic lesions in these cases (Mair and Lane, 1989). Unlike *Streptococcus equi*, the agent of the contagious upper respiratory tract disease commonly referred to as "strangles", *S. zooepidemicus* is a ubiquitous bacterium in the upper respiratory tract and a secondary invader when host susceptibility to respiratory infection is increased, possibly by stress such as transport. It is suggested that transport predisposes the upper respiratory tract and the lower airways to invasion by the bacterium, with episodic pyrexia and acute pneumonia (Oikawa et al., 1994).

The initial clinical signs of shipping fever can be insidious; there is fever, depression, and possibly stiffness; however, lack of cough or nasal discharge is not uncommon, so these clinical signs can mimic other shipping problems. The stiffness and shortening of gait from initial chest pain is similar to laminitis, but it is a respiratory emergency and needs to be diagnosed and treatment initiated as soon as possible (Mansmann and Woodie, 1995).

Factors that may contribute to transport-related respiratory disease in horses are (1) presence of subclinical respiratory diseases, (2) restraint in the “head-up” posture, leading to impairment of pulmonary clearance mechanisms, (3) stress-related impairment of the immune response, (4) presence of noxious gases and high concentrations of airborne dust and bacteria, (5) length and duration of journey, and (6) body orientation during transport (Oikawa et al., 1995).

Moreover, in the traditional hauling of horses hay is placed in a net at the horse's nostrils to allow for some nutrition and keeping the horse entertained. Unfortunately, normal, good quality hay has many dust particles and small mold spores that can be inhaled, consequently having hay near the nostrils increases inhalation of potentially damaging particles and this condition should be avoided.

During transport for preventing respiratory disease, it could be useful to allow the horses' heads more freedom during travel variations, securing a horse by the "log and rope" method. With this method instead of the horse being cross-tied, the horse's halter is attached to one rope that can slide up and down through a secured opening with tension applied by a weight, so head movement can be up and down as well as side to side. Using the "log and rope" method it would be also possible to provide hay in a hay bag on the floor, allowing the horse to eat in the natural position (lowering the head) and all bags, dust particles and water to drop through to the floor.

Other preventives for reducing shipping fever include not allow to travel to horses, that are under therapy with drugs such as phenylbutazone, which could mask the early signs of pneumonia and corticosteroids that would further decrease the horse's defense mechanisms and may also increase the risk of laminitis (Mansmann and Woodie, 1995).

Finally, Oikawa et al. (2004) suggested that increasing the rest time and cleaning the interior of the vehicle during rest stops reduced transportation stress and respiratory insults, factors that may lead to respiratory disease.

Dehydration, Laminitis and Colic Syndrome

As mentioned before, during transport, particular a long one, horses loose body weight and develop dehydration. After transport dehydration status could be from light to severe in accordance with the travel situation (i.e. environmental condition and duration), because despite an increased requirement for water intake, drinking behaviour is generally suppressed by the travel stress in horses (Mars, 1992).

The earliest stages of dehydration are difficult to clinically determine, because a horse could have up to 5% dehydration without showing significant clinical signs. For the athletic horse, 2-3% dehydration can affect performance, so prevention of slight dehydration may be extremely important for the competition horse.

Dehydration can also cascade into more serious metabolic situations: moderate dehydration could initiate blood flow abnormalities to the hooves, inducing laminitis. This problem could be accentuated by the inflammation caused by removing shoes from a horse that normally wears shoes. Other compounding factors that may enhance the risk of laminitis could be the length of shipment relative to the state of fitness of the horse, the level of carbohydrate diet maintained during shipment, and any potential endotoxic disorders initiated due to travel. Preventive measures would include not changing the shoeing status of the horse, adding frog support to those higher risk horses, and reducing carbohydrate intake before and during transport.

Severe dehydration could induce the development of large colon impaction. Tinker et al. (1997) reported that there is a higher risk of colic in the horse that has one to six transports/year, as compared to the horse that is not transported, or one that is transported more than six times per year.

Another potential problem with dehydration would be decreased renal function particularly in horse during medicament treatments (MacAllister and Taylor-MacAIlister, 1994).

There are several methods of preventing dehydration that can be considered. Preparing horses with an aqueous normalizing substance such as apple flavoring has been suggested to offset the differences in water taste during the transport and at the new stall (Mars, 1992). Mineral oil or electrolyte-enriched water via nasogastric tube has been accomplished pre-transport for the prevention of gastrointestinal impaction. The recommendations for stopping during transport are every 4-6 hours and overnighting horses at least every 12-16 hours. During stops and overnighting, to prevent dehydration and correlated pathologies, the horses should have the possibility to do some exercise and to be examined by veterinarians who could administer fluids. Oral electrolytes and water could be easily given via nasogastric tube to the mild to moderate dehydrating horse or intravenously to the severe one. A 450-500 kg horse's stomach can tolerate 6-8 liters of electrolyte enriched water every 15 minutes for 1 to 2 hours (Mansmann and Woodie, 1995).

TRAVEL BEFORE COMPETITION: WHEN SHOULD THE HORSE ARRIVE TO THE RACETRACK?

Despite many horses being transported specifically for performance purpose, surprisingly little is known about the impact transportation has on performance and the results are often conflicting. Beaunoyer and Chapman (1987) suggested that for experienced horses, transport over short distance had little impact on performance. Shale (1987) investigated the effects of forward facing transport on post transit racing performance and reported any negative effects. The effect of transport on jumping horses competition was also studied and in this case only less experienced horses showed major signs of stress and lower performances (Colalesky et al., 1991; Russoniello et al., 1991). Fazio et al. (2008b) carryed out a study about the effects of competition experience and transportation on the adrenocortical and thyroid responses in jumping horses and no statistically change were found apart from a lower basal concentration of free triiodothyronine concentration in the group of experienced jumpers, which did not travel before competition. There is no study that correlates the effects of short transport with performance results, probably why one of the problem in this area is being able to develop good scientific methodology for assessing the effect of transportation on performance, because there are many variables from the individual effect (some horses cope better with transport than others) to the position in the truck and from the fit level of each horse to the driver ability.

Tateo et al. (2012) suggested a recovery period of two hours after a three hours travel on the basis of the muscle enzyme concentration, that showed an increased after transport for that period, but the trial did not include the race in the protocol. Linden et al. (1991) discouraged long travel, longer than 8 hours, before a competition because they could compromise racing performance and suggested some days of recovering.

EFFECT OF TRAINING ON HEMATOLOGICAL PARAMETERS

This field had been deeply studied and many books about sport medicine and exercise equine physiology have been written, so it is well known that exercise induce a physical stress and has an impact on all physiological parameters (Marlin and Nankervis, 2002). Exercise causes, for example, an adrenocortical and thyroid responses (Fazio et al., 2008b), but also change in haematological and haematochemical profiles, as the increase of packet cell volume and the change in neutophils and lymphocytes ratio (Piccione et al., 2010).

Exercise for horses is not only a physiological stress, but also an emotional stress and it must be good manage to improve the equine welfare.

Quaranta et al. (2006) reported that the race induced more change in haematological and physiological parameters than a maximal exercise at the same speed, probably because of the presence of others horses induces competition emotions. In the same way Becker-Birck et al. (2012) pointed that the presence of audit induced a significant increase in the cortisol salivary concentration both in riders and horses during a horse manifestation.

Emotional stress is difficult to study and interpreter in addition to a physical stress (Rietmann et al., 2004), but equine technicians should bear in mind this matter during any competitions and Scientists should try to better study this area.

EFFECT OF EXERCISE ON BEHAVIOUR

Studies in this area are still few. Caaniz et al. (1991) conducted a study to examine the effect of short periods of strenuous training (treadmill exercise) on the subsequent behaviour of Standardbred horses; they found through a focal animal sampling that the horses spent significantly more time drinking and less time resting after the exercise. Those data were confirmed by Padalino (under review): trotters after a race or an intensive physical exercise increased eating and drinking behaviour, whereas decreased resting. Latter authors, on the basis of their results, recommend that horses should be offered food and fresh water in their boxes after cooling down, to guarantee a favorable physiological and behavioural restore. Overall, the assessment of horse behaviour after physical exercise by means of an ethogram could represent a useful tool to monitor equine welfare and its use should rise in the equine praxis.

CONCLUSION

It is important to induce adaptation to the transport, Broom and Johnson (1993) described that a horse which is disturbed when first coaxed into a transport vehicle may show various signs of disturbance, but most of these signs will disappear by, for example, the tenth transportation. These data were confirmed by Schmidt et al (2010), who conducted a study to understand the effect of repeated transports, as happens for race and competitions horses in their life. The authors showed that a transport-induced stress response decreased with repeated transport, indicating that horses were habituated to the situation, but an increased

cortisol secretion remained detectable, probably connected with the loading fear and the first adaptation period to the lorry environment.

Overall, loading fear could be managed by training horses, in particular through the application of the learning theory in equitation science (McGreevy and McLean, 2007) that could help the owner to avoid the refuse to load in the trailer. As mentioned before in the chapter, actually, the use of positive reinforcement seems to be the best training method.

In addition to reduce the stressors during the travel, the better position seems to be the facing backward and for horses, that must travel alone, the use a mirror could decrease the isolation stress. Moreover, to avoid health problems, such as respiratory illness or colic, it could be useful to give some electrolytes and examine the health status of the horses before traveling and check the environmental condition inside the truck. For long distance journey it seems essential to plan some resting period allowing physical exercise, watering and feeding.

To bear in mind is that the first hour travel seems to be the most stressful for the horse, so also after short journey animals need a resting time to adapt at the new stall or condition. Adaptation could be improved offering at the unloading fresh water and food to the horses, that seems to be their first behavioural needs after a transport.

Horses, that must travel for 3 or 4 hours before a race, should arrive at their destination at least 4 hours before beginning physical activity. Further studies are needed in this area to really improve the welfare status of horses transported before competition.

Finally, it is important to underline that vehicles and facilities have been used for animal transport they may harbor infectious agents that could cause the spread of major disease; hence proper disinfection and disinfestations are needed.

REFERENCES

American Horse Council., (1996). The economic impact of the horse industry in the Unites States. National Summary. *American Horse Council Foundation report prepared by Polity Economics Practice. Barents Group LLC*. 1, 1-20.

Anderson, N.V., DeBowes, R.M., Nyrop, K.A., Dayton, A.D., (1985). Mononuclear phagocytes of transport-stressed horses with viral respiratory tract infection. *Am. J. Ve.t Res.* 46, 2272–2277.

Austin, S.M., Foreman, J.H., Hungerford, L.L., (1995) Casecontrol study of risk factors for development of pleuropneumonia in horses. *J. Am. Vet. Med. Assoc.* 207, 325–328.

Baron, A., (1991). *Avoidance and punishment.* In: Iversen IH, Lattal KA editors. *Experimental analysis of behaviour*. New York: Elsevier; 173-217.

Baucus, K.L., Ralston, S.L., Nockels, C.F., McKinnon, A.O., Squires, E.L., (1990). Effects of transportation on early embryonic death in mares. *J Anim Sci.* 68, 345–351.

Beaunoyer, D.E., Chapman, J.D., (1987) Trailering stress on subsequent maximal exercise performance. in: *Proceedings of 11th Equine Nutrition and Physiology Symposium*. Oklahoma States University, Stillwater, Oklaoma, USA, 1987, 379-384.

Becker-Birck, M., Biau, S., Ill, N., Aurich, J., Mostl, E., Aurich, C., (2012). Heart rate, heart rate variability and cortisol release in the horse and ist rider: different response to training and a public equestrian performance. In: *Proceeding of the 8th Equitation Science Conference*, 18-20 July 2012, Edinburgh, 92.

Broom, D.M., (2005). The effect of land transport on animal welfare. *Rev. Sci.Tech.* 24, 683-691.

Broom, D.M., Johnson, K.G., (1993). *Stress and Animal Welfare*. Dordrecht, Netherlands: Kluwer Academic Publishers, 1-7.

Caanitz, H., O'Leary, L., Houpt, K., Petersson, K., Hintz, H., (1991). Effect of exercise on equine behaviour. *Appl. Anim. Beha. Sci.* 31, 1-12.

Casamassima, D., Palazzo, M., Presutti, T., Cinone, M., (2008). Effects of two tame system on physiological parameters of arab horses subjected to load in the trailer. *Ippologia* 3, 13-19.

Cavallone, E., Di Giancamillo, M., Secchiero, B., Belloli, A., Pravettoni, D., Rimoldi, E.M., (2002). Variations of serum cortisol in Argentine horses subjected to ship transport and adaptation stress. *J. Eq.Vet.Sci.* 22, 541-545.

Clark, D.K., Friend, T.H., Dellmeier, G., (1993). The effect of orientation during trailer transport on heart rate, cortisol and balance in horses. *Appl. Anim. Beha. Sci.* 38, 179–189.

Codazza, D., Maffeo, G., Redaelli, G., (1974). Serum enzyme changes and hemato-chemical levels in Thoroughbreds after transport and exercise. *J. S. Afr. Vet. Assoc.* 45, 331–334.

Collins, M.N., Friend, T.H., Jousan, F.D., Chen, S.C., (2000). Effects of density on displacement, falls, injuries, and orientation during horse transport. *Appl. Anim. Beha. Sci.* 67, 169-179.

Covalsky, M., Rossiello, C., Malinowski, K., (1991) Effects of show-jumping performance stress on plasma cortisol and lactate concentrations and heart rate and behaviour in equine. in: *Proceedings 12th Equine Nutrition and Physiology Symposium*, University of Calgary, Canada, 1991, 171-172.

Cregier, S,E,. (1982). Reducing equine hauling stress: a review. *J. Eq.Vet.Sci.* 2, 186-198.

Cross, N., van Doorn, F., Versnel, C., Cawdell-Smith, J., Phillips, C. (2008). Effects of lighting conditions on the welfare of horses being loaded for transportation. *J. Vet. Beha.* 3, 20-24.

Curcio-Wolfe, J., (1996). Tellington-touch. In *Proceedings of the 1996 American Holistic Veterinary Medical Association Annual Conference*. Bel Air, MD: American Holistic Veterinary Medical Association, 1996, 12-13.

Fazio, E., Ferlazzo, A., (2003). Evaluation of Stress During Transport. *Vet. Res. Com.* 27, 519–524.

Fazio, E., Medica, P., Aronica, V., Grasso, L., Ferlazzo, A., (2008a) Circulating b-endorphin, adrenocorticotropic hormone and cortisol levels of stallions before and after short road transport: stress effect of different distance. *Acta. Vet. Scand.* 6, 1-6.

Fazio, E., Medica, P., Cravana, C., Ferlazzo, A., (2008b). Effects of competition experience and transportation on the adrenocortical and thyroid responses of horses. *Vet. Rec.* 163, 713-716.

Ferguson, D.L., Rosales Ruiz, J., (2001). Loading the problem loader: the effects of target training and shaping on trailer loading behaviour of horses. *J. Appl. Behav. Anal.* 34, 409–424.

Friend, T.H., (2001). A review of recent research on the transportation of horses. *J. Anim. Sci.* 79, 32-40.

Gibbs, P.G., Benefield, M.R., Potter, G.D., McNeill, J., Johnson, B.H., Moyer, W., (1997). Profile of horse ownership and use in 8 Texas counties - Phase 2 of Texas horse industry

quality audit. In: *Proc 15th Equine Nutrition and Physiology Society*, Ft Worth, 1997, 321-325.

Gibbs, P.G., Potter, G.D., Jones, L.L., Benefield, M.R., McNeill, J.W., Johson, B.H., Moyer, W., (1998). *The Texas horse Industry: Texas Agricultural Extension Service*, Texas A&M University System, College Station, 1-27.

Gibbs, A.E., Friend, T.H., (1999). Horse preference for orientation during transport and the effect of orientation on balance ability. *J. Appl. Behav. Sci.* 63, 1-9.

Giovagnoli, G., (2008). *Manuale teorico-pratico sul trasporto del cavallo*. Milano: Mursia Editore; 2008, 17-26.

Hendriksen, P., Elmgreen, K., Jan Ladewig, J., (2011). Trailer-loading of horses: Is there a difference between positive and negative reinforcement concerning effectiveness and stress-related signs? *J. Vet. Beha.* 6, 261-266.

Herzolz, A., Fussel, A.E., Timoney, P., Schwermer, H., Bruckner, L., Leadon, D., (2008). Equine travellers to the Olympic Games in Hong Kong: a review of worldwide challenges to equine health, with particular reference to vector-borne disease. *Equine Vet. J.* 40, 87-95.

Houpt, K.A., (1982). Misbehaviour of horses: trailer problems. *Equine Prac.* 4, 12-16.

Houpt, K.A., Leib, S., (1993) Horse handling and transport. In: Houpt, KA, editor. *Livestock handling and transport*. Wallingford: CAB international; 1993, 233-252.

Iacono, C.M., Friend,T.H., Johnson, R.D., Krawezel, P.D., Archer, G.S., (2007a). A preliminary study on the utilization of an onboard watering system by horses during commercial transport. *Appl. Anim. Beha. Sci.* 105, 227-231.

Iacono, C., Friend, T., Keen, H., Martin, T., Krawczel, P. (2007b). Effects of density and water availability on the behaviour, physiology and weight loss of slaughter horses during transport. *J. Equine Vet. Sci.* 8, 355-361.

Jones, W.E., (2003). Transporting horses: minimizing the stress. *J. Equine Vet. Sci.* 12, 543-545.

Judge, NG., (1969). Transport of Horses. *Aust. Vet.J.* 45, 465-469.

Kay, R., Hall, C., (2009). The use of a mirror reduces isolation stress in horses being transported by trailer. *Appl. Anim. Beha. Sci.* 116, 237–243.

Laegreid, W.W., Huston, L.J., Basaraba, R.J., Crisman, M.V., (1988). The effects of stress on alveolar macrophage function in the horse : An overview. *Equine Prac.* 10, 9-16.

Leadon, D., Daykin, J., Blackhouse, W., Frank, C., Attock, M.A., (1990). Enviromental haematological and blood biochemistry changes in equine transit stress. *Proc. Am. Assoc. Equine Pract.* 36, 485-490.

Leadon, D., Waran, N., Herzholz, C., Klay, M., (2008). Veterinary management of horses transport. *Vet. Italiana* 44, 149-163.

Lee, J., Houpt, K., Doherty, O., (2011). A survey of trailering problems in horses. *J. Equine Vet. Sci.* 21, 235-238.

Linden, A., Art, T., Amory, D., Desmecht, D., Lekeux, P., (1991). Effect if 5 different exercise, transportation and ACTH administration on plasma cortisol concentration in sport horses. *Equine exercise physio.* 3, 191-196.

MacAllister, C.G., Taylor-MacAIlister, C., (1994). Treating and Preventing the Adverse Effects of Non Steroidal Antiinflammatory Drugs in Horses. *Veterinary Med.* 3, 241-246.

Mair, T.S., Lane, J.G., (1989). Pneumonia, lung abscesses and pleuritis in adult horses: a review of 51 cases. *Equine Vet. J.* 21, 175-180.

Mal, M.E., Friend, T.M., Lay, D.C., Vogelsang, S.G., Jenkins, O.C., (1991). Physiological responses of mares to short term confinement and isolation *J. Equine Vet. Sci.* 11, 96-102.

Mansmann, R.A., Woodie, B., (1995). Equine transportation problems and some preventives: a review. In: *Proceeding of the 2nd International Conference on equine rescue*. Southern Pines, North Carolina. February 11-12 1995, 141-144**.**

Marlin, D., Nankervis, K., (2002). *Equine exercise physiology*. Malden, USA: Backwell Publishing, 2002, 133-150.

Mars, M.E., Keisling, H.E., Ross, T.T., Armstrong, J.B., Murray, L., (1992). Water acceptance and intake in horses under shipping stress. *J. Equine Vet. Sci.* 12, 17-21.

McGreevy, P.D., McLean, A.N., (2007). Roles of learning theory and ethology in equitation. *J. Vet. Beha.* 2, 108–118.

Munsters, C.C.B.M., de Gooijer, J.W., van den Broek, J., Sloet van Oldruitenborgh-Oosterbaan, M.M., (2013). Heart rate, heart rate variability and behaviour of horses during air transport. *Vet. Rec.* 172, 1-10.

Ohmura, H., Hobo, S., Hiraga, A., Jones, J.H., (2012). Changes in heart rate and heart rate variability during transportation of horses by road and air. *Am. J. Vet. Res.* 73, 515-521.

Oikawa, M., Hobo, S., Oyamada, T., Yoshikawa, H., (2005). Effects of Orientation, Intermittent Rest and Vehicle Cleaning During Transport on Development of Transport-related Respiratory Disease in Horses. *J. Comp.Pathol.* 132, 153–168.

Oikawa, M., Takagi, S., Anzai, R., Yoshikawaw, H., Yoshikawaw, T., (1995). Pathology of Equine Respiratory Disease Occurring in Association with Transport. *J. Comp. Pathol.* 113, 29-43.

Oikawa, M., Kamada, M., Toshikawa, Y., Yoshikawa, T., (1994). Pathology of equine pneumonia associated with transport and isolation of *Streptococcus equi* subsp. *zooepidemicus*. *J. Comp. Pathol.* 111, 205–212.

Padalino, B., Maggiolino, A., Boccaccio, M., Tateo, A., (2012). Effects of different positions during transport on physiological and behavioural changes of horses*. J. Vet.Beha.* 7, 135-141.

Padalino, B., Zacchino, P., Celi, P. Effect of different types of physical exercise on behavioural and physiological parameters of Standardbred horses housed in single stalls. *Inter. Vet. Med.*, accepted.

Piccione, G., Casella, S., Giannetto, C., Messina, V., Monteverde, V., Caola, G., Guttadauro, S., (2010). Haematological and haematochemical responses to training and competition in standardbred horses. *Comp. Clin. Path.* 19, 95-101.

Pritchard, J.C., Burn, C.C., Barr, A.R.S., Whay, H.R., (2009). Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. *Res.Vet. Sci.* 87, 389–395.

Quaranta, A., Tateo, A., Siniscalchi, M., Padalino, B., Iacoviello, R., Centoducati, P., (2006). Influenza di diversi tipi di allenamento su cortisolo ematico ed emocromo in cavalli trottatori. *Ippologia* 17, 5-10.

Randal, J.M., Patel, R., (1994). Thermally induced ventilation of livestock transportes. *Agricult. Engi. Res.* 57, 99-107.

Reece, V.P., Friend, T.H., Stull, C.H., Grandin, T., Cordes, T., (2000). Equine slaughter transport: Update on research and regulation**.** *J. Am.Vet.Med. Asso.* 216, 1253-1257.

Rietmann, T.R., Stuart, A.E.A., Bernasconi, P., Stauffacher, M., Auer, J.A., Weishaupt, M.A., (2004). Assessment of mental stress in warmblood horses: hear rate variability in comparation to hear rate and selected behavioural parameters. *Appl. Anim. Beha. Sci.* 88, 121-136.

Ritter, M.J., Ellis, M., Bowman, R., Brinkmann, J., Curtis, S.E., DeDecker, J.M., Mendoza, O., Murphy, C.M., Orellana, D.G., Peterson, B.A., Rojo, A., Schlipf, J.M., Wolter, B.F., (2008). Effects of season and distance moved during loading on transport losses of market-weight pigs in two commercially available types of trailer. *J. Anim.Sci.* 86, 3137-3145.

Robert, T.D.M., (1990). Staying in a moving trailer. *Equine Athlete* 3, 1-8.

Russoniello, C., Racis, S.P., Ralston, S.L., Maliniwski, K, (1991). Effects of show-jumping performance stress in haematological parameters and cell-mediated immunity in horses. in: *Proceedings 12th Equine Nutrition and Physiology Symposium*, University of Calgary, Canada, 1991, 145-147.

Schmidt, A., Hödl, S.A., Möstl, E., Aurich, J., Müller, J., Aurich, C., (2010). Cortisol release, heart rate, and heart rate variability in transport-naive horses during repeated road transport. *Domest. Anim. Endocrinol.* 39, 205–213.

Scoggins, R.D., (1996). Horses and claustrophobia. *AVSAB Newsletter* 18, 2–3.

Shanahan, S., (2003). Trailer Loading Stress in Horses:Behavioural and Physiological Effects of Nonaversive Training (TTEAM). *J. appl. anim. welfare sci. 6*, 263–274.

Slade, L.M.J., (1987) Trailer transportation and Racing performance. *Proceedings 10th Equine Nutrition and Physiology Symposium.* Fort Collins, CO, Texas, USA, 511-514.

Slater, C., Dymond, S., (2011). Using differential reinforcement to improve equine welfare: Shaping appropriate truck loading and feet handling. *Behav.Process.* 86, 329–339.

Stewart, M., Foster, T.M., Waas, J.R., (2003). The effects of air transport on the behaviour and heart rate of horses. *Appl. Anim. Beha. Sci.* 80, 143-160.

Stull, C.L., (2001). Evolution of the proposed federal slaughter horse transport regulations. *J. Anim. Sci.* 79,12-15.

Stull, C.L., Rodiek, A.V., (2000). Physiological responses of horses to 24 hours of transportation using a commercial van during summer conditions. *J. Anim. Sci.* 78, 1458-1466.

Tarrant, P., (1990). Transportation of cattle by road. *Appl. Anim. Beha. Sci.* 28, 153-170.

Tateo, A., Padalino, B., Boccaccio, M., Maggiolino, A., Centoducati, P. (2012). Transport stress in horses: Effects of two different distances. *J. Vet.Beha.* 7, 33-42.

Tinker, M.K., White, N.A., Lessard, P., Thatcher, C.D., Pelzer, K.D., Davis, B., Carmel, D.K., (1997). Prospective study of equine colic risk factors. *Equine vet. J.* 29, 454–458.

Toscano, M.J., Friend, T.H., (2001). A note on the effects of forward and rear-facing orientations on movement of horses during transport. *Appl. Anim. Beha. Sci.* 73, 281-287.

Traub-Dargatz, J.L., Mckinnon, A.O., Bruyninckx, W.J., Thrall, M.A., Jones, R.L., Blancquaert, A.M.B. (1988) Effect of transport stress on broncoalveolar lavage fluid analysis in female horses. *Am. J. Vet.Res.* 49, 1026-1029.

USDA. 1999. *Livestock slaughter annual summary*. Washington, DC: National Agricultural Statistics Services,1998, 1-100.

Waran, N.K., Leadon, D., Friend, T., (2007). The effect of transportation on the welfare of horses. In: Waran, NK editor. *The welfare of horses*. Netherlands: Kluwer Academic Publishers, Springer; 2007, 125-150.

Waran, N.K., Cuddeford, D., (1995). Effects of loading and transport on the heart rate and behaviour of horses. *Appl. Anim. Beha. Sci.* 43, 71-81.

Waran, N.K., (1993). The behaviour of horses during and after transport by road. *Equine Vet. Edu.* 5, 129-132.

Waran, N.K., Robertson, V., Cuddeford, D., Kokoszko, A., Marlin, D.J., (1996). Effects of transporting horses facing either forward or backward on their behaviour and heart rate. *Vet. Res.*, 139, 7-11.

Week, C.A., McGreevy, P.D., Waran, N.K. (2012). Welfare issue related to transport and handling of both trained and unhandled horses and ponies. *Equine Vet. Edu.* 24, 423-430.

Werner, M., Gallo, C., (2008). Effects of transport, lairage and stunning on the concentrations of some blood constituens in horses destinated for slaughter. *Livestock Sci.* 115, 94-98.

White, A., Reyes, A., Godoy, A., Martinez, R. (1991). Effects of transport and racing on ionic changes in thoroughbred race horses. *Comp. Biochem. Physiol* 3, 343-346.

Whiting, T., (1999). Maximum loading density of loose horses. *Can. J. Anim.Sci.* 79; 115-118.

Websites

http://www.faostat.fao.org
http://www.animalwelfarestandards.net.au/files/2011/02/Land-transport-of-livestock-Standards-and-Guidelines-Version-1.-1-21-September-2012.pdf
http://www.iata.org/Pages/default.aspx
http://www.iata.org/ps/publications/Pages/live-animals.aspx

In: Horses
Editors: A. Paz-Silva, M. S. Arias Vázquez et al.
ISBN: 978-1-63117-560-2

Chapter 3

CLINICAL INTERPRETATION OF QUANTITATIVE PARAMETERS OF THE HEMOGRAM IN THE HORSE

Katy Satué[1], Ana Muñoz[2] and Juan Carlos Gardón[3]
[1]Department of Animal Medicine and Surgery, Cardenal Herrera University, Spain
[2]Department of Animal Medicine and Surgery, Equine Sport Medicine Center, CEMEDE, University of Córdoba, Spain
[3]Department of Experimental Sciences and Mathematics, Catholic University of Valencia San Vicente Mártir, Spain

ABSTRACT

The objective of study of the hemogram is to generate information that would assist the clinician in diagnostic processes, formulation of prognosis, patient management and control. For an accurate interpretation of hematologic data in horses, some characteristics should be considered, such as age, breed, sex, venipunction method, season, reproductive status, feeding, exercise, administration of sedatives, the circadian biological rhythms, etc.). The erythrocyte report data is focused on the possible presence of anemia or polycythemia and their causes, with major relevance in sport horses. The study of white blood cells refers to immunity against infectious microorganisms, through the production of antibodies or by participating in the destruction of microorganisms or allergic responses. Indeed, the leukogram study refers to the inflammatory response in neoplasias. Major alterations that occur in the count of blood platelets are thrombocytosis or thrombocytopenia which may be due to various mechanisms in a large number of inflammatory and ischemic disorders in foals and adult animals. Due to the clinical importance of studying equine hematology, this chapter describes the common quantitative changes affecting erythrocyte, leukocyte and platelet counts, as well as plasma proteins.

Keywords: erythrogram, leukogram, hematology, horse, platelets, total proteins

1. INTRODUCTION

The main function of laboratory tests, among which the hemogram is included, is to generate information that would assist the clinician in the diagnostic process, decision making, formulation of prognosis and patient management and control. The hemogram is one of the most widely used laboratory tests, due to the easy and speed of implementation, and the excellent relationship between the cost and the information it provides. However, it should be kept in mind that it is uncommon to use hematology as diagnostic for a particular disease. Hematologic alterations often reflect the condition of the individual or overall response to a pathological situation. Furthermore, the results should be interpreted taking into account different patient data such as age, race, gender, venipunction method, season, reproductive status, feeding, training, exercise, administration of sedatives and tranquilizers, circadian biological rhythms, altitude… and the information provided by clinical examination (McGowan, 2008; May et al., 2010; Muñoz et al., 2010a; Trigo et al., 2010; Satué et al., 2009; 2010; 2011). The way in which the sample has been collected and managed, and the methodology used in measuring the different parameters may also influence the results and thus, their interpretation (reviewed by Satué et al., 2012). The erythrogram includes quantification of blood components and the observation of changes in morphology. This study includes erythrocyte count (RBC), packed cell volume (PCV), hemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total and differential counts of leukocytes (WBC), platelets (PLT) and the study of cell morphology. For the interpretation of hematological data in horses, it must take into account the following characteristics of this species that are different from others (Morris, 1990; Messer, 1995):

PCV unstable: PCV in horses is highly variable, due to the significant innervation of the spleen and its performance as a reservoir of blood, which could store more than one third of the blood volume. Therefore any adrenergic stimulation, as occurs during exercise, and in response to excitation, causes a splenocontraction, releasing a large amount of blood cells into the peripheral circulation. For this reason, the PCV in the horse at rest should be carefully evaluated according to the excitation level (Schalm and Carlson, 1982). The intensity of the changes in circulating RBC in relation to the spleen activity depends on individual variations, age, breed, and fitness level and in the case of exercise, duration and intensity (Kline and Foreman, 1991). The time required for RBCs to return to resting values is dependent on the degree of the excitement, and may vary from 40 to 60 minutes to up to several hours (Revington, 1983).

Rouleaux formation is a characteristic of the blood of horses, with a marked tendency to form stacks. This implies a rapid separation of the formed elements of plasma with a high erythrocyte sedimentation rate (Schalm and Carlson, 1982). Rouleaux formation can be accentuated by some diseases associated with hyperproteinemia, because high concentrations of plasma proteins, particularly fibrinogen and immunoglobulins, have an insulating effect that reduces the RBC surface membrane charge, promoting RBC aggregation (Schalm and Carlson, 1982; Spengler et al., 2008).

Absence of peripheral signs of regeneration: equine erythrocytes remain in the bone marrow during the process of formation and are not discharged into the blood circulation until maturation is completed. For this reason the morphological characteristics related to regeneration, described in other species, such as polychromasia or reticulocytosis,

macrocytosis, or other signs of peripheral regeneration are rarely found in horse blood smears. Therefore, in anemic horses, the erythrogram is not an accurate measurement test using only peripheral blood. Life span of equine RBC in the circulation is approximately 140 to 150 days. RBCs are released from bone marrow as mature cells and the horse is unique in failing to release reticulocytes into peripheral blood when there is a regenerative response to hemorrhage or hemolysis (Cooper et al., 2005). The reticulocyte count can be performed on marrow aspirates in anemic horses. Normal equine bone marrow contains approximately 3% reticulocytes, then, values greater than 5% are consistent with accelerated erythropoiesis, and an increase as high as 66% in response to severe blood loss (Malikides et al., 1999; Tornquist, 2008). Increases in MCV are inconsistent but slightly more common after hemolysis than after acute blood loss (Radin et al., 1986; Malikides et al., 1999; Pearson et al., 2005). The only change in equine peripheral blood after acute hemorrhage or hemolysis may be a slight anisocytosis that is quantitatively assessed through changes in RBC distribution (Schalm and Carlson, 1982; Radin et al., 1986; Sellon, 2004). The most practical and reliable method of assessing an erythrocyte regenerative response in an anemic horse is the bone marrow analysis. A myeloid erythroid (M/E) ratio less than 0.5 is considered an evident sign of erythrocyte regeneration (Sellon, 2004; Tornquist, 2008).

The *Howell-Jolly bodies* are basophilic nuclear remnants (clusters of DNA) in circulating erythrocytes, which can be found occasionally in equine blood. Unlike what occurs in other species, this presence does not indicate regenerative response in cases of anemia, unless they occur in a significantly increased number (Burrows and Borchard, 1982; Schalm and Carlson, 1982; Pearson et al., 2005; Giardano et al., 2008).

Finally, we must bear in mind that for a proper interpretation of erythrogram is necessary to measure total plasma proteins (TPP) and fractions (albumin, fibrinogen and globulins). The evaluation of these parameters allows us to differentiate between absolute or relative hematological changes, associated with changes in plasma volume (Sneddon et al., 1992; Messer, 1995; Lording, 2008; Grondin and Dewitt, 2010; Trigo et al., 2010).

2. INTERPRETATION OF THE ERYTHROGRAM

Reference data for erythrocyte parameters in horses are presented in table 1.

Table 1. Reference values for the erythrocyte parameters in adult healthy horses (modified from Lassen and Swardson, 1995; Satué et al., 2009; 2010; 2012; Muñoz et al., 2010a) (*RBC, red blood cells; HB, hemoglobin concentration; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets*)

PARAMETERS	UNITS	REFERENCE RANGE
RBC	$10^6/\mu L$	7.0-13.0
HB	g/dL	9.0-14.0
PCV	%	32-45
MCV	fl	37-55
MCH	pg	12-20
MCHC	g/dL	31-39
PLT	$10^3/\mu L$	100-350

2.1. Erythrocyte Count

Pathological changes that can be found in the erythrogram are polycythemia or erythrocytosis and anemia.

Erythrocytosis is defined as the absolute or relative increase in the number of circulating RBCs. It is represented by an increase in PCV, HB and RBC counts (Lassen and Swardson, 1995; Sellon, 2004). The erythrocytosis is classified into two main groups, relative and absolute. In the relative erythrocytosis there is not an increase in RBC. It is caused by dehydration and splenic contraction. Dehydration leads to a decreased plasma volume in relation to the cellular component of the blood. Therefore, it refers to a hemoconcentration, which is accompanied by an increase in TPP concentrations, unless there are additional losses (Lassen and Swardson, 1995). Moreover, splenic contraction can substantially increase PCV, HB and RBC. In both cases, the total number of erythrocytes is not modified (Morris, 1990; Sellon, 2004; Grondin and Dewitt, 2010).

Absolute erythrocytosis occurs with the addition of newly formed cells to the peripheral circulation, and it can be subdivided into primary and secondary absolute erythrocytosis. Primary erythrocytosis is considered a myeloproliferative disorder of the bone marrow. This type of erythrocytosis occurs in some neoplasia or functional disorders of the bone marrow (McFarlane et al., 1998; Koch et al., 2006). This condition might be accompanied by thrombocytosis or leukocytosis, and erythropoietin (EPO) concentrations are within normal limits (Morris, 1998; Sellon, 2004; Lording, 2008).

By contrast, secondary erythrocytosis is due to the action of the EPO on the bone marrow, since the concentrations of this hormone raise significantly. According to some authors, it can be distinguished between physiologically correct secondary erythrocytosis and physiologically incorrect secondary erythrocytosis. The physiologically correct secondary erythrocytosis occurs in cases of systemic chronic hypoxia, such as happens in patients with cardiovascular and pulmonary disease or adaptation to high altitude. Hypoxemia ($PaO_2 < 80$ mm Hg and O_2 saturation < 92%) is diagnostic for secondary appropriate erythrocytosis (Belli et al., 2011). The most common anomalies associated with erythrocytosis are of cardiac origin, such as complex defects such tetralogy or pentalogy of Fallot, although other defects, including ventricular septal defect, eventually may result in right to left shunting and secondary erythrocytosis, or respiratory origin, such as chronic pleuropneumonia. Horses with this condition frequently exhibit cyanotic mucous membranes (Sellon, 2004).

The physiologically incorrect secondary erythrocytosis refers to the production of EPO exacerbated either directly, as occurs in renal tumors (renal carcinoma) or indirectly as part of paraneoplasic syndrome, as in hepatocellular carcinoma or hepatoblastoma (Lennox et al., 2000; Axon et al., 2008; Gold et al., 2008). These horses have persistently elevated PCV that does not response to intravenous fluid therapy, normal plasma protein concentration and mild to moderate elevations in hepatic enzymes (Sellon, 2004).

A summary of the classification and the main etiologies of erythrocytosis in horses is shown in table 2.

Table 2. Classification and etiology of equine erythrocytosis

TYPE	SUBTYPE	ETIOLOGY	CHARACTERISTICS
Relative		Spleen contraction Dehydration	Stressful conditions Increased total plasma proteins in dehydration (unless additional protein loss)
Absolute	Primary	Neoplasia or functional disorders of bone marrow	Normal erythropoietin concentrations
	Secondary		
	Physiologically correct secondary	Chronic respiratory disease Chronic cardiovascular disease Living at high altitude	Increased erythropoietin concentrations
	Physiologically incorrect secondary	Renal neoplasia Paraneoplasic syndrome	

Anemia is defined as an absolute or relative decrease in PCV, HB and circulating RBC (Messer, 1995). In relative anemia, there is not a reduction in the total number of RBC. However, hemodilution or erythrocyte sequestration makes the three variables mentioned decline (Mahaffey and Moore, 1986).

Absolute anemia is clinically relevant because there is a decrease in the total number of RBC and PCV. HB is also decreased, except in cases of intravascular hemolysis. Anemias may be classified as regenerative and non-regenerative based on bone marrow response to the decrease in circulating RBC mass. Regenerative anemia results from either acutely or chronically loss of intact RBCs from circulation (hemorrhage) or accelerated destruction of RBCs (hemolysis). Regenerative anemia is characterized by an increase in effective erythropoiesis in the bone marrow. Non-regenerative anemia occurs following systemic abnormalities or because of intrinsic bone marrow disease and results from a lack of appropriate marrow erythropoiesis in response to normal or accelerated RBC senescence or destruction (Sellon, 2004).

Clinical signs of severe anemia are related to decreased tissue oxygenation and to physiological compensatory mechanisms developed in order to alleviate hypoxia. Signs include paleness of the mucous membranes, tachycardia, tachypnea, weakness, lethargy and a systolic heart murmur caused by decreased viscosity and increased turbulence of the blood in the heart and great vessels in cases of severe anemia. Horses with slight to moderate anemia may have no obvious clinical signs or may have only lethargy and slightly pale mucous membranes. Other clinical signs, including fever, icterus and hemoglobinuria may be present in anemic horses, depending on the primary cause of the anemia (Sellon, 2004).

The types and causes of regenerative anemia described in equines are (Lassen and Swardson, 1995; Morris, 1998; Sellon, 2004):

1 Blood loss, acute or chronic
   - -Epistaxis (guttural pouch mycosis, pulmonary abscesses, exercise induced pulmonary hemorrhage, ethmoidal hematoma, paranasal sinus abscess or infection, upper respiratory tract neoplasm, pneumonia or pleuritis….) (Dixon and Head, 1999; Muñoz et al., 2011; Dobesova et al., 2012; Langford et al., 2013)

- -Hemothorax (fractured ribs, lacerated heart or vessels, neoplasia, coagulopathy….) (Perkins et al., 1999; Hassel, 2007; Trigo et al., 2011).
- -Hematuria (pyelonephritis, cystitis, urolithiasis, urethral ulceration, coagulopathy, idiopathic, neoplasias,….) (Patterson-Kane et al., 2000; Vits et al., 2008; Aleman et al., 2011).
- -Hemoperitoneum (splenic or hepatic rupture, mesenteric or uterine vessel rupture, ovarian neoplasias, verminous arteritis, neoplasia….) (Pusterla et al., 2005; Conwell et al., 2010; Pauwels et al., 2012).
- -Gastrointestinal conditions (ulcerations, non-steroidal anti-inflammatory drug toxicity, parasites, granulomatous intestinal disease, neoplasia as squamous cell carcinoma or lymphoma…) (Welch et al., 1992; Schumacher et al., 2000; Taylor et al., 2006; 2009; Brooks, 2008; Cohen et al., 2008).
- -External conditions (trauma, surgical complication, coagulopathy or external parasites) (Welch et al., 1992; Brooks, 2008).

2 Hemolysis, which could be intravascular and/or extravascular

- Hemolysis caused by infectious and parasitic diseases, such as anemia associated with piroplasmosis (*Theileria equi or Babesia caballi*), clostridiosis and equine infectious anemia (Weiss and Moritz, 2003; Muñoz et al., 2013b).
- Hemolysis caused by oxidative injury, in cases of phenothiazine, onion, garlic and red maple leaf toxicosis (Pearson et al., 2005; Alward et al., 2006).
- Immune-mediated hemolytic anemia (IMHA), which could be primary or secondary. The primary IMHA appears in the neonatal isoerythrolysis and incompatible blood transfusions (Boyle et al., 2005; Polkes et al., 2008). Secondary IMHA has been described in association with bacterial infections (*Clostridium perfringens*, streptococcal infections), viral infections (equine infectious anemia), neoplasias (lymphoma)… (Weiss and Moritz, 2003; McGovern et al., 2011).
- Hemolysis caused by iatrogenic conditions (hypotonic solutions, ionophores, trimethoprim-sulphamethoxazole, human erythropoietin, penicillin….), anemia secondary to systemic therapy with organophosphorus compounds, deficiency in glucose-6-P dehydrogenase…(McConnico et al., 1992; Piercy et al., 1998; Thomas and Livesey, 1998; Harvey, 2006).
- Hemolysis caused by miscellaneous conditions (hepatic disease, hemolytic uremic syndrome, disseminated intravascular coagulation…) (Dolente et al., 2002; Dickinson et al., 2008; Ankringa et al., 2012).
- Microangiopathic hemolysis

Acute blood losses are related with disseminated intravascular coagulation, trauma and surgery, angiopathic, plasmopatic and trombopatic hemorrhagic diathesis, rodenticide poisoning, guttural pouch mycosis, equine purpura hemorrhagic and progressive ethmoid hematoma. Chronic blood loss are related to the digestive system: parasitism, neoplasms and gastric ulcers, losses on the respiratory system (tumors and pulmonary hemorrhage) and blood losses on the genitourinary system (cystitis, urolithiasis, bladder tumors, etc) (Lassen and Swardson, 1995; Morris, 1998; Dixon and Head, 1999; Perkins et al., 1999; Schumacher

et al., 2000; Pusterla et al., 2005; Taylor et al., 2006; 2009; Brooks, 2008; Vits et al., 2008; Conwell et al., 2010; Muñoz et al., 2011; Trigo et al., 2011; Dobesova et al., 2012).

Internal hemorrhage (into body cavities) permits the body to reuse blood components. Approximately two thirds of the erythrocytes lost into the abdomen or thorax are auto transfused back into the circulation within 24 to 72 hours. The other one third of erythrocytes is lysed or phagocytized and the iron and protein are reused. Accelerated bone marrow erythropoiesis is usually evident by 3 days after acute hemorrhage and is maximal by 7 days (Sellon, 2004).

Initially, RBC count appears normal because all blood components have been lost in equal volumes. Physiologic compensatory mechanisms induce redistribution of interstitial fluid into the vasculature and decreased RBC and TPP in peripheral blood. This redistribution of interstitial fluid into the vasculature 24 hours after acute hemorrhage makes impossible to assess the severity of the blood loss. In addition, many erythrocytes stored in the spleen can be released into the circulation as a consequence of endogenous catecholamine release. After vascular equilibration, hematologic parameters should reveal a decrease in PCV, RBC and HB without changes in RBC indices (MCV, MCH and MCHC). This type of anemia usually is accompanied by panhypoproteinemia through proteins loss. A neutrophilic leukocytosis is apparent by 3 hours after hemorrhage and PLT may increase if have not been consumed by excessive coagulation (Sellon, 2004).

Regarding to hemolytic anemias, they can be produced by intravascular or extravascular causes. During intravascular hemolysis, HB released from destroyed erythrocytes combines with plasma haptoglobin, and the tissue mononuclear phagocytes remove the haptoglobin-hemoglobin complex. As a consequence, plasma haptoglobin levels decrease as intravascular hemolysis increases (Pearson et al., 2005). When plasma haptoglobin binding is exceeded, free HB accumulates in the plasma and is eliminated by kidneys. Thus, intravascular hemolysis is characterized by hemoglobinemia and hemoglobinuria. Hemolysis induces a more regenerative response than blood loss (Sellon, 2004).

IMHA (Immune Mediated Hemolytic Anemia) develops by type II hypersensivity mechanisms with antibodies that attach to the surface of RBCs. Primary IMHA is an autoimmune process in which antibodies act directly against the membrane surface antigens of RBC. Secondary IMHA is more common than autoimmune disease. Antibodies attach to the surface of erythrocyte for one or more reason: 1.-Alterations in the RBC membrane produced by a primary viral, bacterial or neoplastic process (Weiss and Moritz, 2003; Johns et al., 2011; McGovern et al., 2011); 2.-Antigen-antibodies complex deposition on the surface of RBCs; 3.-Drugs that cause immunoproteins to react indirectly with RBCs. Drug-induced immune-mediated hemolysis may occur via three mechanisms: 1.-The drug might combine with RBC membranes and might be recognized as foreign by the body. An antibody to this new antigen develops and destroys the drug-coated RBC. These animals have a positive direct Coombs´ test; 2.-The drug can bind to a carrier molecule in the blood and induce an immune response and the drug-carrier complex-antibody binds erythrocyte membranes in a process mediated by the complement leading to hemolysis; 3.-Drug may induce true autoantibody production and causes RBC destruction (Piercy et al., 1998; Thomas and Livesey, 1998).

Antibodies-coated RBCs are unable to pass through the microcirculation of the spleen, become sequestered and destroyed or phagocytized. If RBC membrane is lost in excess, spherocytes with an increase of osmotic fragility can be formed. Most of the IMHAs are

extravascular, but if the antibody fixes and activates complement, intravascular complement-mediated hemolysis may result (Sellon, 2004).

The types and causes of non-regenerative anemia described in equines are the following:

1 Iron deficiency (chronic hemorrhage, nutritional deficiency….) (Fleming et al., 2006; Prins et al., 2009).
2 Anemia of chronic disease (chronic infection / inflammation: pleuritis, pneumonia, peritonitis, enteritis, bacterial endocarditis, internal abscessation, chronic viral disease (equine infectious anemia)….This is one of the most common cause of anemia in horses (Schumacher et al., 2000; Borges et al., 2007; Pritchard et al., 2009; Taylor et al., 2009; Muñoz et al., 2010a; 2012).
3 Bone marrow failure (myelophthisis, myeloproliferative disease, bone marrow toxins: phenylbutazone, chloranphenicol…), radiation, Standardbred horse family hypoplasia, idiopathic pancytopenia….(Meyer et al., 2006; Kelton et al., 2008; Muñoz et al., 2009; Forbes et al., 2011)
4 Miscellaneous conditions (chronic hepatic o renal diseases, endocrine diseases….) (Messer and Johnson, 2007).

Dietary factors as folic acid or cobalamin (vitamin B12) deficiencies are rare, but severe protein deprivation and iron deficiency by chronic external blood loss and chronic disease may result in decreased erythropoiesis in horses. The mechanisms implicated in anemia of chronic diseases and bone marrow failure are selective erythroid hypoplasia, block of iron release from reticuloendothelial storage (ferritin and hemosiderin) resulting in an unavailability of iron for heme synthesis and production of antibodies that cross-react with EPO, that can interfere with erythropoiesis, among others (Sellon, 2004).

2.2. Hemoglobin Concentration and Packed Cell Volume-Hematocrit

The HB concentration is a parameter that, in isolation, has no clinical significance, so it should be evaluated together with the PCV (Messer, 1995). PCV measurement in the horse is especially useful, and in conjunction with TPP allows the assessment of the patient's hydration status (Muñoz et al., 2010b,c; Trigo et al., 2010).

Usually both parameters, PCV and TPP develop parallel. However, there are many clinical situations in which there is a disparity between the two parameters. Such situations can be considered as follows (Morris, 1998; Lording, 2008):

- ✓ An increase in the PCV value in the case of spleen contraction without simultaneous TPP elevations. This clinical scenario can be found in horses with pain or excitation (Trigo et al., 2010).
- ✓ Clinical situations with loss of proteins (enteritis, colitis, renal disease with proteinuria, hepatic failure). In this case, it is possible to find hypoproteinemia with normal PCV or normal proteinemia with increased PCV due to fluid losses that often accompany some of the above mentioned disorders (Reed et al., 2006; Metcalfe et al., 2013).

- ✓ Intravascular hemolytic anemia with a decrease of PCV and depending on the etiology, hypoproteinemia, hyperproteinemia or normal TPP (Weiss and Moritz, 2003).
- ✓ A reduction in TPP and PCV in hemorrhagic processes (Galvin et al., 2004; Pusterla et al., 2005).

Decreases in PCV have been reported as a consistent finding in horses with viral respiratory tract disease and in gastric ulcers (McGowan, 2008).

2.3. Erythrocyte Indices

The three red cell indices, MCV, MCH and MCHC are particularly useful in assessing and categorizing anemic processes. Clinically, the most used are the MCV and MCHC, reporting the average size of the red cells and the amount of HB that have respectively (Messer, 1995, Morris, 1998). In relation to MCV, erythrocytes can be classified as normal or normocytic, smaller than normal or microcytic or larger than normal or macrocytic. According to the MCHC, erythrocytes are classified as normochromic, if they have a normal amount of HB, or hypochromic, if the amount of hemoglobin is below normal (Lording, 2008). Normocytic normochromic anemia accompanies many chronic systemic diseases, including renal and hepatic failure, endocrine abnormalities, neoplastic conditions and chronic infection (Monteith and Cole, 1995; Saulez et al., 2004; Valdes and Johnson, 2005; Muñoz et al., 2010a; Aleman et al., 2011). Microcytic hypochromic anemia (low MCV and MCHC) is associated with iron deficiency (Fleming et al., 2006). Macrocytic anemia (increased MCV) occasionally occurs in horses after a severe hemolytic or hemorrhagic crisis and less commonly, in other regenerative causes of anemia (Sellon, 2004).

3. INTERPRETATION OF QUANTITATIVE VARIATIONS IN THE EQUINE LEUKOGRAM

The reference values for the leukogram in horses are presented in table 3.

Table 3. Reference values for the leukocyte parameters in adult healthy horses, in absolute values and in percentage (modified from Lassen and Swardson, 1995; Satué et al., 2009; 2010; 2012; Muñoz et al., 2010a) (W*BC, white blood cells*)

PARAMETERS	UNITS	REFERENCE RANGE
WBC	$10^3/\mu L$	6,000-14,300
BAND NEUTROPHILS	$10^3/\mu L$ (%)	0-0.100 (0-8)
SEGMENTED NEUTROPHILS	$10^3/\mu L$ (%)	2,500-7,500 (22-72)
TOTAL NEUTROPHILS	$10^3/\mu L$ (%)	2,500-7,500
LYMPHOCYTES	$10^3/\mu L$ (%)	2,500-5,000 (17-68)
MONOCYTES	$10^3/\mu L$ (%)	0,000-1,000 (0-14)
EOSINOPHILS	$10^3/\mu L$ (%)	0,000-0,500 (0-10)
BASOPHILS	$10^3/\mu L$ (%)	0,000- (0-4)

3.1. Neutrophils

3.1.1. Neutrophilia

The neutrophilia or increased number of circulating neutrophils, can occur in physiological (stress response, spleen contraction) and pathological situations (inflammation, infection, neoplasia). Endogenous or exogenous glucocorticoids or epinephrine, excitement, exercise or stress may result in neutrophilia (Rossdale et al., 1982; Trigo et al., 2010). In main lines, neutrophilia is considered when the number of neutrophils exceeds 6.000/mm^3 of blood. A deviation of the left denotes a value greater than 300/mm^3 of immature forms (Grondin and Dewitt, 2010).

Clinical neutrophilia occurs when the output of the neutrophils formed in the bone exceeds migration into tissues. In this circumstance, the total pool of neutrophils undergoes an expansion and the half-life of circulating population is normal or slightly increased. The magnitude of the neutrophilia is higher in infectious processes localized, with abscess formation, compared to widespread inflammatory disorders (Mair and Hillyer, 1992; Mair and Brown, 1993; Saulez et al., 2004; White et al., 2009; Watts et al., 2011; Muñoz et al., 2010a; 2012; Viu et al., 2012).

The presence and magnitude of the deviation to the left depends on three factors: 1. -Number of neutrophils in the bone marrow pool, 2. -Release from the bone marrow and 3. -The flow velocity of neutrophils from the mitotic compartment of the bone marrow towards the non-mitotic compartment of maturation and storage. Therefore, the severity of the condition is reflected in the intensity of the left shift and the presence of toxic changes in neutrophils (Latimer, 1999; Hollbrook et al., 2003; Grondin and Dewitt, 2010).

In some horses, neutrophilia can be observed but the diagnosis of the affected tissue can be tricky. Under these clinical conditions it should be excluded widespread affection of dermal tissues, gastrointestinal and genitourinary systems, joints, tissue planes or hidden abscesses (White et al., 2009; Watts et al., 2011). On the other hand, we must bear in mind that hemorrhagic and hemolytic anemias present with neutrophilia (Dickinson et al., 2008; Patton et al., 2009).

The convalescence of a pathological condition is characterized by a decrease in the intensity of the shift to the left, because the production of neutrophils from the bone marrow fills the non-mitotic pool. Furthermore, the reduction in tissue demands promotes regression toward baseline values. The persistence of neutrophilic leukocytosis with a minimum deviation to the left reflects the existence of a chronic suppurative disease, usually associated with non-regenerative anemia of chronic disease and hyperfibrinogenemia (Torkelson, 2002; Porter et al., 2008; Wong et al., 2008; Grondin and Dewitt, 2010; Muñoz et al., 2010a; 2012).

3.1.2. Neutropenia

Reduced number of neutrophils in peripheral blood, below 3.000/mm^3, also called neutropenia, is a consequence of certain pathological processes. Neutropenia can derive from the individual or simultaneous actuation of one of these three mechanisms: defective production in bone marrow, temporary exchange between the marginal and peripheral pools, and faster migration to the tissues, with a speed greater than the ability of the bone of replacing those cells. Always bear in mind that the neutropenia is a serious clinical problem that can result from a severe bacterial process (Latimer, 1999).

Reduced neutrophil production in the bone marrow has been reported in horses and ponies. Exposure to ionizing radiation and chemotherapy might result in severe neutropenia (Taintor and Schleis, 2011). In bone marrow diseases, the neutropenia is a manifestation of pancytopenia, but the etiology is often difficult to establish, despite the comprehensive assessment of medical record, physical examination, laboratory diagnosis and even pathological study. Furthermore, neutropenia secondary to core mass reduction is rare, but can be seen in myelophthisis (Kelton et al., 2008; Latimer, 1999; Grondin and Dewitt, 2010), immune-mediated neutropenia and myeloproliferative disorders, including granulocytic leukemia, leading to chronic neutropenia (Mair and Hillyer, 1992; Monteith and Cole, 1995; Sellon, 2004; Muñoz et al., 2009). Although medullary destruction and pancytopenia are found in cattle after mastitis and metritis, this fact has not been reported in horses (Weiss and Miller, 1985). However neutropenia arising from medullary destruction must be considered in any equine patient, after excluding other causes of neutropenia, especially if there is a concomitant sepsis of severe intensity (Latimer, 1999; Grondin and Dewitt, 2010).

Medullary space obliteration appears secondary to stromal reactions or infiltrative diseases, even though neutropenia is accompanied by pancytopenia. Stromal reactions include myelofibrosis or replacement of normal bone marrow cells by fibrous connective tissue and osteopetrosis or osteoid deposition. The most common infiltrative diseases are disseminated granulomatous inflammation and cancer (Latimer and Meyer, 1989). In horses, most of myelophthisic processes with secondary neutropenia are attributed to neoplastic conditions, mainly lymphoma and leukemia (Latimer, 1999; Kelton et al., 2008; Ringer et al., 2008; Taintor and Schleis, 2011).

The temporary exchange between the circulating and marginal pools is reflected in the leukogram by neutropenia, although the total amount of blood neutrophils may remain unchanged. This mechanism together with increased tissue demand, are the primary considerations in the differential diagnosis of neutropenia in horses. This happens mainly in cases of septicemia in neonate foals and endotoxemia in adult horses, in a variety of gastrointestinal disorders such as right dorsal colitis syndrome, strangulating obstruction, peritonitis, enteritis and salmonellosis (Schumacher et al., 2000; Davis and Jones, 2003; Sellon, 2004; Johns et al., 2006; Poulin Braim et al., 2009; Braumwart et al., 2011). In these cases, the presence of neutropenia suggests a diminished lifetime of the neutrophils and a migration from blood vessels into the tissues severely damaged. Intravascular or intrasinusoidal destruction or sequestration of these cells is found (Latimer, 1999). In addition, neutropenia below 1.500/mm^3, is a common finding in hyperadrenocorticism, by administration of exogenous corticosteroids or acute infections of diverse etiology, as bacterial, rickettsial and viral infections, associated with left deviation, toxic cellular degeneration and leukocyte changes (Skowronek et al., 1995; Latimer, 1999; Franzén et al., 2005; Harless and Pusterla, 2006; Siska et al., 2013).

3.2. Lymphocytes

Lymphocytosis, an increased number of circulating lymphocytes, implies an elevation above 5.000 cells/mm^3 and is attributed to physiological stress, chronic antigenic stimulation or lymphoid malignancies. As mentioned above, physiological lymphocytosis comes from sympathetic increased release of hormones, mainly adrenaline, whose release induces an

increase in circulating lymphocytes up to 14.000 cells/mm^3 (Rossdale et al., 1982; Muñoz et al., 2010a).

Moreover, chronic infections and inflammatory lesions occur with lymphocytosis. Typical examples of these circumstances are bacterial infections and alterations following immunization (Mair et al., 1989; James et al., 2010). This increase in the number of lymphocytes is related to the presence of immune cells in blood smears. Lymphoid neoplasms are not a frequent cause of lymphocytosis in horses. However, it can be seen in lymphoma in the leukemic phase, in lymphocytic leukemia and in multiple myeloma or plasmocytoma (Mair, 1996; McConkey et al., 2000; Meyer et al., 2006; Muñoz et al., 2009; 2013b; Mendes et al., 2011).

Lymphopenia is associated with glucocorticoid administration, stress and *Anaplasma* infections. It is also a common finding in the early days of viral infections, such as herpes virus type I (HV-1) (Rossdale et al., 1982; Van der Kolk, 1997; Nolen-Walston et al., 2004; Franzén et al., 2005; Dynon et al., 2007; Muñoz et al., 2010a). Other causes to consider in the differential diagnosis of lymphopenia are immunodeficiency such as combined immunodeficiency syndrome in the Arabian foal (Latimer, 1999; Jelinek et al., 2006; Grondin and Dewitt, 2010).

3.3. Eosinophils

Eosinophilia, defined as the increase in the number of eosinophils over 800 cells/mm3, suggests an antigen-antibody interaction in tissues in which there is a large number of mastocytes, such as skin and lung, or a parasitic problem that produces sensitization. Foals do not have circulating eosinophils and display a substantial increase by the age of 3 months. It is assumed that this fact results from exposure to parasites or other antigens. The chance of eosinophilia becomes more likely and its intensity is more marked in cases of nematodosis, due to the necessity of tissue migration to complete its life cycle (Latimer, 1999).

Furthermore, the inflammation in the skin, gastrointestinal and genitourinary tracts may cause eosinophilia secondary to mastocyte cell degranulation (Gibson and Alders, 1987). However, it seems paradoxical that many of these diseases may appear with a severe eosinophilic tissue infiltration without concomitant presence of peripheral eosinophilia (Pass and Bolton, 1982). The increased presence of these cells in the damaged tissue is associated with the degranulation of basophils or mast cells that release chemotactic factors derived from complement, with the synthesis of vasoactive amines and cytokines and immune complex deposition within tissues (Sellon, 2004).

Peripheral eosinophilia most commonly results from allergic reactions, parasitic infections, including habronemosis, strongylosis and pediculosis (Browns et al., 2008; Down et al., 2009). Eosinophilic myeloproliferative leukemia is uncommon, although it has been reported in a horse (Morris et al., 1984). A marked eosinophilia also has been seen in horses with lymphoma and transitional cell carcinoma (Sellon, 2004). Eosinophilia is also found in the equine multisystemic eosinophilic epitheliotropic disease (McCue et al., 2003; Swain et al., 2003; Singh et al., 2006). Eosinopenia or reduction in the number of circulating eosinophils, is difficult to evaluate in horses because leukograms of clinically normal animals contain small amounts of this type of cell. Nevertheless, eosinopenia has been described in association with active infectious or inflammatory processes as well as in response to

increasing concentrations of endogenous or exogenous corticosteroid and stress (Rossdale et al., 1982; Grondin and Dewitt, 2010).

3.4. Monocytes

Monocytosis or increased peripheral monocyte concentration appears with intense phagocytosis processes as tissue necrosis, intravascular hemolysis or chronic suppurative diseases (Kaplan and Moore, 1996; Muñoz et al., 2010a). Monocytosis is often seen in the post-acute or recovery phase following a viral infection (Mair, 1996), in myelomonocytic and granulocytic myeloproliferative disorders and other types of leukemias (Reppas and Canfield, 1996; McCue et al., 2003; Tan et al., 2007; Muñoz et al., 2009). The monocytopenia or decrease in the number of circulating monocytes has no clinical relevance (Sellon, 2004; Grondin and Dewitt, 2010).

3.5. Basophils

The increased number of blood basophils, basophilia, is difficult to assess, since these cells are few times in the circulation. However, the most common causes of basophilia are hypersensitivity reactions, hyperlipemia, allergic dermatitis, and inflammatory or neoplastic diseases. Basophilia has been reported in a horse with basophilic enterocolitis (Pass et al., 1984). A summary of the quantitative alterations of WBC is shown in tables 4 and 5.

Table 4. Summary of the main etiologies of quantitative alterations of equine white polymorphonuclear cells (neutrophils, eosinophils and basophils)

ALTERATION	MECHANISMS	ETIOLOGY
NEUTROPHILS		
Neutrophilia	Release of catecholamines	Stress, spleen contraction, pheochromocytoma
	Increased cortisol concentrations	Exogenous administration, chronic stressful situations, hyperadrenocorticism
	Release from bone marrow and mobilization from marginal pool	Infectious and non-infectious inflammatory disorders
Neutropenia	Defective production in bone marrow	Exposure to ionizing radiation, chemotherapy, bone marrow diseases
	Temporary exchange between marginal and peripheral pools	Septicemia, endotoxemia
	Faster migration to injured tissues	Bacterial, rickettial and virus infections, non-septic inflammation
EOSINOPHILS		
Eosinophilia		Hypersensitivity reactions, parasitic infections, eosinophilia leukemia, multisystemic eosinophilic epitheliotropic disease
Eosinopenia	Increased cortisol concentrations	Exogenous administration, chronic stressful situations, hyperadrenocorticism
BASOPHILS		
Basophilia		Hypersensitivity reactions, parasitic infections, inflammatory and neoplasias

Table 5. Summary of the main etiologies of quantitative alterations of lymphocytes and monocytes in horses

ALTERATION	MECHANISMS	ETIOLOGY
LYMPHOCYTES		
Lymphocytosis	Spleen contraction	Stress
	Chronic antigenic stimulation	Immunization, chronic bacterial infections
	Lymphoid neoplasias	Lymphoma (leukemic phase), lymphocytic leukemia, multiple myeloma or plasmacytoma
Lymphopenia	Increased cortisol concentrations	Exogenous administration, chronic stressful situations, hyperadrenocorticism
		Onset of viral infections
		Combined immunodeficiency syndrome
MONOCYTES		
Monocytosis	Intense phagocytic and necrotic processes	Intravascular hemolysis Chronic suppurative diseases Abscess
		Recuperation after a viral insult
		Myeloproliferative neoplasia

4. INTERPRETATION OF THE QUANTITATIVE VARIATIONS OF PLATELETS

Quantitative changes in platelet numbers include thrombocytosis and thrombocytopenia. Thrombocytosis can be physiological or pathological. Physiological thrombocytosis is produced by the mobilization of the platelets from the spleen and from other non-splenic compartments as lung or bone marrow, in response to exercise or excitement (Messer, 1995; Morris, 1998).

Pathologic thrombocytosis can be primary, when derived from bone marrow disease, or secondary when derived from fractures, surgery or infectious and inflammatory diseases or follows acute hemorrhage (Messer, 1995; Sellon et al., 1997; Morris, 1998; Sellon, 2004).

Thrombocytopenia is defined as a peripheral platelet count lesser than 100,000 platelets/μl. Clinical signs of thrombocytopenia in the horse reflect abnormal primary hemostasis and include petechial and ecchimotic hemorrhages of mucous membranes, epitasis or increased bleeding after venipuncture (Humber et al., 1991). Adequate sampling and platelet count are essential before a diagnosis of true thrombocytopenia in horses (Sellon et al., 1997). The platelets of some horses form aggregates in EDTA, resulting in an inaccurate count or pseudothrombocytopenia (Hinchcliff et al., 1993). Blood smear examination or sodium citrate anticoagulant may help distinguish true thrombocytopenia from pseudothrombocytopenia in the horse (Sellon, 2004).

Thrombocytopenia might result from various mechanisms, such as reduction of thrombocytopoiesis, increased peripheral destruction of platelets, increased consumption, platelet sequestration in the spleen and idiopathic origin (Sellon, 1998). The main causes of thrombocytopenia in horses are those:

A. Reduced thrombocytopoiesis. Any disease and/or medication that affect the bone marrow function might originate thrombocytopenia, sometimes accompanied by anemia and leukopenia. Diseases of the bone marrow with thrombocytopenia in horses are: hereditary defects, myelophthisis, myelofibrosis, myelodysplasia and idiopathic medullary aplasia. Other causes of reduced thrombocytopoiesis are the administration of myelosupressive drugs as, phenylbutazone, chloramphenicol, estrogens and trichloroethylene extracted soybean meal and irradiation (Edwards et al., 1993; Sellon et al., 1996; Ringger et al., 1997; Muñoz et al., 2009; 2011).
B. Increased peripheral destruction of platelets. It occurs in primary and secondary immune-mediated thrombocytopenia. In a primary immune-mediated thrombocytopenia, there is an increased production of antibodies against platelet membrane antigens. As a consequence, platelets are removed by the mononuclear phagocyte system in liver, spleen and bone marrow (autoimmune disease; systemic lupus erythrematosus or idiopathic) (Crawford et al., 1996; Buechner-Maxwell et al., 1997). The secondary immune-mediated thrombocytopenia is due to nonspecific binding of circulating immune complexes to platelet surface receptors. In the horse, it has been described associated with viral infections (herpes, influenza, African horse sickness, equine infectious anemia, equine viral arteritis), bacterial infections (sepsis neonatal, *Anaplasma phagocytophilum*), neoplasias (lymphoma) and drugs (penicillin) or toxins (Reef et al., 1984; Humber et al., 1991; Edens et al., 1993; Kimberley et al., 2004).
C. Increased consumption and loss of platelets. This mechanism is associated with bleeding, disseminated intravascular coagulation and localized activation of coagulative and fibrinolytic processes as occurs in vasculitis (purpura hemorrhagic equine), in vascular neoplasia (hemangiosarcoma) and renal diseases (hemolytic uremic syndrome) (Welch et al., 1992; Southwood et al., 2000; Dolente et al., 2002; Monreal et al., 2000; Dallap et al., 2003; Johns et al., 2005).
D. Sequestration in the spleen. This mechanism is associated with the great storage capacity of platelets in the spleen and anti-megakaryocyte antibody production, especially in cases of splenomegaly. In human and in small animal medicine, splenic diseases are associated with thrombocytopenia. This circumstance is much less common in horses.

5. Interpretation of Plasma Protein Concentrations in the Horse

Although plasma/serum protein concentrations are not a part of the hemogram, their assessment is of great important for an accurate interpretation of RBC, HB and PCV (Eckersall, 2008). The variations of total plasma protein (TPP) are associated with the changes in their fractions, i.e., albumin, globulin and fibrinogen. Reference values for total plasma proteins and fractions are presented in table 6 for horses.

Table 6. Reference values for total plasma proteins and fractions in adult healthy horses (modified from Lassen and Swardson, 1995; Muñoz et al., 2010a)

PARAMETERS	UNITS	REFERENCE RANGE
TOTAL PLASMA PROTEINS	g/dL	6-7.5
ALBUMIN	g/dL	2.5-3.5
FIBRINOGEN	mg/dL	200-500
GLOBULINS	g/dL	2.1-3.8
α1-GLOBULINS	g/dL	0.06-0.7
α2-GLOBULINS	g/dL	0.31-1.31
β-GLOBULINS	g/dL	0.69-2.47
Ɣ-GLOBULINS	g/dL	0.55-1.90

5.1. Albumin

Hyperalbuminemia does not derive from an excessive production of albumin. On the contrary, it is a secondary process to dehydration or laboratorial error in cases of lipemia or hemolysis.

However, this last cause depends on the analytical procedure (Muñoz et al., 2010b,c; Trigo et al., 2010).

Hypoalbuminemia is a common laboratorial finding in equine medicine and it is associated with the following pathological conditions:

- Secondary to hemodilution, after excessive fluid therapy.
- Reduced synthesis in chronic states of malnutrition, liver diseases or acute phase reaction. Although in liver disease, a reduced albumin concentration is expected, it is not a common finding, because of the prolonged half-life of this protein in the blood (Parraga et al., 1995; Durham et al., 2003; Camus et al., 2010; Muñoz et al., 2010a). In addition, it is well known a release of cytokines appears in inflammatory, infectious, neoplastic and traumatic diseases. Increased cytokines production leads to a down-regulation in the synthesis of albumin in the liver. Therefore, albumin is a negative acute-phase protein (Patterson et al., 1988; Jacobsen et al., 2009).
- Increased loss of albumin in glomerulopathies (nephrotic syndrome), protein-losing enteropathies, severe bleeding, and scald burns... (Kemper et al., 2000; Mcsloy et al., 2007; McConnico et al., 2008; Aleman et al., 2011).
- Sequestration of albumin in body cavities, as in pleuropneumonia or peritonitis (Patterson-Kane et al., 2001; McConnico et al., 2008).
- Increased protein catabolism in cases of negative energy balance, such as fever, trauma, surgery and neoplasias.

Table 7. Summary of the etiologies of altered albumin concentrations in horses

ALTERATION	MECHANISMS	ETIOLOGIES
Hyperalbuminemia	Decreased plasma volume (panhyperproteinemia)	Dehydration
	Laboratory error	
Hypoalbuminemia	Hemodilution (panhypoproteinemia)	Excessive fluid therapy. Congestive diseases (congestive heart failure)
	Reduced hepatic synthesis	Chronic malnutrition, chronic liver failure, acute phase reaction
	Increased albumin loss	Glomerulopathy, protein-losing enteropathies, bleeding, burns, accumulation into cavities
	Increased catabolism	Fever, trauma, surgery, neoplasia

The alterations found in albumin concentrations in equine medicine are presented in table 7.

5.2. Globulins

Globulins are divided into three fractions, according to their electrophoretic mobility, α, β and δ globulins. The α globulins are subdivided into subunits α-1 and α-2. Globulins α-1 include α-1 antitrypsin, the α-1-antichymotrypsin, the prosomucoide (acid glycoprotein), serum amyloid A and lipoprotein α-1 (HDL). Globulins α-2 include α-2 macroglobulin (protease inhibitor), haptoglobin (binds to free hemoglobin), protein C inhibitor (clotting factors VIII and V on), ceruloplasmin (copper transporter) and lipoprotein α-2. Likewise, β globulins are divided into β1 and β-2 subfractions. Within β1 globulins, one can distinguish transferrin (iron-binding) and hemopexin. β-2 globulins include complement factor 3, C-reactive protein, plasminogen, β-2 lipoprotein (LDL), β-2-microglobulin, portion of IgA and IgM. Fibrinogen also migrates to this region. Thirdly, δ-globulins include immunoglobulins IgM, IgA and IgG (Eckersall, 2008; Flaminio et al., 2009).

Clinical hypoglobulinemia is related to the decline of δ-globulins, since the reduction of the other two fractions is not clinically significant. Hypoglobulinemia is found in cases of hypoproteinemia. Furthermore, the reduction of δ-globulin may be due to hereditary factors such as combined primary immunodeficiency, pony's agammaglobulinemia, selective IgM deficiency, transitional hypogammaglobulinemia or acquired conditions, such as failure of passive transfer of immunity and neoplastic processes (Perryman, 2000; Crisman et al., 2008; Eckersall, 2008; Flaminio et al., 2009).

Hyperglobulinemia derives from the increase of one or more fractions of the globulins. The increase of α-globulins indicates an acute phase protein response or appears as a laboratory finding in some cases of nephrotic syndrome. The β-globulins are increased in acute and chronic inflammatory processes, liver diseases, suppurative skin diseases and nephrotic syndrome. Finally, an elevation of the δ-globulin concentrations is found in those neoplastic processes that affect plasma cells and lymphocytes, such as multiple myeloma, lymphocytic leukemia and lymphoma. In these cases, an isolated type of immunoglobulin is synthesized, leading to a monoclonal gammopathy (McConkey et al., 2000; Barton et al., 2004; Pusterla et al., 2004; Kim et al., 2005; Muñoz et al., 2009; 2013a).

Additionally, a δ-hyperglobulinemia might represent an exaggerated active immune response against antigenic stimulation, as happens in chronic inflammation, in chronic liver disease, liver abscesses and highly suppurative diseases. In these cases, several types of δ globulins could be increased (polyclonal gammopathy) (Crisman et al., 2008).

The main alterations in globulin concentrations in equine medicine are summarized in table 8.

Table 8. Summary of alterations of globulins in horses

ALTERATION	MECHANISM	ETIOLOGY
Hypoglobulinemia		
α	Panhypoproteinemia	
β	Panhypoproteinemia	
γ	Hereditary immunodeficiencies	Combined immunodeficiency, Agammaglobulinemia, selective IgM deficiency
	Acquired immunodeficiencies	Failure of passive transfer of immunity, neoplasias
Hyperglobulinemia		
α	Acute phase response, nephrotic syndrome	
β	Acute and chronic inflammatory disorders, liver disease, suppurative skin disease, nephrotic syndrome	
γ	Neoplastic conditions affecting lymphocytes and plasma cells	Multiple myeloma, lymphocytic leukemia, lymphoma
	Increased immune response	Persistent inflammations, chronic liver disease, abscess

5.3. Fibrinogen

Fibrinogen is a high molecular weight molecule produced by the liver. Its main function is to act as a substrate for thrombin to form fibrin during hemostasis. Moreover, fibrinogen is an acute-phase protein, and therefore, in the horse is a marker of inflammatory response (Crisman et al., 2008).

Hyperfibrinogenemia suggests inflammatory processes, although the concentration of fibrinogen is not directly correlated with the severity of the disease. Fibrinogen is considered a 'minor' acute phase protein, with small increases in response to an inflammatory insult. Nevertheless, it is commonly used in equine medicine, because the technique to measure is easy and inexpensive (Borges et al., 2007; Jacobsen et al., 2009).

Hypofibrinogenemia may be due to a reduced synthesis, in liver dysfunction or to an increased consumption, in coagulopathies, such as disseminated intravascular coagulation (Dallap et al., 2003; 2009; Stokol et al., 2005).

REFERENCES

Aleman, M., Nieto, J.E., Higgins, J.K., 2011. Ulcerative cystitis associated with phenylbutazone administration in two horses. *J. Am. Vet. Med. Assoc*. 239, 499-503.

Alward, A., Corriher, C.A., Barton, M.H., Sellon, D.C., Blikslager, A.T., Jones, S.L., 2006. Red maple (Acer rubrum) leaf toxicosis in horses: a retrospective study of 32 cases. *J. Vet. Intern. Med*. 20, 1197-1201.

Ankringa, N., Wijnberg, I.D., Boerman, S., Ijzer, J., 2012. Copper-associated hepatic cirrhosis in a Friesian horse. *Tijdschr. Diergeneeskd.* 137, 310-314.

Axon, J.E., Russell, C.M., Begg, A.P., Adkins, A.R., 2008. Erythrocytosis and pleural effusion associated with a hepatoblastoma in a Thoroughbred yearling. *Aust. Vet. J*. 86, 329-333.

Barton, M.H., Sharma, P., LeRoy, B.E., Howerth, E.W., 2004. Hypercalcemia and high serum parathyroid hormone-related protein concentration in a horse with multiple myeloma. *J. Am. Vet. Med. Assoc*. 225, 409-413.

Belli, C.B., Baccarin, R.Y., Ida, K.K., Fernandes, W.R., 2011. Appropriate secondary absolute erythrocytosis in a horse. *Vet. Rec.* 169, 609.

Borges, A.S., Divers, T.J., Stokol, T., Mohammed, O.H., 2007. Serum iron and plasma fibrinogen concentrations as indicators of systemic inflammatory diseases in horses. *J. Vet. Intern. Med.* 21, 489-494.

Boyle, A.G., Magdesian, K.G., Ruby, R.E., 2005. Neonatal isoerythrolysis in horse foals and a mule foal: 18 cases (1988-2003). *J. Am. Vet. Med. Assoc*. 227, 1276-1283.

Braumwart, C.A., Doherty, T.J., Schumacher, J., Willis, R.S., Adair, H.S. III, Rohrbach, B.W., 2011. Effects of hyperbaric oxygen treatment on horses with experimentally induced endotoxemia. *Am. J. Vet. Res*. 72, 1266-1275.

Brooks, M.B., 2008. Equine coagulopathies. *Vet. Clin. North Am.: Equine Pract.* 24, 335-355.

Browns, H.M., Cuttino, E., LeRoy, B.E., 2008. A subcutaneous mass on the neck of a horse. *Vet. Clin. Pathol.* 36, 119-123.

Buechner-Maxwell, V., Scott, M.A., Godber, L., Kristensen, A., 1997. Neonatal alloimmune thrombocytopenia in a Quarter Horse foal. *J. Vet. Intern. Med*. 11, 304-308.

Burrows, G.C., Borchard, R.E., 1982. Experimental lead toxicosis in ponies: comparison of the effects of smelter effluent-contaminated hay and lead acetate. *Am. J. Vet. Res*. 43, 2129-2133.

Camus, M.S., Krimer, P.M., Leroy, B.E., Almy, F.S., 2010. Evaluation of the positive predictive value of serum protein electrophoresis beta-gamma bridging for hepatic disease in three domestic animal species. *Vet. Pathol.* 47, 1064-1070.

Cohen, N., Kent Carter, G., Mealey, R.H., Taylor, T.S., 2008. Medical management of right dorsal colitis in 5 horses: a retrospective study (1987-1993). *J. Vet. Intern. Med*. 9, 272-276.

Conwell, R.C., Hillyer, M.H., Mair, T.S., Pirie, R.S., Clegg, P.D., 2010. Haemoperitoneum in horses: a retrospective review of 54 cases. *Vet. Rec.* 167, 514-518.

Cooper, C., Sears, W., Bienzle, D., 2005. Reticulocyte changes after experimental anemia and erythropoietin treatment of horses. *J. Appl. Physiol.* 99, 915-921.

Crawford, T.B., Wardrop, K.J., Tornquist, S.J., Reilich, E., Meyer, K.M., McGuire, T.C., 1996. A primary production deficit in the thrombocytopenia of equine infectious anemia. *J. Virol*. 70, 7842-7850.

Crisman, M.V., Scarratt, W.K., Zimmerman, K.L., 2008. Blood protein and inflammation in the horse. *Vet. Clin. North Am. Equine Pract.* 24(2): 285-297.

Dallap Schaer, B.L., Epstein, K., 2009. Coagulopathy of the critically ill equine patient. *J. Vet. Emerg. Crit. Care* 19, 53-65.

Dallap, B.L., Dolente, B., Boston, R., 2003. Coagulation profiles in 27 horses with large colon volvulus. *J. Vet. Emerg. Crit. Care* 13, 215-225.

Davis, J.L., Jones, S.L., 2003. Suppurative cholangiohepatitis and enteritis in adult horses. *J. Vet. Intern. Med.* 17, 583-587.

Dickinson, C.E., Gould, D.H., Davidson, A.H., Avery, P.R., Legare, M.E., Hyatt, D.R., Debroy C, 2008. Hemolytic-uremic syndrome in a postpartum mare concurrent with encephalopathy in the neonatal foal. *J. Vet. Diagn. Invest.* 20, 239-242.

Dixon, M., Head, K.W., 1999. Equine nasal and paranasal sinus tumours: part 2. A contribution of 28 case reports. *Vet. J.* 157, 279-294.

Dobesova, O., Schwarz, B., Velde, K., Jahn, P., Zert, Z., Bezdekova, B., 2012. Guttural pouch mycosis in horses: a retrospective study of 28 cases. *Vet. Rec*. 171, 561. Epub ahead of print.

Dolente, B.A., Wilkins, P.A., Boston, R.C., 2002. Clinicopathologic evidence of disseminated intravascular coagulation in horses with acute colitis. *J. Am. Vet. Med. Assoc.* 220, 1034-1038.

Down, S.S., Hughes, I., Henson, F.M.D., 2009. Cutaneous habronemiasis in a 9-year-old Arab gelding in the United Kingdom. *Equine Vet. Educ*. 21, 4-8.

Durham, A.E., Newton, J.R., Smith, K.C., Hillyer, M.H., Hillyer, L.L., Smith, M.R., Carr, C.M., 2003. Retrospective analysis of historical, clinical, ultrasonographic, serum biochemical and haematological data in prognostic evaluation of equine liver disease. *Equine Vet. J*. 35, 542-547.

Dynon, K., Black, W.D., Ficorilli, N., Hartley, C.A., Studdert, M.J., 2007. Detection of viruses in nasal swab samples from horses with acute febrile, respiratory disease using virus isolation, polymerase chain reaction and serology. *Aust. Vet. J*. 85, 46-50.

Eckersall, P., 2008. Proteins, proteomics, and the dysproteinemias. In: *Clinical biochemistry of domestic animals.* Kaneko, J., Harvey, J., Bruss, M. (eds). Boston: Academic Press Elsevier, pp. 117-155.

Edens, L.M., Robertson, J.L., Feldman, B.F., 1993. Cholestasic hepatopathy, thrombocytopenia and lymphopenia associated with iron toxicity in a Thoroughbred gelding. *Equine Vet. J.* 25, 81-84.

Edwards, D.F., Parker, J.W., Wilkinson, J.E., Gayman Helman, R., 1993. Plasma cell myeloma in the horse. *J. Vet. Intern. Med.* 7, 169-176.

Flaminio, M.J.B.F., Tallmadge, R.L., Salles-Gomes, C.O.M., Matychak, M.B., 2009. Common variable immunodeficiency in horses is characterized by B cell depletion in primary and secondary lymphoid tissues. *J. Clin. Immunol.* 29, 107-116.

Fleming, K.A., Barton, M.H., Latimer, K.S., 2006. Iron deficiency anemia in a neonatal foal. *J. Vet. Intern. Med*. 20, 1495-1498.

Forbes, G., Feary, D.J., Savage, C.J., Nath, L., Church, S., Lording, P., 2011. Acute myeloid leukaemia (M6B: pure acute erythroid leukaemia) in a Thoroughbred foal. *Aust. Vet. J.* 89, 269-272.

Franzén, P., Aspan, A., Egenvall, A., Gunnarsson, A., Aberg, L., Pringle, J., 2005. Acute clinical, hematologic, serologic and polymerase chain reaction findings in horses experimentally infected with a European strain of Anaplasma phagocytophilum. J. Vet. *Intern. Med.* 19, 232-239.

Galvin, N., Dillon, H., McGovern, F., 2004. Right dorsal colitis in the horse: minireview and reports on three cases in Ireland. *Ir. Vet. J*. 57, 467-473.

Giardano, A., Rossi, G., Pieralisi, C., Paltrinieri, S., 2008. Evaluation of equine hemograms using the ADVIA 120 as compared with an impedance counter and manual differential count. *Vet. Clin. Pathol.* 37, 21-30.

Gibson, K.T., Alders, R.G., 1987. *Eosinophilic* enterocolitis and dermatitis in two horses. *Equine Vet J*. 19(3): 247–252.

Gold, J.R., Warren, A.L., French, T.W., Stokol, T., 2008. What is your diagnosis?. Biopsy impression smear of a hepatic mass in a yearling Thoroughbred filly. *Vet. Clin. Pathol*. 37, 339-343.

Grondin, T.M., Dewitt, S.F., 2010. Normal hematology of the horse and donkey. In:Schalm´s Veterinary Hematology. Weiss, D.J., Wardrop, K.J. (eds.), Wiley Blackwell Inc., pp. 821-828.

Harless, W., Pusterla, N., 2006. Equine herpesvirus 1 and 4. Respiratory disease in the horse. *Clin. Techn. Equine Pract.* 5, 197-202.

Harvey, W., 2006. Pathogenesis, laboratory diagnosis and clinical implications of erythrocyte enzyme deficiencies in dogs, cats, and horses. *Vet. Clin. Pathol*. 35, 144-156.

Hassel, D.M., 2007. Thoracic trauma in horses. *Vet. Clin. North. Am.: Equine Pract*. 23, 67-80.

Hinchcliff, R.W., Kociba, G.J., Mitte, LA., 1993. Diagnosis of EDTA-dependent pseudothrombocytopenia in a horse. *J. Am. Vet. Med. Assoc*. 203, 1715-1716.

Hollbrook, T.C., Munday, J.S., Brown, C.A., Glover, B., Schlievert, M.P., Sanchez, S., 2003. Toxic shock syndrome in a horse with Staphylococcus aureus pneumonia. *J. Am. Vet. Med. Assoc.* 222, 620-623.

Humber, K.A., Beech, J., Cudd, T.A., Palmer, J.E., Gardner, S.Y., Sommer, M.M., 1991. Azathioprine for treatment of immune-mediated thrombocytopenia in two horses. *J. Am. Vet. Med. Assoc.* 224, 83-87.

Jacobsen, S., Nielsen, J.V., Kjelgaard-Hansen, M., Toelboell, T., Fjeldborg, J., Halling-Thomsen, M., Martinussen, T., Thoefner, M.B., 2009. Acute phase response to surgery of varying intensity in horses: a preliminary study. *Vet. Surg.* 38, 762-769.

James, F.M., Engiles, J.B., Beech, J., 2010. Meningitis, cranial neuritis and radiculoneuritis associated with Borrelia burgdorferi infection in a horse. *J. Am. Vet. Med. Assoc.* 237, 1180-1185.

Jelinek, F., Faldyne, M., Jasurkova-Mikutova G., 2006. Severe combined immunodeficiency in a Fell pony foal. *J. Vet. Med. A Physiol. Pathol. Clin. Med*. 53, 69-73.

Johns, I.C, Stephen, J.O., Del Piero, F., Richardson, D.W., Wilkins, P.A., 2005. Hemangiosarcoma in 11 young horses. *J. Vet. Intern. Med*. 19, 564-570.

Johns, I.C., Desrochers, A., Wotman, K.L., Sweeney, R.W., 2011. Presumed immune-mediated hemolytic anemia in two foals with Rhodococcus equi infection. *J. Vet. Emerg. Crit. Care* 21, 273-278.

Johns, I.C., Jesty, S.A., James, F.M., 2006. Pseudomonas aeruginosa sepsis in an adult horse with enteric salmonellosis. *J. Vet. Emerg. Crit. Care* 16, 219-223.

Kaplan, N.A., Moore, B.R., 1996. Streptococcus equi endocarditis, meningitis and panophthalmitis in a mature horse. *Equine Vet. J.* 8, 313-316.

Kelton, D.R., Holbrook, T.C., Gilliam, L.L., Rizzi, T.E., Brosnahan, M.M., Confer, A.W., 2008. Bone marrow necrosis and myelophthisis: manifestations of T-cell lymphoma in a horse. *Vet. Clin. Pathol.* 37, 403-408.

Kemper, D.L., Perkins, G.A., Schumacher, J., Edwards, J.F., Valentine, B.A., Divers, T.J., Cohen, N.D., 2000. Equine lymphocytic plasmacytic enterocolitis: a retrospective study of 14 cases. *Equine Vet. J.* 32, 108-112.

Kim, D.Y., Taylor, H.W., Eades, S.C., Cho, D.Y., 2005. Systemic AL amyloidosis associated with multiple myeloma in a horse. *Vet. Pathol.* 42, 81-84.

Kimberley, M., McGurrin, J., Arroyo, L.G., Bienzle, D., 2004. Flow cytometric detection of platelet-bound antibody in three horses with immune-mediated thrombocytopenia. *J. Am. Vet. Med. Assoc.* 199, 591-594.

Kline, H., Foreman, J.H., 1991. Heart and spleen weights as a function of breed and somatotype. *Equine Exerc. Physiol.* 3, 17-21.

Koch, T.G., Wen, X., Bienzle, D., 2006. Lymphoma, erythrocytosis, and tumor erythropoietin gene expression in a horse. *J. Vet. Intern. Med.* 20, 1251-1255.

Langford, J., Thomson, P., Knight, P., 2013. Epistaxis in racehorses: risk factors and effects on career. *Equine Vet. J.* 91, 198-203.

Lassen, E.D., Swardson, C.J., 1995. Hematology and hemostasis in the horse: normal functions and common abnormalities. *Vet. Clin. North Am.: Equine Pract.* 11(3), 351-389.

Latimer, K.S., 1999. Leukocytic hematopoiesis. In: *Equine Medicine and Surgery.* King, C. (ed.), Mosby, St. Louis, USA, pp. 1992-2001.

Latimer, K.S., Meyer, D.J., 1989. Leukocytes in health and disease. In: *Textbook of Veterinary Internal Medicine*. Etinger (ed.), Saunders, Philadelphia, pp. 2181-2224.

Lennox, T.J., Wilson, J.H., Hayden, D.W., Bouljihad, M., Sage, A.M., Walser, M.M., Manivel, J.C., 2000. Hepatoblastoma with erythrocytosis in a young female horse. *J. Am. Vet. Med. Assoc.* 216, 718-721.

Lording, P.M., 2008. Erythrocytes. *Vet. Clin. North Am.: Equine Pract.* 24, 225-237.

Mahaffey, E.A., Moore, J.N., 1986. Erythrocyte agglutination associated with heparin treatment in three horses. *J. Am. Vet. Med. Assoc.* 189(11), 1478-1480.

Mair, T.S., Brown, P.J., 1993. Clinical and pathological features of thoracic neoplasia in the horse. *Equine Vet. J.* 25, 220-223.

Mair, T.S., Hillyer, M.H., 1992. Clinical features of lymphosarcoma in the horse: 77 cases. *Equine Vet. Educ.* 4, 108-113.

Mair, T.S., 1996. Update on infectious respiratory diseases of the horse. *Equine Vet. Educ.* 8, 329-335.

Mair, T.S., Taylor, F.G.R. Pinsent, Mr. P.J.N., 1989. Fever of unknown origin in the horse: a review of 63 cases. *Equine Vet. J.* 21, 260-265.

Malikides, N., Kessell, A., Hodgson, J.L., Rose, R.J., Hodgson, D.R., 1999. Bone marrow response to large volume blood collection in the horse. *Res. Vet. Sci.* 67, 285-293.

May, M.L., Nolen-Walston, R.D., Utter, M.E., Boston, R.C., 2010. Comparison of hematologic and biochemical results on blood obtained by jugular venipuncture as compared with intravenous catheter in adult horses. *J. Vet. Intern. Med*. 24, 1462-1466.

McConkey, S., Lopez, S., Pringle, J., 2000. Extramedullary plasmacytoma in a horse with ptyalism and dysphagia. *J. Vet. Diagn. Invest.* 12, 282-284.

McConnico, R.S., Morgan, T.W., Williams, C.C., Hubert, J.D., Moore, R.M., 2008. Pathophysiologic effects of phenylbutazone on the right dorsal colon in horses. *Am. J. Vet. Res.* 69, 1496-1505.

McConnico, R.S., Roberts, M.C., Tompkins, M., 1992. Penicillin-induced immune-mediated hemolytic anemia in a horse. *J. Am. Vet. Med. Assoc.* 201, 1402-1403.

McCue, M.E., Davis, E.G., Rush, B.R., Cox, J.H. , Wilkerson, M.J. 2003. Dexamethasone for treatment of multisystemic eosinophilic epitheliotropic disease in a horse. *J. Am. Vet. Med. Assoc.* 223, 1320-1323.

McFarlane, D., Sellon, D.C. , Parker, B. 1998. Primary erythrocytosis in a 2-year-old Arabian gelding. *J. Vet. Intern. Med.* 12, 384-388.

McGovern, K.F., Lascola, K.W., Davis, E., Fredrickson, R., Tan, R., 2011. T-cell lymphoma with immune-mediated anemia and thrombocytopenia in a horse. *J. Vet. Intern. Med.* 25, 1181-1185.

McGowan, C., 2008. Clinical pathology in the racing horse: the role of clinical pathology in assessing fitness and performance in the racehorse. *Vet. Clin. North Am.: Equine Pract.* 24, 405-421.

Mcsloy, A., Poulsen, K., Fisher, P.J., Armien, A., Chilton, J.A., Peek, S., 2007. Diagnosis and treatment of a selective immunoglobulin M glomerulonephropathy in a Quarter Horse gelding. *J. Vet. Intern. Med.* 21, 874-877.

Mendes, L.C.N., De Araujo, M.A., Bovino, F., Rozza, D.B., Machado, G.F., Cadioli, F.A., Feitosa, F.F.L., Peiró, J.R., 2011. Clinical, histological and immunophenotypic findings in a mare with a mammary lymphoma associated with anaemia and pruritus. *Equine Vet. Educ.* 23, 177-183.

Messer, N.T. 4th, Johnson, P.J., 2007. Evidence-based literature pertaining to thyroid dysfunction and Cushing's syndrome in the horse. *Vet. Clin. North Am.: Equine Pract.* 23, 329-364.

Messer, NT., 1995. The use of laboratory tests in equine practice. *Vet. Clin. North Am.: Equine Pract.* 11, 345-350.

Metcalfe, L.V., More, S.J., Duggan, V., Katz, L.M., 2013. A retrospective study of horses investigated by weight loss despite a good appetite (2002-2011). *Equine Vet. J.* 45, 340-345.

Meyer, J., Delay, J., Bienzle, D., 2006. Clinical, laboratory and histopathological features of equine lymphoma. *Vet. Pathol.* 43, 914-924.

Monreal, L., Anglés, A., Espada, Y., Monasterio, J., Monreal, M., 2000. Hypercoagulation and hypofibrinolysis in horses with colic and DIC. *Equine Vet. J.* 32, 19-25.

Monteith, C.N., Cole, D., 1995. Monocytic leukemia in a horse. *Can. Vet. J.* 36, 765-766.

Morris, D. D., 1998. Diseases of the hemolymphatic system. In: Equine Internal Medicine. Reed, S.M., Bayly, W.M.,(eds.). WB Saunders Co, pp. 558-601.

Morris, D.D.,1990. Alterations in the erythron. In: *Large Animal Internal Medicine*. Smith, B.P.,(ed). St. Louis. The C.V. Mosby Company, pp. 418-424.

Morris, D.D., Bloom, J., Roby, K.A., Woods, K., Tablin, F., 1984. Eosinophilic myeloproliferative disorder in a horse. *J. Am. Vet. Med. Assoc.* 185, 993-996.

Muñoz, A., Riber, C., Satué, K., Trigo, P., Gómez-Díez, M., Castejón, F.M., 2013a. Multiple myeloma in horses, dogs, and cats: a comparative review focused on clinical signs and pathogenesis. In: *Multiple Myeloma: a quick reflection on the fast progress.* In tech, pp. 289-326.

Muñoz, A., Riber, C., Trigo, P., Castejón, F., 2009. Hematopoietic neoplasias in horses: myeloproliferative and lymphoproliferative disorders. *J. Equine Sci.* 20, 59-72.

Muñoz, A., Riber, C., Trigo, P., Castejón, F., 2010a. Hematology and clinical pathology data in chronically starved horses. *J. Equine Vet. Sci*. 10, 581-589.

Muñoz, A., Riber, C., Trigo, P., Castejón, F., 2010b. Muscle damage, hydration, electrolyte balance and vasopressin concentrations in successful and exhausted endurance horses. *Pol. J. Vet. Sci.* 13(2), 373-379.

Muñoz, A., Riber, C., Trigo, P., Castejón-Riber, C., Castejón, F.M., 2010c. Dehydration, electrolyte imbalances and renin-angiotensin-aldosterone-vasopressin axis in successful and unsuccessful endurance horses. *Equine Vet. J.* 38, 83-90.

Muñoz, A., Riber, C., Trigo, P., Gómez-Díez, M., Castejón, F., 2012. Bacterial endocarditis in two Spanish foals after neonatal septicemia. *J. Equine Vet. Sci.* 32, 760-766.

Muñoz, A., Rodríguez, R.G.M., Riber, C., Trigo, P., Gómez-Díez, M., Castejón, F.M., 2013b. Subclinical Theileria equi infection and rhabdomyolysis in three endurance horse. *Pak. Vet. J*. 33, 257-259.

Muñoz, A., Trigo, P., Riber, C., Castejón, F.M., 2011. Spontaneous bilateral epistaxis associated with the administration of phenylbutazone in a horse. *Revue Med. Vet*. 162, 421-424.

Nolen-Walston, R.D., D'Oench, S.M., Hanelt, LM., Sharkey, L.C., Paradis, M.R., 2004. Acute recumbency associated with Anaplasma phagocytophilum infection in a horse. *J. Am. Vet. Med. Assoc.* 224, 1964-1966.

Parraga, M.E., Carlson, G.P., Thurmond, M., 1995. Serum protein concentrations in horses with severe liver disease: a retrospective study and review of the literature. *J. Vet. Intern. Med.* 9, 154-161.

Pass, D.A., Bolton, J.R., 1982. Chronic *eosinophilic* gastroenteritis in the horse. *Vet. Pathol.* 19(5), 486–496.

Pass, D.A., Bolton, J.R., Mills, J.N., 1984. Basophilic enterocolitis in a horse. *Vet. Pathol.* 21, 362-364.

Patterson, S.D., Auer, D., Bell, K., 1988. Acute phase response in the horse: plasma protein changes associated with adjuvant induced inflammation. *Biochem. Int.* 17, 257-264.

Patterson-Kane, J.C., Donahue, J.M., Harrison, L.R., 2001. Septicemia and peritonitis due to Actinobacillus equuli infection in an adult horse. *Vet. Pathol.* 38, 230-232.

Patterson-Kane, J.C., Tramontin, R.R., Giles, R.C. Jr, Harrison, L.R., 2000. Transitional cell carcinoma of the urinary bladder in a Thoroughbred with intra-abdominal dissemination. *Vet. Pathol.* 37, 692-695.

Patton, K., Wright, A., Kuroki, K., Beard, L., 2009. Hemorrhagic gastritis associated with renal failure, hemoglobinuria and isolation of Clostridium perfringens in a horse. *J. Equine Vet. Sci*. 29, 633-638.

Pauwels, F.E., Wigley, S.J., Munday, J.C., Roc, W.E., 2012. Bilateral ovarian adenocarcinoma in a mare causing haemoperitoneum and colic. *N. Z. Vet. J.* 60, 198-202.

Pearson, W., Boermans, H.J., Bettger, W.J., McBride, B.W., Lindinger, M.I., 2005. Association of maximum voluntary dietary intake of freeze-dried garlic with Heinz body anemia in horses. *Am. J. Vet. Res.* 66, 457-465.

Perkins, G., Ainsworth, D.M., Yeager, A., 1999. Hemothorax in 2 horses. *J. Vet. Intern. Med.* 13, 375-378.

Perryman, LE., 2000. Primary immunodeficiencies of horses. *Vet. Clin. North Am.: Equine Pract.* 16, 105-116.

Piercy, R.J., Swardson, C.J., Hinchcliff, K.W., 1998. Erythroid hipoplasia and anemia following administration of recombinant human erythropoietin to two horses. *J. Am. Vet. Med. Assoc.* 212, 244-247.

Polkes, A.C., Giguère, S., Lester, G.D., Bain, F.T., 2008. Factors associated with outcome in foals with neonatal isoerythrolysis (72 cases, 1988-2003). *J. Vet. Intern. Med.* 22, 1216-1222.

Porter, S.R., Saegerman, C., Van Galen, G., Sandersen, C., Delguste, C., Guyot, H., Amory, H., 2008. Vegetative endocarditis in equids (1994-2006). *J. Vet. Intern. Med.* 22, 1411-1416.

Poulin Braim, A.E., MacDonald, M.H., Bruss, M.L., Grattendick, K.J., Giri, S.N., Margolin, S.B., 2009. Effects of intravenous administration of pirfenidone on horses with experimentally induced endotoxemia. *Am. J. Vet. Res.* 70, 1031-1042.

Prins, M., Van Leeuwen, M.W., Teske, E., 2009. Stability and reproducibility of ADVIA 120-measured red blood cell and platelet parameters in dogs, cats, and horses, and the use of reticulocyte haemoglobin content (CHCR) in the diagnosis of iron deficiency. *Tijdschr. Diergeneeskd.* 134, 272-278.

Pritchard, J.C., Burn, C.C., Barr, A.R., Whay, H.R., 2009. Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. *Res. Vet. Sci.* 87, 389-395.

Pusterla, N., Fecteau, M.E., Madigan, J.E., Wilson, W.D., Magdesian, K.G., 2005. Acute hemoperitoneum in horses: a review of 19 cases (1992-2003). *J. Vet. Intern. Med.* 19, 344-347.

Pusterla, N., Stacy, B.A., Vernau, W., De Cock, H.E., Magdesian, K.G., 2004. Immunoglobulin A monoclonal gammopathy in two horses with multiple myeloma. *Vet. Rec*. 155, 19-23.

Radin, M.J., Eubank, M.C., Weiser, M.G., 1986. Electronic measurement of erythrocyte volume and volume heterogeneity in horses during erythrocyte regeneration associated with experimental anemia. *Vet. Pathol.* 23, 656-660.

Reed, S.K., Messer, N.T., Tessman, R.K., Keegan, K.G., 2006. Effects of phenylbutazone alone or in combination with flunixin meglumine on blood protein concentrations in horses. *Am. J. Vet. Res.* 67, 398-402.

Reef, V.B., Dyson, S.S., Beech, J., 1984. Lymphosarcoma and associated immune-mediated hemolytic anemia and thrombocytopenia in horses. *J. Am. Vet. Med. Assoc.* 184, 313-317.

Reppas, G.P., Canfield, P.J., 1996. Malignant mast cell neoplasia with local metastasis in a horse. *N. Z. Vet. J*. 44, 22-25.

Revington, M., 1983. Haematology of the racing Thoroughbred in Australia: 1: reference values and the effect of excitement. *Equine Vet. J.* 15, 141-144.

Ringer, N.C., Edens, L., Bain, P., Raskin, R.E., Larock, R., 2008. Acute myelogenous leukaemia in a mare. *Aust. Vet. J*. 75, 329-331.

Ringger, N.C., Edens, L., Bain, P., Raskin, R.E., Larock, R., 1997. Acute myelogenous leukaemia in a mare. *Aust. Vet. J*. 75, 329-331.

Rossdale, P.D., Burguez, P.N., Cash, G., 1982. Changes in blood neutrophil/lymphocyte ration related to adrenocortical function in the horse. *Equine Vet. J*. 14, 293-298.

Satué, K., Blanco, O., Muñoz, A., 2009. Age-related differences in the hematological profile of Andalusian broodmares of Carthusian strain. *Vet. Med*. 54), 175–182.

Satué, K., Hernández, A., Lorente, C., O´Coonor, J.E., 2010. Immunophenotypical characterization in Andalusian horse: variations with age and gender. *Vet. Immunol. Immunopathol*. 133, 219-227.

Satué, K., Hernández, A., Muñoz, A., 2012. Physiological factors influencing equine hematology. In: *Hematology Science and Practice.* Open Acces Publisher. Pp. 573-596.

Satué, K., Muñoz, A., Montesinos, P., 2011. Seasonal variations in the erythrogram in pregnant Carthusian mares. *Proceeding of 13th conference of the ESVCP/ECVCP, 9TH conference of AECCP, 12TH ACCP and ASVCP,* 31 Aug-3 Sept, Dublin (Ireland), pp. 24.

Saulez, M.N., Schlipf, J.W., Cebra, C.K. McDonough, S.P., Bird, K.E., 2004. Use of chemotherapy for treatment of a mixed-cell thoracic lymphoma in a horse. *J. Am. Vet. Med. Assoc.* 224, 733-738.

Schalm, O.W., Carlson, G.P., 1982. The blood and the blood forming organs. In: Equine Medicine and Surgery, American Veterinary Publications, pp. 377-414.

Schumacher, J., Edwards, J.F., Cohen, N.D., 2000. Chronic idiopathic inflammatory bowel diseases in the horse. *J. Vet. Intern. Med.* 14, 258-265.

Sellon, D.C., 1998. Thrombocytopenia in horses. *Equine Vet. Educ.* 10(3), 133-139.

Sellon, D.C., 2004. Disorders of the hematopoietic system. In: *Equine Internal Medicine.* Reed, S.M., Bayly, W.M., Sellon, D.C., (eds.), Saunders, pp. 721-768.

Sellon, D.C., Levine, J., Millikin, E., Palmer, K., Grindem, C., Covington, P., 1996. Thrombocytopenia in horses: 35 cases (1989-1994). *J. Vet. Intern. Med.* 10, 127-132.

Sellon, D.C., Levine, J.F., Palmer, K., Millikin, E., Grindem, C., Covington, P., 1997. Thrombocytosis in 24 horses (1989-1994). *J. Vet. Intern. Med.* 11, 24-29.

Singh, K., Holdbrook, T.C., Gilliam, L.L., Cruz, R.J., Duff, J., Confer, A.W., 2006. Severe pulmonary disease due to multisystemic eosinophilic epitheliotropic disease in a horse. *Vet. Pathol.* 43, 189-193.

Siska, W.D., Tottle, R.E., Messick, J.B., Bisby, T.M., Toth, B., Kritcheusky, J.E., 2013. Clinicopathologic characterization of six cases of equine granulocytic anaplasmosis in a non-endemic area (2008-2011). *J. Equine Vet. Sci.* 33, 653-657.

Skowronek, A.J., LaFranco, L., Stone-Marschat, M.A., Burrage, T.G., Rebar, A.H., Laegreid, W.W., 1995. Clinical pathology and hemostatic abnormalities in experimental African horse sickness. *Vet. Pathol.* 32, 112-121.

Sneddon, J.C., Van der Walt, J., Mitchell, G., 1992. Effects of dehydration and rehydration on the intravascular space in horses. *Comp. Biochem. Physiol. Comp. Physiol*. 103, 163-167.

Southwood, L.L., Schott H.C. II, Henry, C.J., Kennedy, F.A., Hines, M.T., Geor, R.J., Hassel, D.M., 2000. Disseminated hemangiosarcoma in the horse: 35 cases. *J. Vet. Intern. Med.* 14, 105-109.

Spengler, M.I., Bertoluzzo, S.M., Catalani, G., Rasia, M.L., 2008. Study on membrane fluidity and erythrocyte aggregation in equine, bovine and human species. *Clin. Hemorheol. Microcirc*. 38, 171-176.

Stokol, T., Erb, H.N., De Wilde, L., Tornquist, S.J., Brooks, M., 2005. Evaluation of latex agglutination kits for detection of fibrin(ogen) degradation products and D-dimer in healthy horses and horses with severe colic. *Vet. Clin. Pathol.* 34, 375-382.

Swain, J.M., Licka, T., Rhind, S.M., Hudgson, N.P., 2003. Multifocal eosinophilic enteritis associated with a small intestinal obstruction in a Standardbred horse. *Vet. Rec*. 24, 648-651.

Taintor, J., Schleis, S., 2011. Equine lymphoma. *Equine Vet. Educ.* 23, 205-213.

Tan, R.H.H., Crisman, M.V., Clark, S.P., Gagea, M., Zimmerman, K., 2007. Multicentric mastocytoma in a horse. *J. Vet. Intern. Med.* 21, 340-343.

Taylor, S.D. Haldorson, G.J., Vaughan, B., Pusterla, N., 2009. Gastric neoplasia in horses. *J. Vet. Intern. Med.* 23, 1097-1102.

Taylor, S.D., Pusterla, N., Vaughan, B., Whitcomb, M.B., Wilson, W.D., 2006. Intestinal neoplasia in horses. *J. Vet. Intern. Med.* 20, 1429-1436.

Thomas, H.L., Livesey, M.A., 1998. Immune-mediated hemolytic anemia associated with trimethoprim-sulphamethoxazole administration a in a horse. *Can. Vet. J*. 39, 171-173.

Torkelson, J., 2002. Perirectal abscess, colic, and dyschezia in a horse. *Can. Vet. J*. 43, 127-128.

Tornquist, S.J., 2008. Bone marrow and lymph node evaluation. *Vet. Clin. North Am. Equine Pract.* 24, 261-283.

Trigo, P., Castejón, F., Riber, C., Muñoz, A., 2010. Use of biochemical parameters to predict metabolic elimination in endurance rides. *Equine Vet. J*. 38, 142-146.

Trigo, P., Muñoz, A., Castejón, F., Riber, C., Hassel, D.M., 2011. Rib fracture in a horse during an endurance race. *Can. Vet. J*. 52, 1226-1227.

Valdes, A., Johnson, J.R., 2005. Septic pleuritis and abdominal abscess formation caused by Rhodococcus equi in a foal. *J. Am. Vet. Med. Assoc.* 227, 960-963.

Van der Kolk, J.H., 1997. Equine Cushing's disease. *Equine Vet. Educ.* 9, 209-214.

Vits, L., Araya, O., Bustamante, H., Mohr, F, Galecio, S., 2008. Idiopathic renal haematuria in a 15-year-old Arabian mare. *Vet. Rec*. 162, 251-252.

Viu, J., Monreal, L., Jose-Cunilleras, E., Cesarini, C., Añor, S., Armengou, L., 2012. Clinical findings in 10 foals with bacterial meningoencephalitis. *Equine Vet. J*. 41, 100-104.

Watts, A.E., Johnson, A.L., Felippe, M.J., Divers, T.J., 2011. Recurrent Actinobacillus peritonitis in an otherwise healthy thoroughbred horse. *Aus. Vet. J*. 89, 143-146.

Weiss, D.J., Miller, D.C., 1985. Bone marrow necrosis associated with pancytopenia in a cow. *Vet. Pathol.* 22(1), 90-92.

Weiss, D.J., Moritz, A., 2003. Equine immune-mediated hemolytic anemia associated with Clostridium perfringens infection. *Vet. Clin. Pathol.* 32, 22-26.

Welch, R.D., Watkins, J.P., Taylor, T.S., Cohen, N.D., Carter, G.K., 1992. Disseminated intravascular coagulation associated with colic in 23 horses (1984-1989). *J. Vet. Intern. Med.* 6, 29-35.

White, S.D., Affolter, V.K., Dewey, J., Kass, P.H., Outerbridge, C., Ihrke, P.J., 2009. Cutaneous vasculitis in equines: a retrospective study of 72 cases. *Vet. Dermatol*. 20, 600-606.

Wong, D.M., Belgrave, R.L., Williams, K.J., Del Piero, F., Alcott, C.J., Bolin, S.R., Marr, C.M., Nolen-Walston, R., Myers, R.K., Wilkins, P.A., 2008. Multinodular pulmonary fibrosis in five horses. *J. Am. Vet. Med. Assoc.* 232, 898-905.

In: Horses
Editors: A. Paz-Silva, M. S. Arias Vázquez et al.
ISBN: 978-1-63117-560-2

Chapter 4

HORSE REARING CONDITIONS, HEALTH STATUS AND RISK OF SENSITIZATION TO GASTROINTESTINAL PARASITES

C. Cazapal-Monteiro[1], J. A. Hernández[1], M. S. Arias[*1], J. L. Suárez, S. Miguélez[1], I. Francisco[1], P. Lago[2], M. I. Rodríguez[1], F. J. Cortiñas[1] and A. Romasanta[1]

[1]Equine Diseases Study Group (COPAR-2129), Animal Pathology Department, Veterinary Faculty, Santiago de Compostela University, Lugo, Spain
[2]Piensos NANTA, Padrón, A Coruña, Spain

ABSTRACT

Horses can be maintained outdoors or indoors. When managed outdoors, they routinely feed on domestic pastures, and are supplemented with grain and roughage when necessary. Other possibilities consist of horses grazing freely on natural pastures in woodland areas, a regime known as silvopasturing. They are never supplemented, and sufficient feeding and veterinary attention is not provided. They are used to reduce unwanted vegetation, and very few economic benefits can be achieved by meat production. In most cases, horses are focused to breeding, farming, silvopasturing or sport (aptitude). With the aim for gaining knowledge on the possible influence of horse rearing conditions on their health status and the risk of sensitization to gastrointestinal parasites also, a survey was conducted in NW Spain. Two blood samples were individually collected from each animal: samples preserved with anticoagulant were examined by means of an automated Coulter-Counter for determining the values of red (erythrocytes, haemoglobin and haematocrite) and white parameters (leukocytes, lymphocytes and granulocytes). Sera were faced to the excretory/secretory antigens of gastrointestinal parasites (cyathostomins and stomach bots). Results were analyzed according to the equine aptitude.

Significantly lower records for erythrocytes, haemoglobin and haematocrit were obtained in silvopasturing horses, while no aptitude-differences in the white blood cells

* Corresponding Author address: Email: mariasol.arias@usc.es.

were demonstrated. Horses focused to farming and/or silvopasturing reached the highest percentages of sensitization against cyathostomins, whereas the equines dedicated to sport did it against the stomach bots. The presence of antibodies against strongylids was correlated to erythropenia, anaemia and elevated levels of granulocytes and lymphocytes. Seropositivity to stomach bots was associated with low haematocrite counts and increased numbers of leukocytes. The possible influence of rearing conditions is discussed. Horses feeding on forests reached the highest percentages of strongyle-sensitization among silvopasturing equids, probably due these are generally autochthonous and indigenous breeds difficult to immobilize for administering any parasiticide. One surprisingly result was the observation of horses dedicated to sport and/or leisure had the greatest counts of exposure to *Gasterophilus*. The possible explanation could be linked to their participation in sporting events held outdoors, where different substances in the sweat, perspiration or smell could serve as attractants for the adult flies.

Keywords: Horses, strongyles, gastric bot, rearing conditions

INTRODUCTION

Horse husbandry covers different aspects related with their care and their breeding. By paying attention to raising and rearing, all the features concerning nutrition, stable cleanliness, deworming, grooming and hoof care should be satisfied (Frape, 1998). One interesting point to observe regarding equine management are the five basic needs to fulfill: possibilities of movement (exercise), balanced feed (minerals, vitamins, fruits and vegetables), light, friends and certain logical work (Nielsen, 1998).

As originally travelers possibly to ensure foraging, horses require enough space for stretching, walking and cantering. Opposite to a very highly extended belief, equines have a small stomach. Consequently, they should not be given big feed portions a day, and should ingest small feed portions several times a day (Purcell, 2001).

It has been demonstrated the necessity of day light for the production of vitamin D. It is crucial for establishing the circadian rhythms. For avoiding respiratory problems, fresh air is needed (Duren and Crandell, 1999).

Due to their gregarious character, horses band together. Therefore the presence of specimens alone should be avoided (McCall, 1989). Equines are considered smart animals and as a consequence they like to develop intellectual work.

The analysis of these requirements appears to point that most could be satisfied if horses had the opportunity of behave freely, remaining under pasture conditions where the presence of shelters ensures their protection against sun, wind, rain and/or cold (FASS, 2010). Nevertheless, this is not ever possible, and sometimes horses are kept indoors under appropriate conditions.

In the last years, there has been observed an increasingly tendency for maintaining the horses under pasturing conditions, even those belonging to Pure Bred and having an elevated economic value. Among different explanations, the current financial crisis appears to be partly responsible, due to the elevated cost for feeding these animals (Arias et al., 2012).

Horse Husbandry Types

Attending to the possibilities of the horses can freely behave or not, two main types of husbandry could be defined:

a). ***Not freely behaving horses***: they need to be managed for feeding, doing exercise, etc. The extreme case consists of horses are tied on a moveable bar or iron chain, which impedes them to move. Contact with other horses is never possible unless the owner loose him (Figure 1).

Other type frequently observed is horses remaining stabled during the night (Figure 2). The horse owner is responsible for the horse enjoys 2-3 hours a day on the pasture, as well as has contact to other horses. For longer than 20 hours/day horses have no other option but starring on the walls of their stable. In both cases, feeding consists of feedstuff and fresh forage (when possible). In the second case, horses have the opportunity of eating grass while doing exercise.

Figure 1. Not freely behaving horse.

Figure 2. Stabled horse.

b). ***Freely behaving horses*:** the equines can decide to spend time indoors or outdoors. Under a paddock husbandry, horses can openly go from the stable to a small grassland through a direct connection (Figure 3).

Figure 3. Freely behaviour horses from Pure Galician Bred.

Other kind of husbandry consists of horses are kept on the pasture or in an open stable. It is needed a refuge for protecting the horses from adverse weather conditions (wind, rain, too hot, excessive cold) similar to Figure 4.

Figure 4. Example of a shelter for freely behaving horses.

Finally, when the horses are reared in an exercise pen, they have open access to different areas where they can drink, roll, eat, and play with other horses. The purpose is that horses are obliged to a certain intellectual work and movement.

Under these regimes, nutrition is based on grass and a small portion of feedstuff. Horses can directly take the forage, and they can move around freely whilst doing also.

Equine Nutrition

Feed is one of the principal expenses in owning horses, and the fees can be reduced by preserving them healthy and by trying to give them a balanced ration. In order to achieve optimal performance, equine dietary supplies must be adjusted to age, weight, level of activity and life status. For example, a broodmare in lactation has different nutritional requirements than a 10-year-old, non-working horse.

Forages are normally ingested as a primary component of horses' diets, because of the basic necessity for normal functioning of their digestive system. By ingesting pasture and hay, forages can provide varying amounts of the nutrient requirements. Despite of some investigations pointed that forages should supply one half or more of the total weight of the feed consumed daily, it looks preferably that they ingest a minimum of 1 percent of their body weight in hay or pastures each day.

Bearing in mind that feeding can result high-priced, proper management of pastures might help to get good and inexpensive food for horses. Mature horses performing minimal or no work can be maintained on high quality forages without grain supplementation. However, growing, breeding, or working horses require supplementing the forage with grain or concentrate. In contrast, poorly-managed pastures supply little or no feed, and become frequently the source of many internal parasites.

Forage

Besides the factors needing consideration when feeding the horses (age, life status, etc.), the origin of the forage is other feature to contemplate. When owners have extensive grasslands, there is no problem for providing to the horses fresh grass from the pasture. On the other hand, when pasture land is limited, owners can be obliged to give the horses preserved forage, either in long fiber form as hay, haylage (semi-wilted, fermented grass in bags) or in short chopped fiber form commonly known as chaff, which is commonly provided to horses and ponies to bulk out their concentrate feed and to prevent them eating too quickly. However feeding chaff alone can be dusty and can irritate some horses making them cough. Most of the nutrients needed for a mature horse can be obtained by ingesting high quality hay, due to its content in energy, protein, minerals and vitamins.

Husbandry, Nutrition and Health Status

Horses are extremely susceptible to molds, fungi, and other sources of toxic substances in forage. Mold problems generally occur in hay that has been baled at a too high moisture level (20% or more) without the use of a preservative. This is especially a problem with first cutting hay because it is harvested during a period of time when it rains frequently and the weather conditions are less than ideal for hay drying.

Among the diseases which can be developed by horses, some infections caused by parasites are important due to its frequency and pathogenicity. This is the case of cestodoses (*Anoplocephala*), gastrointestinal nematodoses or myiasis (*Gasterophilus, Rhinoestrus*) (Mula et al., 2013). In some cases, horses become infected after eating herbage contaminated with

some mites (cestodes), eggs (ascarids) or third stage larvae (strongyles). Myiasis are diseases caused by the larval stages of flies (Sánchez-Andrade et al., 2010).

Parasitic diseases are strongly linked to the horse husbandry, and can be responsible for decrease in the horse health status. Not freely behaving horses have limited or not access to forage, and thus a lower risk of exposure to parasitoses transmitted by feeding grass (Arias et al., 2012). Though stabled specimens (mainly dedicated to breeding and sport/leisure) do not spend time in the grasslands, it is very frequent they are given fresh-cut forage, which can also vehicle parasitic forms. On the contrary, equines maintained in farms or forests (silvopasturing) seem to be at high risk of exposure to forage-transmitted diseases due to the elevated chance of ingesting infective stages of certain parasites (Shimano, 2004; Kornaś et al., 2010).

The horse is host to a great number of gastrointestinal helminthes, of which nematodes of the family Strongylidae, the roundworm *Parascaris equorum* and the cestode *Anoplocephala perfoliata* are the most important (Lind et al., 2007). These parasites are ubiquitous and have been recognized as significant causes of clinical disease in horses.

Anoplocephala perfoliata (Figure 5) is the main tapeworm affecting equids (Rodríguez-Bertos et al., 1999; Bello & Abell, 1999). After ingestion of infected mites present in the soil of grasslands, cysticercoids excyst at the gut level and attach to the intestinal mucosa in the region of the ileo-cecal junction. Gravid proglotids and/or eggs are released in the feces, and once in the soil, ingested by oribatid mites, the intermediate hosts.

Figure 5. *Anoplocephala perfoliata.*

Tapeworms have been related to the appearance of histopathological and gross lesions such as mucosal ulceration and enteritis, which could be responsible for colic, intussusceptions and cecal rupture (Ryu et al., 2001; Traub-Dargatz et al., 2001).

Parascaris equorum (Nematoda: Ascaridoidea) infections occur commonly in foals and yearlings, although specific examination for *Parascaris equorum* revealed that 10% of 20

older horses were infected (Lyons et al., 2000). The horses ingest infective eggs that are dispersed in the surrounding environment by previous years' foals. Infections may cause nasal discharge, coughing, ceased growth, inappetence, rough hair coat and lethargy. At large worm burdens intestinal obstruction may occur (Ryu et al., 2004; Lind & Christensson, 2009). Despite of the extended belief that parascariosis is only important among young horses, recent investigations have demonstrated their presence in adult individuals also (Francisco et al., 2009).

After the decline of large strongyle infections as a result of widespread use of modern anthelmintic compounds, their clinical importance has become underlined and these nematodes recognized as an important cause of digestive diseases in horses including weight loss, hypoalbuminemia and diarrhoea as well as colic, poor growth, anaemia, debilitation and rough hair coat (Murphy and Love, 1997).

Cyathostominae are nematodes infecting virtually all grazing horses. These are nematodes with a direct life cycle, during which the parasites undergo a period of inhibited development as early third stage larvae in the large intestinal wall (Love *et al*., 1999). These larvae can constitute up to 90% of the total cyathostomin burden, and become very significant in cyathostomin-associated disease, due to large numbers of larvae can accumulate and reactivate to provoke a syndrome known as larval cyathostominosis (Figure 6).

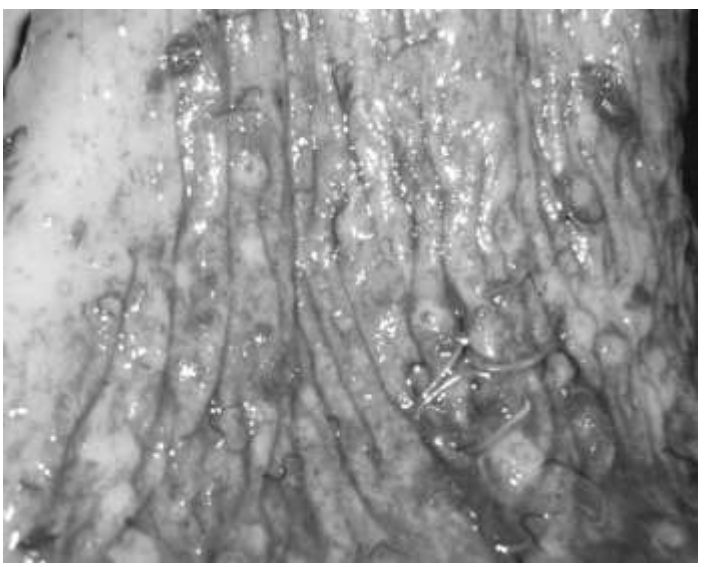


Figure 6. Large intestinal lesions caused by third stage larvae from Cyathostominae.

Adult cyathostomins can be detected by faecal egg count analysis (Dowdall *et al*., 2004). However, horses with high mucosal burdens commonly have low or negative faecal egg counts (Paul, 1998), and the early stages are not detectable diagnostically and following treatments, covert, life-threatening burdens of mucosal larvae may persist (Love and McKeand, 1997). Furthermore, there are no non-invasive methods to assess the effectiveness of anthelmintic treatments administered to horses with large mucosal burdens (Dowdall *et al*., 2002).

Gasterophilosis is a myiasis affecting equid hosts caused by *Gasterophilus* spp. larvae (Diptera: Oestridae) (Figure 7) mainly in the Palaearctic and Afrotropical regions (Colwell *et*

al., 2006). Eggs are deposited by the adult flies during warm seasons when females lay eggs on the horses' forelegs, lips, face and the intermandibular area; an exception being female *G. pecorum*, which lay eggs on the grass (Cogley and Cogley, 2000). The first-stage larvae hatch from the eggs and introduced into the mouth, and after a 5-week period they moult to second-stage larvae (Edwards, 1982). Second instars moved to the stomach and intestine, where they moult into third instars, which can remain attached to the stomach and intestine for 8–10 months (Coles and Pearson, 2000).

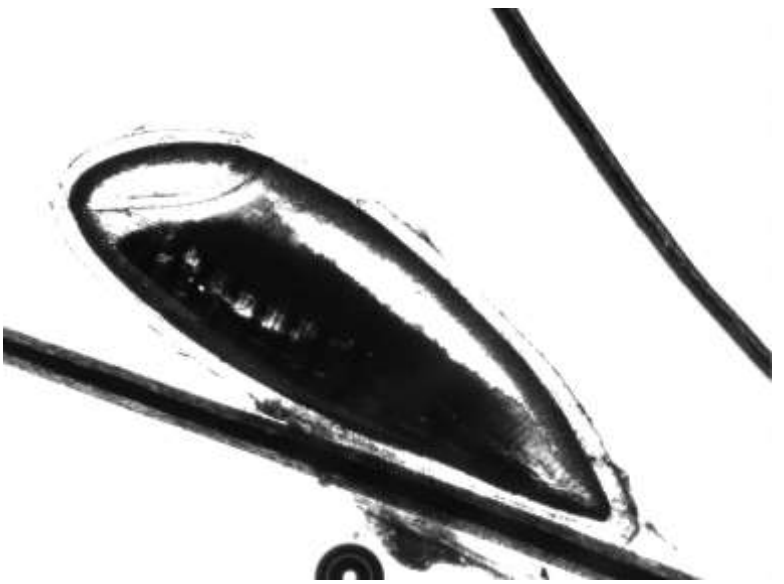


Figure 7. Egg of gastric bot on the hair, containing the 1st stage larva.

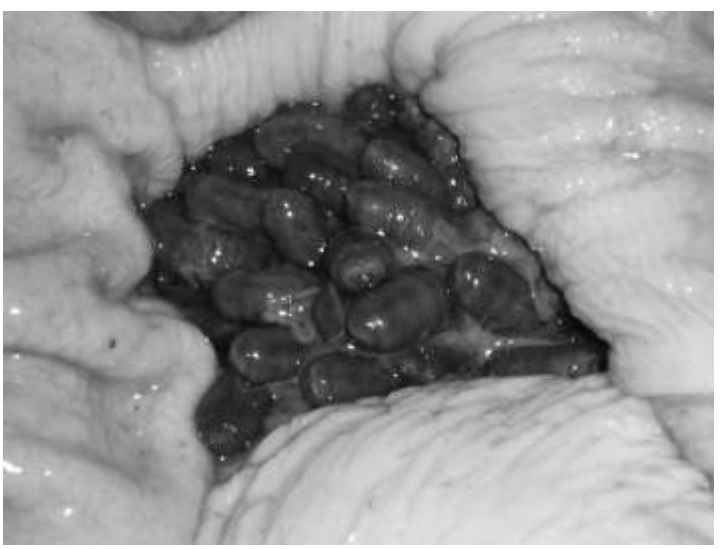


Figure 8. 2nd and 3rd larvae stages from gastric bot in a horse stomach.

Gasterophilosis is associated with impaired swallowing, gastrointestinal ulcerations, gut obstructions or volvulus, rectal prolapse, anaemia, diarrhoea, and digestive disorders (Gökçen et al., 2008). High larval burdens, like the one in Figure 8, have been implicated in gastric ulceration and rupture, intramural gastric suppuration, peritonitis following gastroduodenal perforation, and gastro-oesophageal reflux (Edens and Murray, 1992). Immature *Gasterophilus nasalis* may burrow into the spaces around the teeth and can cause necrosis of the gums. There are a few reports of human myiasis associated with *Gasterophilus* larvae causing subcutaneous crawling or ophthalmo-myiasis (Royce et al., 1999; Chen, 2001).

The presence of hooked mouthparts and spines in the 3rd stage *Gasterophilus* larvae can provoke haemorrhages, chronic gastritis, ulcerated stomach or even stomach rupture (Sánchez-Andrade et al., 2010).

Accordingly to the previous stated it seems very interesting and helpful to analyze the possible relationship among horse health status and the risk of forage transmitted diseases (FTDs) and insect-borne diseases (IBDs). With this purpose, a research was conducted among horses under different husbandry regimes in NW Spain. Hematological parameters were monitored and analyzed regarding the presence of patent infection and exposure to strongyle parasites (as etiological agents of FTDs). Equine sensitization to *Gasterophilus intestinalis* was also determined for gaining information on the threat of IBDs.

METHODS

Field Trial for Analyzing the Influence of Husbandry on Horse Health Status: Blood Parameters

Experimental Design

The current trial was conducted on 325 horses from NW Spain. Several breeds of horses are involved in a wide variety of activities throughout this area. Many of these horses are owned and managed for recreation or sport, and less for owners' income. According to their husbandry, four categories were considered: breeding (104), farming (66), silvopasturing (126) and sport/leisure (29).

Under the category of ***breeding*** there were considered horses dedicated to get specimens with the purpose of selling them before the breeding maturity is reached, i.e. as foals or yearlings (Figure 9). The most frequent horse bred involved are those with high economic value (Pure Blood, Arabian, Spanish Sport) but in occasions crossbreds are also included. These are horses kept indoors during night at least, and when weather conditions are adverse, and receiving appropriate nutrition and veterinary care. Feeding is based on feedstuff and hay, whereas fresh forage is seldom given.

The term ***farming horses*** (Figure 10) designates those individuals normally feeding in small grasslands near to the proprietor house. They are stabled only under very unfavorable climate conditions. Nowadays, these horses develop an essential role for keeping pastures free of brushes and unwanted vegetation, especially those formerly grazed by other livestock species as ruminants (cattle). Most commonly are crossbred horses, feeding on pasture. Supplementation is observed when forage is scarce.

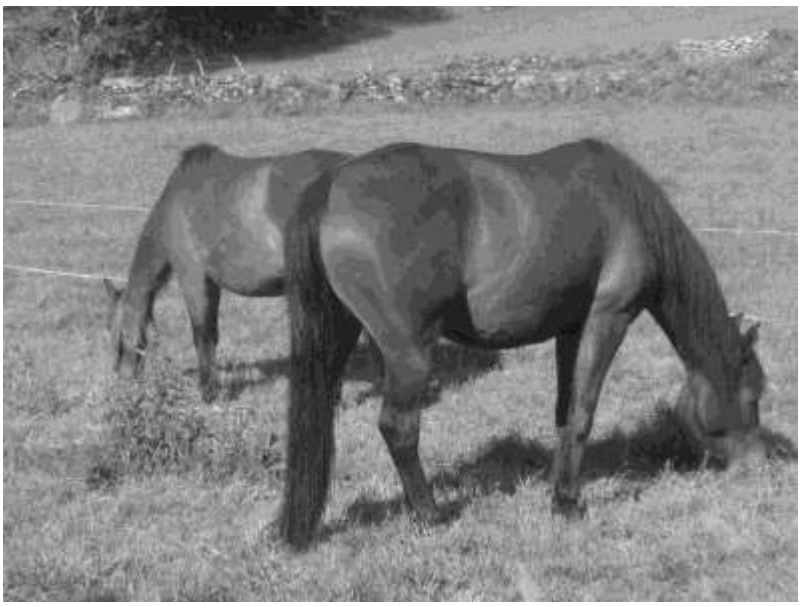


Figure 9. Breeding horses.

Figure 10. Farming horses.

Silvopasturing integrates the production of timber and livestock. The trees are handled for high-value saw logs and at the same time provide shade and shelter for livestock and forage. The horses (Figure 11) are raised to offer income through sales and subsidies (Husak

& Grado, 2002), providing a biological wee control thus decreasing the need for herbicides (Sharrow, 1999).

This land-management strategy is becoming very important on areas where coniferous trees exist. The autochthonous breeds seem the most adequate ones for grazing under a silvopastoral system due to their natural adaptation ability (Francisco et al., 2009).

Figure 11. Silvopasturing horses.

Figure 12. Horses dedicated to sport.

Horses dedicated to ***sport*** are managed indoors, but they have access to pasture for exercise as can be seen in Figure 12. Only individuals having high economic value are involved, most frequently Arabian Pure Bred, Spanish Sport Horse and English Pure Bred. Feeding consists of feedstuff and conserved forage, but fresh grass can be also ingested when doing exercise. Excellent care is provided to these specimens, including veterinary attention. Similar conditions are observed for ***leisure*** horses, used by their owners for periodically riding them.

Analysis of Body Performance and Condition

With the aim for gaining knowledge on their health status, all the horses were bled. Two blood samples were individually collected, one was preserved with anticoagulant, and the other without it for getting the serum.

Samples with anticoagulant were examined by means of an automated Coulter-Counter, the Abacus Junior Vet hematology analyzer (Spain). Sera were faced to the excretory/secretory antigens of gastrointestinal parasites (cyathostomins and stomach bots).

There were assessed the values of the red blood cells (RBC), Hemoglobin (HB) and Hematocrite (HCT), as well as the white blood cells (WBC), lymphocytes (LYM) and granulocytes (GRA).

In table 1 the reference values provided by the manufacturer of the hematology analyzer are summarized.

Table 1. Reference values of the hematology analyzer

Red cell parameters			White cell parameters		
RBC ($x10^{12}$/L)	HB (g/dL)	HCT (%)	WBC ($x10^{9}$/L)	LYM ($x10^{9}$/L)	GRA ($x10^{9}$/L)
6.8 - 12.9	11 - 19	32 - 53	5.4 - 14.3	1.5 - 7.7	2.3 - 9.5

Examination of the Exposure to Helminth Parasites

The presence of IgG antibodies against gastrointestinal parasites (cyathostomins and stomach bots) was evaluated by means of an ELISA and their respective excretory/secretory antigens.

Exposure to Gastrointestinal Nematodes (Cyathostomins)

Antigen preparation was done according to Paz-Silva et al. (2011). After collecting a large quantity feces from horses passing strongyle eggs, droppings were maintained during 15 days at 20-22ºC (Francisco et al., 2009). Then the coprological cultures were examined under the microscope, and only cyathostomins were detected. Once the third larval stage was attained, they were washed in PBS (phosphate buffered saline, pH 7.2) and incubated for 24 h at 37°C and 5% CO2 in RPMI (Roswell Park Memorial Institute) culture medium, using a ratio of approximately 1,000 larvae/1.5 ml of RPMI medium. During a 3-day period, the medium was removed every 6 h and then centrifuged, dialyzed extensively against water, concentrated and kept lyophilized as CES.

ELISAs were performed by adding 0.5 µg mL^{-1} CES to the wells of U-bottom microtiter plates (Costar, Barcelona, Spain). After an incubation period of 8 hours at 4ºC, sera (tested in duplicated) diluted at 1:100 in 10% PTL (PBS–0.3% Tween 20 and 10% skimmed milk) were

added to the wells and maintained at 37ºC for 1h. Finally, horseradish peroxidase (HRP) conjugated with rabbit anti-Horse IgG (Nordic Immunology Laboratories, The Netherlands) was used at a 1:1000 dilution and incubated for 1 h. Substrate consisting of 10 mg of ortho-phenylenediamine in 12 mL of citrate buffer and 10 µL of 30% H_2O_2 were then placed into each well, and the absorbance was read using a spectrophotometer (Titertek Multiskan) at 492 nm. The sensitivity and specificity of the CES-ELISA was 82% and 80%, respectively (Francisco, 2009).

Exposure to Gastric Bots (Gasterophilus Intestinalis)

Excretory/secretory antigens of *Gasterophilus intestinalis* second instars (L2) were prepared in accord to Roelfstra et al. (2009) and Sánchez-Andrade et al. (2010). In brief, L2 larvae obtained from the stomachs of slaughtered horses were washed in PBS and then incubated in RPMI at 37ºC and 5% CO2 atmosphere for 3 days, with changes every 8-10 hours. Subsequently, the medium was collected, dialyzed exhaustively against water, and kept lyophilized as GES.

For the analysis of the IgG antibodies, GES were diluted to a concentration of 2.5 mg mL^{-1} in PBS to coat the wells of the ELISA plates (Costar, Corning Inc.). After 8 h at 4ºC, sera diluted at 1:250 in PTL were tested in duplicate. HRP conjugated goat anti-horse IgG (Sigma-Aldrich Co., Madrid, Spain) was used at 1:2500 dilutions. Absorbances were read using a spectrophotometer (Titertek Multiskan, Hämmelina) at 492 nm.

The values of sensitivity and specificity for the GES-ELISA were 89% and 78% (Sánchez-Andrade et al., 2010).

Statistical Analysis

The seroprovelance values were expressed as percentages and the 95% confidence interval, and analyzed by use of the χ^2 test. Significance was considered when $P < 0.05$. The Pearson's correlation test was applied to evaluate the existence of correlation among the different variables considered. All tests were performed with the statistical package SPSS, version 20 (SPSS Inc., Chicago, IL, USA).

RESULTS

Relationship among Husbandry, Health Status and Risk of Sensitization to Gastrointestinal Parasites

Red Blood Cell (RBC) Values

The analysis of the RBCs regarding the horse aptitude is summarized in table 2. The specimens maintained in forests reached the highest percentages of erythropenia, whereas the lowest were obtained in the equines dedicated to breeding and/or sport and leisure. The percentage of horses showing erythrocytosis were very low.

Table 2. Red blood cell values according to the handling of horses

	Breeding	Farming	Silvopasturing	Sport / Leisure
Erythropenia (<6.8 x 10^9/L)	1%	12%	***26%***	3%
Normal (6.8 – 12.9 x 10^9/L)	96%	88%	71%	93%
Erythrocytosis (> 12.9 x 10^9/L)	3%	0%	2%	3%
Statistics	χ^2= 36.477, *P*= 0.001			

Table 3. Hemoglobin values regarding the horse aptitude

	Breeding	Farming	Silvopasturing	Sport / Leisure
Low HB (< 11 g/dL)	1%	12%	***21%***	3%
Normal (11 – 19 g/dL)	93%	80%	76%	86%
High HB (> 19 g/dL)	6%	8%	2%	10%
Statistics	χ^2= 28.987, *P*= 0.001			

As shown in table 3, an overall percentage of 21% silvopasturing horses showed reduced values of hemoglobin, and 12% farming equines. Higher concentrations than normal were obtained in breeding, farming and sport/leisure horses.

Almost 4 out of each 10 horses feeding on brushes in forests (silvopasture) presented haematocrite values lower than normal (table 4).

Table 4. Hematocrite values regarding the horse aptitude

	Breeding	Farming	Silvopasturing	Sport / Leisure
Reduced HCT (< 32%)	7%	14%	***39%***	7%
Normal (32 – 53%)	88%	83%	58%	86%
Elevated HCT (> 53%)	5%	3%	3%	7%
Statistics	χ^2= 43.940, *P*= 0.001			

White Cell Values

As drawn in table 5, the highest percentages of horses with leucopenia were observed among the horses dedicated to farming and sport/leisure. Leukocytosis was mainly detected among the horses under forest conditions.

Despite of significant differences were not obtained, very low counts of horses with lymphocytosis were observed in NW Spain (table 6). Farming horses reached the highest percentages of equines with reduced values of lymphocytes.

Horses maintained in forests exhibited the highest counts of granulocytosis, followed by farming equines as we can see in table 7. One unexpected result was the observation of granulocyte levels lower than normal among the horses focused to sport and/or leisure.

Table 5. White cell values regarding the horse aptitude

	Breeding	Farming	Silvopasturing	Sport / Leisure
Leukopenia (<5.4 x 10^9/L)	6%	***15%***	2%	14%
Normal (5.4 - 14.3 x 10^9/L)	93%	83%	82%	86%
Leukocytosis (> 14.3 x 10^9/L)	1%	1%	***17%***	0%
Statistics	χ^2= 41.879, *P*= 0.001			

Table 6. Lymphocytes values regarding the horse aptitude

	Breeding	Farming	Silvopasturing	Sport / Leisure
Lymphopenia (<1.5 x 10^9/L)	7%	***15%***	5%	7%
Normal (1.5 - 7.7 x 10^9/L)	92%	83%	94%	93%
Lymphocytosis (> 7.7 x 10^9/L)	1%	1%	2%	0%
Statistics	χ^2= 7.478, *P*= 0.279			

Table 7. Granulocytes values regarding the horse aptitude

	Breeding	Farming	Silvopasturing	Sport / Leisure
Granulocytopenia (<2.3 x 10^9/L)	19%	17%	2%	***31%***
Normal (2.3 - 9.5 x 10^9/L)	80%	77%	80%	69%
Granulocytosis (> 9.5 x 10^9/L)	4%	15%	***18%***	0%
Statistics	χ^2= 44.264, *P*= 0.001			

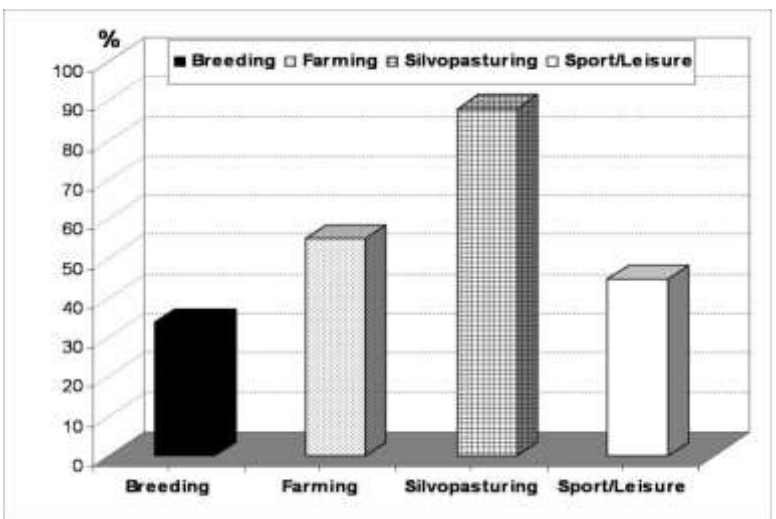


Figure 13. Prevalence of horses with patent infection by strongyle parasites.

Patent Infection by Strongyles

The occurrence of patent infection by strongyles was detected in 60% of the sampled specimens, as demonstrated by the observation of eggs in their stools. The highest numbers were observed in the silvopasturing horses (88%) and the lowest in the breeding equines (Figure 13). These differences were significant (χ^2= 75.433, *P*= 0.001).

Sensitization against Parasites (Cyathostomins and Gastric Bots)

An overall prevalence of 53% horses exposed to gastrointestinal nematodes (cyathostomins) was obtained, ranging from 41% in the breeding horses and those focused to sport/leisure, and 64% in the equines under silvopasture conditions (χ^2= 13.934, *P*= 0.003) (Figure 14).

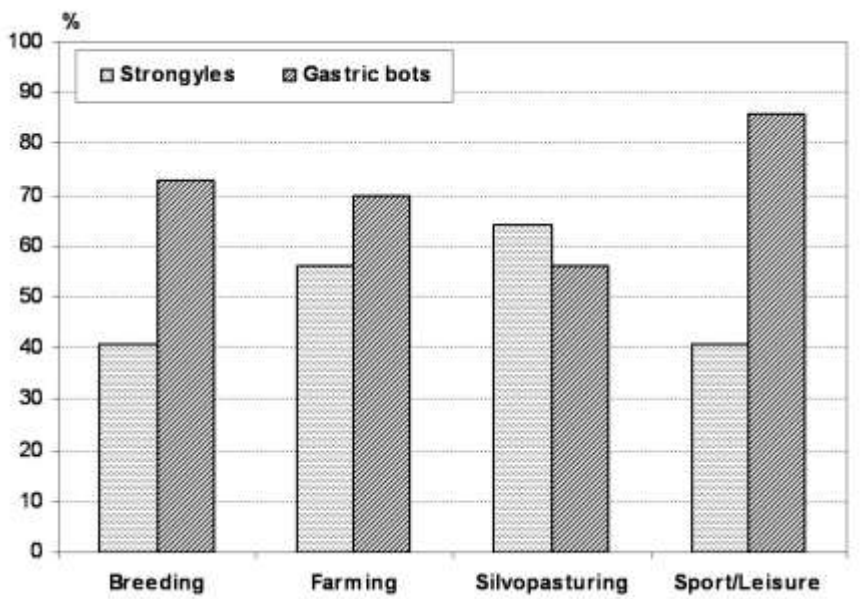


Figure 14. Prevalence of horses exposed to parasites.

Sixty-seven percent horses had antibodies against the gastric bot *Gasterophilus intestinalis*. Equines focused to sport and/or leisure achieved the highest percentages (86%) of sensitization, while silvopasturing ones exhibited the lowest counts (56%) (χ^2= 13.272, *P*= 0.004).

As drawn in Figure 2, horses managed for sport and/or leisure exhibited the lowest counts of exposure to the strongyles and the highest to the gastric bots.

By considering the simultaneous analysis of the presence of antibodies against both parasites, it was demonstrated mixed exposure mainly in breeding and sport/leisure horses, whereas farming and silvopasturing equines presented sensitization to gastric bots chiefly as shown in table 8.

Table 8. Presence of antibodies against strongyles, gastric bots and mixed exposure

	Breeding	Farming	Silvopasturing	Sport / Leisure
Strongyles	14%	18%	28%	3%
Gastric bots	28%	**38%**	**36%**	38%
Strongyles + Gastric bots	**45%**	32%	20%	**48%**
Statistics	χ^2= 26.439, *P*= 0.002			

Relationship between Variations in Hematic Parameters, Patent Infection By Strongyles and Sensitization against Cyathostomins and Gastric Bots

With the aim to determine the explain the appearance of hematic disorders, the possible relationship between patent infection or exposure to parasites, and the development of anemia, erythrocytosis, leukocytosis, leukopenia, granulocytopenia and granulocytosis was investigated. Anemia was defined as the occurrence of lower values than normal for the RBC, hemoglobin and PCV (Messer, 1995).

As summarized in table 9, a total of 13% horses presented anemia, 7% reduced values of leukocytes and 13% of granulocytes. The percentage of equines with erythrocytosis was 2% only, 7% leukocytosis and 8% granulocytosis.

Table 9. Relationship between digestive parasite and blood disorders

	Total	Strongyle patent infection	Sensitization to strongyles	Sensitization to gastric bots
Anemia	13%	5%	7%	3%
Erythrocytosis	2%	1%	1%	2%
Leukopenia	7%	3%	3%	4%
Leukocytosis	7%	5%	4%	4%
Granulocytopenia	13%	5%	6%	11%
Granulocytosis	8%	5%	5%	5%

The presence of adult strongyles was weakly related to hematic alterations (1-5%). Sensitization to strongyles and hematic disorder occurred in 1-7% cases, and 2-11% when analyzing exposure to gastric bots and values of hematic parameters different than normal. It should be underlined that infection and/or exposure to strongyle nematodes was both associated to changes in red and white blood parameters, while sensitization against gastric bots was mainly linked to variations in granulocytes.

A possible explanation to these results could be these horses develop a shorter period in the grasslands (Getachew et al., 2012). By opposite, the finding of the highest percentages of positivity among the autochthonous equines appears to be attributable to these are individuals maintained on forests where non-agricultural measures are applied to the soil, plus a wrong therapy is provided.

CONCLUSION

The health status of horses can be influenced by their rearing conditions, due to the association with feeding availability and appropriate care. The finding of anemia among horses maintained in forests is expected, because they are feeding on bushes in rangelands, and the food is scarce during some period, such as winter or summer. Nevertheless, at least 1 out of 10 horses under farming regimes did not reach the standard values of RBCs, despite being well-kept horses receiving proper care and nutrition. In the same way, the observation of near 10% of the horses belonging to the category of farming or sporting with leucopenia seems difficult to explain, especially 31% of sport horses presenting granulocytopenia.

To discard or confirm the role of some parasites over these results, there was investigated if horses have been exposed to gastrointestinal nematodes, strongyles and gastric bots. Horses feeding on forests belong generally to autochthonous and indigenous breeds, difficult to immobilize for administering any parasiticide. This explains the highest percentages of strongyle-sensitization among silvopasturing equids, and the presence of patent infection also. One surprisingly result was the observation of horses dedicated to sport and/or leisure reached the greatest counts of exposure to *Gasterophilus*. The possible explanation could be linked to their participation in sportive events held outdoors, where different substances in the sweat, perspiration or smell could serve as attractants for the adult flies. It should be taken into consideration that the flies do not enter the stables, thus infestation of horses occurs when they are spending time outdoors only.

Finally, there has been demonstrated an association among exposure to strongyle nematodes and reduced values for the red blood cell parameters, while sensitization against gastric bots decreases the count of granulocytes.

REFERENCES

Arias, M.S., Piñeiro, P., Hillyer, G.V., Francisco, I., Cazapal-Monteiro, C.F., Suárez, J.L., Morrondo, P., Sánchez-Andrade, R., Paz-Silva, A. (2012). Enzyme-linked immunosorbent assays for the detection of equine antibodies specific to a recombinant *Fasciola hepatica* surface antigen in an endemic area. *Parasitol. Res.* 110, 1001-1007.

Bello, T.R., Abell, J.E. (1999). Are equine tapeworms an emerging disease? A retrospective study. *J Equine Vet Sci.* 19, 11.

Chen, X.N. (2001). A case of skin myiasis caused by *Gasterophilus nigricornis*. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 19, 60.

Cogley, T.P., Cogley, M.C. (2000). Field observations of the host-parasite relationship associated with the common horse bot fly, *Gasterophilus intestinalis*. *Vet. Parasitol*. 88, 93–105.

Coles, G.C., Pearson, G.R. (2000). *Gasterophilus nasalis* infection: prevalence and pathological changes in equids in south-west England. *Vet. Rec*. 146, 222–223.

Colwell, D.D., Hall, M.J.R., Scholl, P.J. (2006). A synopsis of the biology, hosts, distribution, disease significance and management of the genera. In: D.D. Colwell, M.J.R. Hall, P.J. Scholl (Eds.), the *Oestrid Flies: Biology, Host-Parasite Relationships, Impact and Management*. CAB International.

Dowdall, S.M., Matthews, J.B., Mair, T., Murphy, D., Love, S., Proudman, C.J. (2002). Antigen-specific IgG(T) responses in natural and experimental cyathostominae infection in horses. *Vet. Parasitol*. 106, 225–242.

Dowdall S. M. J., Proudman C. J., Klei T. R., Mair T., Matthews J. B. (2004). Characterisation of IgG (T) serum antibody responses to two larval antigen complexes in horses naturally- or experimentally-infected with cyathostomins. *Int. J. Parasitol.* 34: 101–108.

Duren, S.E., Crandell, K. (1999). The role of vitamins in growth of horses. Advances in Equine Nutrition II. *Kentucky Equine Research*. *http://www.ker.com/library /advances/227.pdf*

Edens, L.M., Murray, M.J. (1992). Gastro-oesophageal reflux in a weanling filly: association with *Gasterophilus* spp. infestation. *Equine Vet. J*. 11, 18–23.

Edwards, G.T. (1982). The prevalence of *Gasterophilus intestinalis* in horses in northern England and Wales. *Vet. Parasitol.* 11, 215–222.

FASS (Federation of Animal Science Societies) (2010*). Guide for the care and use of agricultural animals in research and teaching.* Champagne, IL: FASS.

Francisco, I., Arias, M., Cortiñas, F.J., Francisco, R., Mochales, E., Sánchez, J.A., Uriarte, J., Suárez, J.L., Morrondo, P., Sánchez-Andrade, R., Díez-Baños, P., Paz-Silva, A., (2009). Silvopastoralism and autochthonous equine livestock: analysis of the infection by endoparasites. *Vet. Parasitol.* 164, 357–362.

Francisco, R. (2009). *"Diagnóstico de estrongilosis equina mediante ELISA y antígenos de excreción/secreción".* Trabajo de Investigación Tutelado. Facultad de Veterinaria de Lugo. Universidad de Santiago de Compostela.

Frape, D. (1998). *Equine Nutrition and Feeding*. (2nd Ed). Blackwell Science, London. p. 43-71.

Getachew, A.M., Innocent, G., Proudman, C.J., Trawford, A., Feseha, G., Reid, S.W., Faith, B., Love, S. (2012). Equine cestodosis: a sero-epidemiological study of *Anoplocephala perfoliata* infection in Ethiopia. *Vet. Res. Commun*. 36(2), 93-98.

Husak, A.L., Grado, S.C. (2002). Monetary benefits in a southern silvopastoral system. *South. J. Appl. Forestry* 26, 159-164.

Kornaś, S., Cabaret, J., Skalska, M., Nowosad, B. (2010). Horse infection with intestinal helminths in relation to age, sex, access to grass and farm system. *Vet. Parasitol*. 174(3-4), 285-291.

Lind, E.O., Christensson, D. (2009). Anthelmintic efficacy on *Parascaris equorum* in foals on Swedish studs. *Acta Vet. Scand.* 22, 51-45.

Lind, E.O., Rautalinko E., Uggla, A., Waller, P.J., Morrison, D.A., Höglund, J. (2007). Parasite control practices on Swedish horse farms. *Acta Vet. Scand*. 49, 25.

Love, S., McKeand, J.B. (1997). Cyathostomosis: practical issues of treatment and control. *Equine Vet. J.* 9, 253-256.

Love, S., Murphy, D., Mellor, D. (1999). Pathogenicity of cyathostome infection. *Vet. Parasitol.* 85, 113-121.

Lyons, E.T., Swerczek, T.W., Tolliver, S.C., Bair, H.D., Drudge, J.H., Ennis, L.E. (2000). Prevalence of selected species of internal parasites in equids at necropsy in central Kentucky (1995–1999). *Vet. Parasitol*. 92, 51-62.

McCall, C.A. (1989). The effect of body condition of horses on discrimination learning abilities. *Appl. Anim. Behav. Sci*. 22, 327-334.

Mula, P., Pilo, C., Solinas, C., Pipia, A.P., Varcasia, A., Francisco, I., Arias, M.S., Paz-Silva, A., Sánchez-Andrade, R., Morrondo, P., Díez-Baños, P., Scala, A. (2013). Epidemiology, chronobiology and taxonomic updates of *Rhinoestrus* spp. infestation in horses of Sardinia Isle, Western Mediterranean (Italy). *Vet. Parasitol.* Feb 18, 192(1-3), 240-246.

Murphy, D., Love, S. (1997). The pathogenic effects of experimental cyathostome infections in ponies. *Vet. Parasitol*. 70, 99-110.

Nielsen, B.D. (1998). Nutrient requirements of the young equine athlete. Advances in Equine Nutrition II. *Kentucky Equine Research*. *http://www.ker.com/library/advances/232.pdf*

Paul, J.W. (1998). Equine larval cyathostomosis. *Comp. Cont. Educ. Pract. Vet.*. 20, 509-515.

Paz-Silva, A., Francisco, R., Rodríguez, I., Francisco, I., Cazapal-Monteiro, C.F., Arias, M.S., Suárez, J.L., Sánchez-Andrade, R. (2011). Isolation of potentially useful antigens from cyathostomin third-stage larvae by using a fast protein liquid chromatography one-step method. *Clin. Vaccine Immunol.* 18(9), 1462-1466.

Purcell, K. (2001). An ounce of prevention: Feeding management to minimize colic. Advances in Equine Nutrition III. *Kentucky Equine Research http://www.ker.com /library/advances/311.pdf*

Rodríguez-Bertos, A., Corchero, J., Castaño, M., Peña, M.L., Luzón, M., Gómez-Bautista, M., Meana, A. (1999). Pathological alterations caused by *Anoplocephala perfoliata* infection in the ileocaecal junction of equids. *Zentralbl Veterinarmed A*. 46(5), 261-269.

Roelfstra, L., Deeg, C.A., Hauck, S.M., Buse, C., Membrez, M., Betschart, B., Pfister, K. (2009). Protein expression profile of *Gasterophilus intestinalis* larvae causing horse gastric myiasis and characterization of horse immune reaction. *Parasit. Vectors* 2, 6.

Ryu, S.H., Bak, U.B., Kim, J.G., Yoon, H.J., Seo, H.S., Kim, J.T., Park, J.Y., LeeM C.W. (2001). Cecal rupture by *Anoplocephala perfoliata* infection in a thoroughbred horse in Seoul Race Park, South Korea. *J. Vet. Sci*. 2(3), 189-193.

Ryu, S.H., Jang, J.D., Bak, U.B., Lee, C.W., Youn, H.J., Lee, Y.L. (2004). Gastrointestinal impaction by *Parascaris equorum* in a Thoroughbred foal in Jeju, Korea. *J. Vet. Sci.* 5(2), 181–182.

Sánchez-Andrade, R., Cortiñas, F.J., Francisco, I., Sánchez, J.A., Mula, P., Cazapal, C., Vázquez, L., Suárez, J.L., Francisco, R., Arias, M.S., Díez-Baños, P., Scala, A., Paz-Silva, A. (2010). A novel second instar *Gasterophilus* excretory/secretory antigen-based ELISA for the diagnosis of gasterophilosis in grazing horses. *Vet. Parasitol*. 171(3-4), 314-320.

Sharrow, S.H. (1999). Silvopastoralism: competition and facilitation between trees, livestock and improved grass-clover pastures on temperate rainfed lands. *In*: *Agroforestry in Sustainable Agricultural Systems*. L.E. Buck, J.P. Lassoie, E.C.M. Fernandes (Eds). CRC Press LLC. Boca Raton, Florida. 111-130.

Shimano, S. (2004). Oribatid mites (Acari: Oribatida) as an intermediate host of *Anoplocephalid* cestodes in Japan. *Appl Entomol Zool*. 39, 1-6.

Traub-Dargatz, J.L., Kopral, C.A., Seitzinger, A.H., Garber, L.P., Forde, K., White, N.A. (2001). Estimate of the national incidence of and operation-level risk factors for colic among horses in the United States, spring 1998 to spring 1999. *J Am Vet Med Assoc.* 219(1), 67-71.

In: Horses
Editors: A. Paz-Silva, M. S. Arias Vázquez et al.
ISBN: 978-1-63117-560-2

Chapter 5

STRONGYLES SHED IN FAECES AS A MEANS OF MONITORING THE PARASITE SCENARIO IN HORSE STUD FARMS

L. M. Madeira de Carvalho,[1*] S. Sousa,[2]
M. Cernea,[3] L. C. Cernea,[3] M. Arias[4] and A. Paz-Silva[4]
[1] Centro de Investigação Interdisciplinar em Sanidade Animal,
Faculdade de Medicina Veterinária, Universidade de Lisboa
(CIISA-FMV-UL), Pólo Universitário do alto da Ajuda,
Avenida da Universidade Técnica, Lisboa, Portugal
[2] Escola Universitária Vasco da Gama, Coimbra, Portugal
[3] University of Agricultural Sciences and Veterinary Medicine,
Faculty of Veterinary Medicine, Cluj-Napoca, Romania
[4] Equine Diseases Study Group (COPAR), Parasitology Diseases,
Animal Pathology Department, Veterinary Faculty,
Santiago de Compostela University, Lugo, Spain

ABSTRACT

Horse production is a major animal industry in Portugal with horses being bred and trained for for several purposes, including sport (jumping, dressage, horseball), leisure riding, bullfighting, working as draft animals, etc. Strongyles are a very important group of equine parasites, and there are some seventy different species of helminths in this group that are found in horses. The identification of adult strongyles to species is important because it provides information on population of these worms within a horse and suggests the potential risk of the helminths present producing diarrhoea or colic, being associated with anthelmintic resistance, and the effects of animal hunsbandry on their control. Studies comparing worms present within the intestine at *post mortem* with the adult and larval stages shed in faecal samples of horses on the same farms have

* Research funded by FCT-CIISA-FMV-UL, Project CIISA. 8. Strongylosis; Corresponding Author: madeiradecarvalho@fmv.ulisboa.pt.

shown high degrees of corrleation. Thus, for clinical and epidemiological surveys, along with the assessment of deworming efficacy programmes, a great deal can be learned without the need to recover worms from horses at necropsy.

Over several years, 23 horses were selected that had diarrhoea with the natural shedding of adult strongyles in faeces (N=3) or after deworming (N=20). A faecal sample was collected after defecation or 24-48 h after deworming. All 23 horses were found positive for adult stages of strongyles, 30,4% for *Strongylinae and* 100% for *Cyathostominae*, in a total of 2628 worms belonging to 10 genera and 24 species of strongyles (4 *Strongylinae* and 20 *Cyathostominae*). Nematodes of subfamily *Cyathostominae* contributed to 99,7% of the total number of collected strongyles, while *Strongylinae* comprised only 0,34%. An average of 8 species/host (minimum 1 and maximum 17) and 114 worms/sample/host (minimum 1 and maximum 373) were found. The 10 most prevalent species in subfamily *Cyathostominae* (*Cylicocyclus insigne*, *Cylicocyclus nassatus*, *Cyathostomum catinatum*, *Cylicostephanus longibursatus*, *Cylicocyclus asworthi*, *Cyathostomum pateratum*, *Cylicostephanus calicatus*, *Coronocyclus coronatus*, *Cylicocyclus leptostomum*, *Coronocyclus labiatus*), comprised 93,3 % of total studied strongyles, being *C. nassatus* the most abundant one, contributing to 35 % of the total worm count. There was a marked reduction in the *Strongylinae*, namely *S. vulgaris*, when compared with *Cyathostominae*, which comprised 99,7% of the total number of strongyles. This approach to the study of strongyle populations within horses produced very useful data concerning the dominance of cyathostomins when related to the entire strongyle population, provided for the identification of a large number of strongyle species (24), and represents a potentially valid alternative to parasitological studies requiring euthanasia for the purpose of necropsies for worm recovery.

Keywords: Horse strongyles, faecal samples, adult stages, survey, horse farms, parasitic scenario, Portugal

INTRODUCTION

The existence of interaction between man and horses in the Iberian Peninsula is known since 300,000 years BC, when they were hunted by humans as a food source. In Portugal their presence is documented around 17,000-20,000 BC, but only beginning around 4000 BC is their documentation of domestication. In Portugal, the existing breeds of horses descended from populations that settled in the North (the Garranos), the Center (Sorraia horses), and further south (the Lusitano horse breed), being late influenced by Arab breeds introduced during the sojourn of the Moors within the Iberian Peninsula. One of the products of the breed selections resulted in the production of a war horse with excellent features, which also is ideal for many sporting practices. Although a selection for war purposes was the main goal for thousands of years, bullfighting is currently the main arena wherein this horse is utilized today. The horse production system may involve small farms (with working or leisure mares, using their foals for commercial purposes), or larger stud farms (with mare groups of 20-50 animals, being these last ones concentrated in the Alentejo and Ribatejo regions, in the center and south). In recent decades larger horse farms have appeared further north and more and more, horses have changed from war/working animals to leisure animals, with many around the country being teated as companion animals and pets (Monteiro, 1983; Madeira de Carvalho, 2001, 2006).

Regardless of the size and kind of the horse farm, all horses grazing in early life acquire a mixed infection by different species of gastrointestinal parasites, whose evolution depends on several etiological and epidemiological factors, such as the underlying geographic region and pasture ecosystem, type of farming and husbandry, equine population and of course, the parasitic agents involved (Duncan, 1982, 1985).

The most important parasites of equines, due to their ubiquity, frequency, prevalence and pathogenic effects, are the nematodes of the Strongylidae family, commonly designated by intestinal strongyles or simply strongyles. These are divided into two distinct subfamilies: Strongylinae and Cyathostominae. The subfamily Strongylinae (strongylins) comprises species with complex migration in the host (mesenteric arteries, liver, pancreas, etc.) such as the genus *Strongylus*. The known species of the subfamily Cyathostominae (cyathostomins) develop only in the mucosa and submucosa of the cecum and colon (Lichtenfels, 1975; Love & Duncan, 1988; Lichtenfels *et al.*, 1998). For decades, equine practicioners considered highly pathogenic only the representatives of the genus *Strongylu*s, with the nematode species *Strongylus vulgaris* being considered the "Horse Killer", given its constant association with colic syndrome (Kester, 1975). However, the world of parasites is also composed of change and an interesting phenomenon was observed in the early 1990s: the cyathostomins became the predominant group of strongyles in horses and were also considered an important cause of colic in horses (Herd, 1990; Uhlinger, 1990).

Cyathostomins are currently considered one of the most pathogenic parasites of horses. However, although many recent advances have allowed the development of new compounds for their control, there is still much to investigate about their identification, basic biology, pathogenesis of the disease they cause and the application of innovative control programs to arrest the growing development of anthelmintic resistance associated with these nematodes (Love *et al*., 1999; Lyons *et al.*, 1999; Tolliver, 2000; Lichtenfels *et al.*, 2008).

At the farm level, diagnosis of the presence of strongyles is based on faecal tests, namely Faecal Egg Counts (FEC) using McMaster chamber and faecal cultures for L3 isolation. FEC and larval cultures both have value as quantitative and qualitative diagnostic tools for detecting the presence or absence of a given parasite group. However, a major weakness of FEC, is that strongyle eggs cannot be differentiated to genus or species level. The more than 60 equine strongyle species have widely different pathological potentials, the significance of a given egg count can be very difficult to interpret if additional information is lacking. For instance, it is important to know whether *S. vulgaris* is present, since this parasite is regarded as much more pathogenic than cyathostomin species. Larval cultures allow for differentiation of *S. vulgaris* and other large strongyle species from the cyathostomin group, but it remains impossible to differentiate the more than 50 different species of cyathostomins infecting equids. Larval cultures can be performed in most veterinary practice laboratories, but the method is time-consuming and laborious, and requires a level of technical skill from the person reading the samples. Furthermore, there is still limited information on the sensitivity and specificity for detection of different species with the larval culture (Nielsen *et al*., 2010; Nielsen, 2012).

The identification of adult stages of horse strongyles is very important, namely due to the vast number, 64, of species known. Their differentiation is essential since 50 of them are cyathostomins, recognized as major pathogens in the complex of gastrointestinal disease in horses and resistant to the majority of anthelmintics (Lichtenfels et al., 2008; Matthews, 2011). Generally, identification of adult stages is used in epidemiological studies, namely

during necropsies or in organs removed from animals at slaughter houses. The accuracy of species composition estimation can allow the identification of a range of 10 to 25 species of cyathostomins (Collobert-Laugier *et al*., 2002; Chapman *et al*., 2003).

Since the 1980s, nematodes shed in the faeces have been used as an alternative to necropsy or organ isolation in slaughter houses for strongyle genus/species identification. Recent research allowed its use for diagnosis of horses cyathostominosis, the main species recovered after dewormings and the ones involved in anthelmintic resistance, expanding its potential interest as another tool aimed at a better understanding of the parasitic scenario at farm level (Anderson & Hasslinger, 1982; Olsen *et al*., 2003; Osterman Lind *et al*., 2003; Kuzmina *et al*., 2005; Kuzmina & Kharchenko, 2008; Kornas *et al*., 2011).

Based on this methodology, between 1992 and 1998, a research was carried out on a Horse Stud Farm, "Companhia das Lezírias, S.A. (CL), a major horse producer in Portugal. Field and laboratory studies were conducted to assess the genera/species of horse strongyles, adult and larval stages (L4/L5), shed in the faeces after natural expulsion or deworming. This research aimed to evaluate prevalence and relative abundance of genera/species, together with their differential analysis according to the age group, the environment (housed versus grazing animals) and anti-parasitic product used for deworming.

EXPERIMENTAL WORK

Material and Methods

The difficulty of *post mortem* exams for parasitological purposes in stud farm horses (CL is a commercial horse farm, and the sacrifice of animals for scientific purposes is not allowed, except in accidental deaths, always with a delayed report) and the need for a better knowledge of the strongyles species involved at a farm level, led us to seek an alternative means to search and perform the identification of adult and larval stages (L4/L5) of these nematodes. Accordingly, the identification of strongyles eliminated in the faeces could displace the need for necropsies for the purposes of disease prevention and control. In this study, we proceeded to collect specimens eliminated in the faeces of 23 animals. The data for this material, such as the sampling dates, animal husbandry (in the barn or on the pasture) type of shedding (animals with natural elimination of strongyles or after deworming) and dewormer used, are detailed in Table 1 (Madeira de Carvalho, 2001).

The CL horses used in this study were selected from two different situations: a) animals showing diarrheal symptoms and/or natural shedding of strongyles in faeces b) dewormed animals according to the anthelmintic control program of the horse farm. For b) option, horses were selected after first contacting the Veterinary Surgeon for registration of dates for deworming and schedule of the days for faecal sampling. The anthelmintic administration followed the normal deworming program of the farm, using no other compounds than those used at the time of our study (Madeira de Carvalho, 2001).

Table 1. Horses of CL used in faecal sampling for strongyle collection – Collection dates, age groups, sex, husbandry, type of shedding and drugs used in horse deworming

Number	Date	Age	Sex	Husbandry	Shedding	Anthelmintic
1	22-12-92	10 months	Male	Pasture	Natural	Non treated
2	09-03-95	3 years	Male	Barn	Deworming	Ivermectin*
3	09-03-95	3 years	Male	Barn	Deworming	Ivermectin
4	09-03-95	3 years	Male	Barn	Deworming	Ivermectin
5	09-03-95	3 years	Male	Barn	Deworming	Ivermectin
6	11-12-95	3 years	Male	Barn	Deworming	Ivermectin
7	11-12-95	3 years	Male	Barn	Deworming	Ivermectin
8	11-12-95	6 years	Male	Barn	Deworming	Ivermectin
9	11-12-95	15 years	Male	Barn	Deworming	Ivermectin
10	13-03-96	4 years	Female	Barn	Deworming	Ivermectin
11	09-07-96	6 years	Female	Pasture	Deworming	Doramectin**
12	28-01-97	1 year	Male	Pasture	Deworming	Doramectin
13	28-01-97	1 year	Male	Pasture	Deworming	Doramectin
14	28-01-97	1 year	Male	Pasture	Deworming	Doramectin
15	28-01-97	1 year	Male	Pasture	Deworming	Doramectin
16	28-01-97	1 year	Male	Pasture	Deworming	Doramectin
17	28-01-97	1 year	Male	Pasture	Deworming	Doramectin
18	28-01-97	1 year	Male	Pasture	Deworming	Doramectin
19	28-01-97	1 year	Male	Pasture	Deworming	Doramectin
20	28-01-97	1 year	Male	Pasture	Deworming	Doramectin
21	28-01-97	1 year	Male	Pasture	Deworming	Doramectin
22	06-01-98	1 year	Male	Pasture	Natural	Non treated
23	02-03-98	5 years	Female	Pasture	Natural	Non treated

*Eqvalan® Oral Paste, Merial; **Dectomax, Injectable solution® SC/IM, Zoetis (ex-Pfizer).

The sampling methodology was based on previous research directed to the isolation of adult nematodes in faecal samples, modified by the fact that we searched for strongyles eliminated both naturally or after deworming. Animals naturally excreting adult and larval stages L4/L5 of strongyles were selected and a faecal sample of about 1 kg was collected immediately after defecation. In dewormed animals, a faecal sample of about 1 kg was also collected 24-48 h after administration of the anthelmintic (Anderson & Hasslinger, 1982; Madeira de Carvalho, 1991; Madeira de Carvalho, 2001;).

Faecal samples were placed in plastic bags, identified and stored under refrigeration in thermal boxes. Faeces were transported to the laboratory and whenever possible its processing arose up to 24 hours after collection and kept for that purpose in the refrigerator at 4-5°C. In situations where this procedure was not possible,the faecal samples were frozen at -20 º C for a few days before harvesting the strongyles. The nematodes were collected with tweezers and stylus and the remaining faecal mass was subjected to sedimentation in trays with dark background for more detailed search of small strongyles. The nematodes were washed with water or saline and placed in Petri dishes in a refrigerator at 4°C for 6-12 hours, to allow its extension. Adults and larval stages (L4/L5) were stored in sealed plastic containers and preserved in 70% ethanol until their identification. After separation of nematodes under the stereoscopic microscope, their identification was performed in slide and coverslip using lactophenol d'Amman.

The specimens were identified by observing their morphological characters, particularly the anterior end and buccal capsule. Genus and species identification and nomenclature followed several specialized textbooks and papers concerning horse strongyles, namely the ones by Lichtenfels and Lichtenfels and collaborators in USA, although several others, namely from Germany and Russia, were used too (Popova, 1955, 1958; Lichtenfels, 1975; Georgi, 1982; Lanfredi & Honer, 1984; Hartwich, 1986; Dvojnos & Kharchenko, 1994, Bowman, 1995; Lichtenfels et al, 1998; Tolliver et al, 2000; Lichtenfels *et al*., 2008;).

The descriptive statistics was performed with software "Microsoft Office Excel 2010®". For quantitative analysis of infection in this group of hosts (prevalence and average mean intensity and abundance of strongyles per host), terminology and methodology were used according to reference papers (Bush *et al.*, 1997; Rósza *et al.*, 2000). The interspecific comparison between prevalence and intensity of infection was performed, respectively, by the Fisher's Exact test and by the Bootstrap (for means) and Mood tests (for medians), based on the software "Reiczigel J, Rózsa L 2005.

Quantitative Parasitology 3.0, Budapest". Correlation analysis between prevalence and total harvested specimens/host and number of species/host was performed with Pearson test and the software "GraphPad InStat Version 3.5 for Windows 2000, GraphPad Software Inc., San Diego California USA, www.graphpad.com". Results concerning the average number of species/specimens according to the environment, age, type of shedding and dewormer used are displayed and differences between are analysed with Fisher Exact test. Results were considered significant for $p < 0.05$.

Results

In the horses of this stud farm, 2628 strongyle specimens were collected, including nematodes of 10 genera, 24 species (4 belonging to subfamily Strongylinae and 20 to the subfamily Cyathostominae) and L4/L5 larval stages of *Cyathostomum* spp. (Tables 2 and 3). Nematodes of subfamily Cyathostominae represented 99.7 % of the total population of strongyles, while the subfamily Strongylinae corresponded only to 0.3 %.

Most strongyles identified in the sampled material (99.35%) were also represented by adult stages with 1786 females and 668 males (174 adult forms used for other works were only identified based on the cephalic end, not having been sexed). Seventeen specimens of *Cyathostomum* spp. (L4/L5), constituted 0.65% of the total sample. All the 23 horses were parasitized with Cyathostominae (100%) and only seven with Strongylinae (30,4%). In this work, we recorded for the first time in Portugal the representatives of the species *Cylicocyclus brevicapsulatus* and *Cylicostephanus bidentatus*.

The ten species with higher prevalence were, in descending order: *Cylicocyclus insigne* (82.6%), *Cylicocyclus nassatus* (73.9%), *Cyathostomum catinatum* (60.9%), *Cylicostephanus longibursatus* (56.5%), *Cylicocyclus ashworthi* (52.2%), *Cylicostephanus calicatus* (47.8%), *Cyathostomum pateratum* (47.8%), *Cylicocyclus leptostomum* (34.8%), *Coronocyclus coronatus* (34.8%) and *Cylicostephanus goldi* (30.4%). Strongyles species per host ranged 1-17, with a variation of 1-373 specimens strongyles per host. The nematodes of the species *C. nassatus* constituted about 35% of this horse strongyle population, making this cyathostomin species the most abundant in this stud farm. Moreover, the 10 most prevalent species in CL

(all belonging to the subfamily Cyathostominae), represented in this study 93.3% of the total strongyles studied (Figures 1 and 2).

The confidence intervals for the prevalence and intensity infection are listed in Table 3 and significant differences in the prevalence and intensity of infection for each species are recorded in Tables 4 and 5.

Table 2. Prevalence, number of specimens, percentage of the total sample, mean intensity and abundance of different species of horse strongyles in a stud farm

Species	Positive horses (N=23)	Prevalence* (%)	Nº of specimens	% of the total number	Mean Intensity **	Mean Abundance ***
Cylicocyclus insigne	19	82.6	251	9.55	13.21	10.91
Cylicocyclus nassatus	17	73.9	914	34.78	53.76	39.74
Species	Positive horses (N=23)	Prevalence (%)*	Nº of specimens	% of the total number	Mean Intensity **	Mean Abundance ***
Cyathostomum catinatum	14	60.9	159	6.05	11.36	6.91
Cylicostephanus longibursatus	13	56.5	384	14.61	29.54	16.70
Cylicocyclus asworthi	12	52.2	239	9.09	19.92	10.39
Cyathostomum pateratum	11	47.8	334	12.71	30.36	14.52
Cylicostephanus calicatus	11	47.8	31	1.18	2.82	1.35
Coronocyclus coronatus	8	34.8	24	0.91	3.00	1.04
Cylicocyclus leptostomum	8	34.8	80	3.04	10.00	3.48
Coronocyclus labiatus	7	30.4	20	0.76	2.86	0.87
Cylicocyclus elongatus	7	30.4	21	0.80	3.00	0.91
Cylicostephanus goldi	7	30.4	34	1.29	4.86	1.48
Cyathostomum spp.(L4/L5)	7	30.4	17	0.65	2.43	0.74
Coronocyclus labratus	6	26.1	8	0.30	1.33	0.35
Cylicostephanus minutus	6	26.1	54	2.05	9.00	2.35
Triodontophous serratus	5	21.7	22	0.84	4.40	0.96
Cylicocyclus radiatus	5	21.7	6	0.23	1.20	0.26
Petrovinema poculatum	3	13	3	0.11	1.00	0.13
Triodontophorus brevicauda	2	8.7	3	0.11	1.50	0.13
Poteriostomum imparidentatum	2	8.7	5	0.19	2.50	0.22
Craterostomum acuticaudatum	1	4.3	1	0.04	1.00	0.04
Strongylus vulgaris	1	4.3	6	0.23	6.00	0.26
Cylicocyclus brevicapsulatus #	1	4.3	10	0.38	10.00	0.43
Parapoteriostomum mettami	1	4.3	1	0.04	1.00	0.04
Cylicostephanus bidentatus #	1	4.3	1	0.04	1.00	0.04

*Number of positive hosts/number of sampled hosts; **Mean number of parasites/number of infected hosts;***Mean number of parasites/total number of hosts. # First record in Portugal.

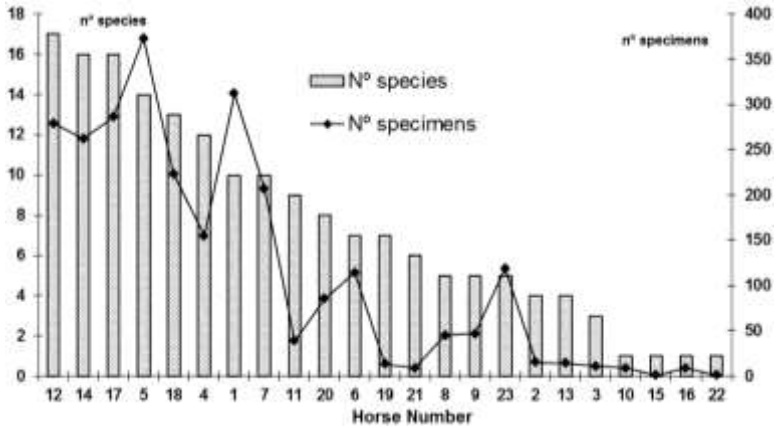


Figure 1. Number of strongyle species and specimens per host in horse stud farm.

Table 3. Strongyle prevalence, mean intensity of infection and confidence intervals (CI)* in 23 sampled horses

	Prevalence	Conf. Inter. 95%	Mean	Conf. Int. 95%
	(%)	Prevalence	Intensity	Mean Intensity
Craterostomum acuticaudatum	4.3	0.11-21.95	1.00	**
Strongylus vulgaris	4.3	0.11-21.95	6.00	**
Triodontophorus brevicauda	8.7	1.07-28.04	1.50	1-1.50
Triodontophous serratus	21.7	7.46-43.71	4.40	1.40-7.80
Cyathostomum catinatum	60.9	38.54-80.30	11.36	7.00-15.79
Cyathostomum pateratum	47.8	26.81-69.42	30.36	8.00-56.00
Coronocyclus coronatus	34.8	16.37-57.27	3.00	1.25-4.88
Coronocyclus labiatus	30.4	13.21-52.92	2.86	1.57-4.14
Coronocyclus labratus	26.1	10.22-48.41	1.33	1-1.50
Cylicocyclus asworthi	52.2	30.58-73.19	19.92	7.42-32.75
Cylicocyclus brevicapsulatus	4.3	0.11-21.95	10.00	**
Cylicocyclus elongatus	30.4	13.21-52.92	3.00	1.43-4.57
Cylicocyclus insigne	82.6	61.21-95.05	13.21	7.32-19.05
Cylicocyclus leptostomum	34.8	16.37-57.27	10.00	5.38-13.88
Cylicocyclus nassatus	73.9	51.59-89.78	53.76	29.24-79.00
Cylicocyclus radiatus	21.7	7.46-43.71	1.20	1.00-1.40
Parapoteriostomum mettami	4.3	0.11-21.95	1.00	**
Cylicostephanus bidentatus	4.3	0.11-21.95	1.00	**
Cylicostephanus calicatus	47.8	26.81-69.42	2.82	1.64-3.82
Cylicostephanus goldi	30.4	13.21-52.92	4.86	1.00-10.43
Cylicostephanus longibursatus	56.5	34.49-76.81	29.54	3.54-66.77
Cylicostephanus minutus	26.1	12.02-45.10	9.00	4.00-13.50
Petrovinema poculatum	13	0.11-21.95	1.00	**
Poteriostomum imparidentatum	8.7	1.07-28.04	2.50	2.00-2.50
Cyathostomum spp.(L4/L5)	30.4	13.21-52.92	2.43	1.00-3.86

*Analysis performed according to the methodology of Rózsa et al. (2000) and "Reiczigel J, Rózsa L 2005. Quantitative Parasitology 3.0, Budapest"; **Small sample size prevented the calculation of the confidence interval.

Table 4. Interspecific differences in strongyle prevalence at a horse stud farm level

	Cra.acu	S.vulg	T.brev	T.ser	Cy.cat	Cy.pat	Co.coro	Cor.labi	Cor.labr	Cc.ashw	Cc.bre	Cc.elo	Cc.insi	Cc.lep	Cc.nas	Cc.rad	Para.met	Cs.bid	Cs.cal	Cs.gol	Cs.lon	Cs.min	Petr.poc	Pot.imp	L4/L5
Cra.acu	ns	ns	ns	***	***	*	*	ns	***	ns	*	***	*	***	ns	ns	ns	**	*	***	ns	ns	ns	*
S.vulg	ns	ns	ns	***	***	*	*	ns	***	ns	*	***	*	***	ns	ns	ns	**	*	***	ns	ns	ns	*
T.brev	ns	ns	ns	***	***	ns	ns	ns	**	ns	ns	***	ns	***	ns	ns	ns	**	ns	***	ns	ns	ns	ns
T.ser	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Cy.cat	ns	ns	ns	*	ns	ns	ns	*	ns	***	ns	ns	ns	ns	*	***	***	ns	ns	ns	*	***	***	ns
Cy.pate	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	*	ns	ns	ns	**	**	ns	ns	ns	ns	*	**	ns
Co.coro	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	***	ns	**	ns	*	*	ns	ns	ns	ns	ns	ns	ns
Cor.labi	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	***	ns	**	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
Cor.labr	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cc.ashw	***	ns	**	ns	ns	ns	ns	ns	ns	ns	***	ns	*	ns	ns	ns	***	**	ns	ns	ns	ns	*	***	ns
Cc.bre	ns	*	ns	ns	ns	***	ns	*	ns	***	ns	*	***	*	***	ns	ns	ns	**	*	***	ns	ns	ns	*
Cc.elo	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	***	ns	**	ns	*	*	ns	ns	ns	ns	ns	ns	ns
Cc.insi	***	***	***	***	***	ns	***	***	***	*	***	***	ns	***	ns	***	***	***	***	***	ns	***	***	***	***
Cc.lep	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	***	ns	*	ns	*	*	ns	ns	ns	ns	ns	ns	ns
Cc.nas	***	ns	***	ns	ns	ns	ns	ns	ns	ns	***	**	ns	*	ns	***	***	***	ns	**	ns	**	***	***	*
Cc.rad	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Para.met	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	***	*	***	ns	ns	**	*	***	ns	ns	ns	ns	ns
Cs.bid	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	***	*	***	ns	ns	***	*	***	ns	ns	ns	ns	*
Cs.cal	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	ns	ns	ns	ns	ns	ns	*	**	ns	ns
Cs. gol	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cs.lon	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	ns	ns	ns	ns	ns	ns	ns	**	***	ns
Cs.min	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Petr.poc	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Pot.imp	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
L4/L5	*	*	ns	ns	ns	ns	ns	ns	ns	***	*	ns	***	ns	*	ns	ns	*	ns	ns	ns	ns	ns	ns	ns

ns - non significant (p>0,05)

*Prevalence with significant differences p<0,05

**Prevalence with significant differences p<0,01

***Prevalence with significant differences p<0,001

Calculations performed based on RÓZSA *et al* (2000) and software "Reiczigel J, Rózsa L 2005. Quantitative Parasitology 3.0, Budapest"

Cra.acut-Craterostomum acuticaudatum; S.vulg-Strongylus vulgaris; T.brev-Triodontophorus brevicauda; T.ser-Triodontophous serratus
Cy.cat-Cyathostomum catinatum; Cy.pate-Cyathostomum pateratum; Co.coro-Coronocyclus coronatus; Cor.labi-Coronocyclus labiatus
Cor.labr-Coronocyclus labratus; Cc.ashw-Cylicocyclus asworthi; Cc.bre-Cylicocyclus brevicapsulatus; Cc.elo-Cylicocyclus elongatus;
Cc.insi-Cylicocyclus insigne; Cc.lep-Cylicocyclus leptostomus; Cc.nas-Cylicocyclus nassatus; Cc.rad-Cylicocyclus radiatus;
Para.met-Parapoteriostomum mettami; Cs.bid-Cylicostephanus bidentatus; Cs.cal-Cylicostephanus calicatus; Cs. gol-Cylicostephanus goldi;
Cs.lon-Cylicostephanus longibursatus; Cs.min-Cylicostephanus minutus; Pet.poc-Petrovinema poculatum; Pot.imp-Poteriostomum imparidentatum; L4/L5-Cyathostomum spp.(L4/L5)

Table 5. Interspecific differences in infection intensity of strongyles at a horse stud farm level

	S.vulg	T.brev	T.ser	Cy.ca	Cy.pa	Co.coro	Cor.lat	Cor.lab	Cc.ash	Cc.bre	Cc.elo	Cc.in	Cc.lep	Cc.nas	Cc.rad	Para.me	Cs.bid	Cs.cal	Cs.go	Cs.lon	Cs.mi	Pet.po	Pot.im	L4/L5
Cra.acut	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S.vulg	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
T.brev	ns	ns	******	ns	ns	ns	ns	ns	ns	ns	ns	******	**	******	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
T.ser	*****	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*****	ns	******	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cy.cat	ns	******	******	****,#**	ns	ns	ns	ns	ns	ns	ns	ns	ns	*****	******	ns	ns	****,##**	ns	ns	ns	******	******	******
Cy.pat	#	#	#	ns	ns	ns	ns	ns	ns	ns	#	ns	ns	ns	#	ns	ns	ns	ns	ns	ns	ns	ns	ns
Co.coro	ns	ns	**#**	ns	ns	ns	ns	ns	ns	ns	***,#**	*,#	****,#**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cor.labi	ns	**##**	ns	ns	ns	ns	ns	ns	ns	ns	*****	*	****,##**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cor.labr	**##**	ns	ns	ns	ns	ns	ns	ns	ns	ns	***,#**	**,##	****,#**	ns	ns	ns	ns	*	ns	#	ns	ns	ns	ns
Cc.ashw	ns	**#**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*****	**#**	ns	ns	ns	**##**	ns	ns	ns	ns	ns	ns
Cc.bre	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cc.elo	*****	*	******	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cc.insi	ns	*****	*****	***,#**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***,#**	ns	ns	ns	*****	*****	*****
Cc.lep	*****	**,#	*****	**,#	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	**	*	*
Cc.nas	****,#**	ns	******	****,##**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	****,##**	******	ns	******	******	******	******
Cc.rad	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
Para.met	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cs.bid	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cs.cal	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
Ct.gol	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cs.lon	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cs.min	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Petr.po	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Pot.imp	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
L4/L5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*Aritmetic mean of mean intensity by the Bootstrap method with significant differences p<0,05

**Aritmetic mean of mean intensity by the Bootstrap method with significant differences p<0,01

#Median of mean intensity by the Mood with significant differences p<0,05

##Median of mean intensity by the Mood with significant differences p<0,01

Calculations performed based on RÓZSA *et al* (2000) and

software "Reiczigel J, Rózsa L 2005. Quantitative Parasitology 3.0, Budapest"

Cra.acut-Craterostomum acuticaudatum; S.vulg-Strongylus vulgaris; T.brev-Triodontophorus brevicauda; T.ser-Triodontophous serratus

Cy.cat-Cyathostomum catinatum; Cy.pate-Cyathostomum pateratum; Co.coro-Coronocyclus coronatus; Cor.labi-Coronocyclus labiatus

Cor.labr-Coronocyclus labratus; Cc.ashw-Cylicocyclus asworthi; Cc.bre-Cylicocyclus brevicapsulatus; Cc.elo-Cylicocyclus elongatus;

Cc.insi-Cylicocyclus insigne; Cc.lep-Cylicocyclus leptostomus; Cc.nas-Cylicocyclus nassatus; Cc.rad-Cylicocyclus radiatus;

Para.met-Parapoteriostomum mettami; Cs.bid-Cylicostephanus bidentatus; Cs.cal-Cylicostephanus calicatus; Cs. gol-Cylicostephanus goldi;

Cs.lon-Cylicostephanus longibursatus; Cs.min-Cylicostephanus minutus; Pet.poc-Petrovinema poculatum; Pot.imp-Poteriostomum imparidentatum; L4/L5-Cyathostomum spp.(L4/L5)

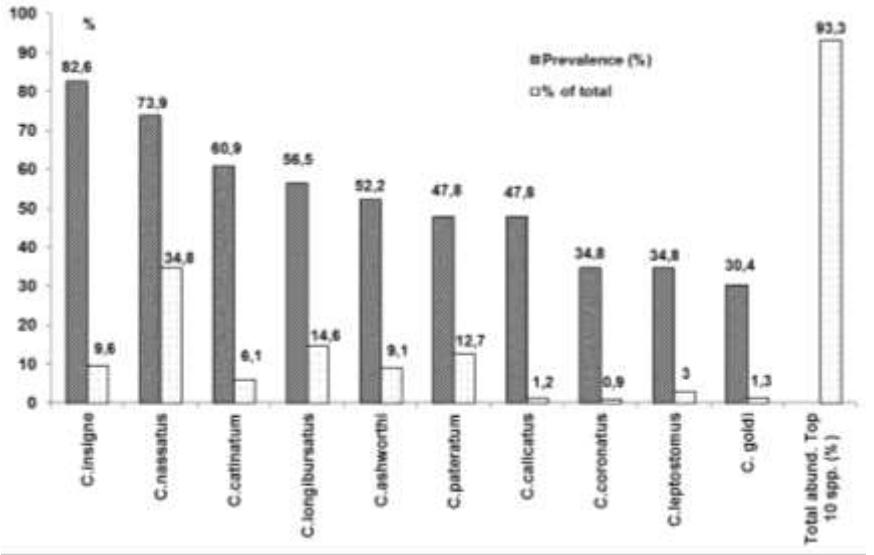


Figure 2. Ten most prevalent species and % of total collected strongyles (Last column represents the sum of individual abundances for these species).

Table 6. Total number and ratio male/female of collected strongyles at a horse stud farm level

	Total Nº Strongyles	Males	Females	Ratio males/females
Craterostomum acuticaudatum	1	0	1	*
Strongylus vulgaris	6	2	4	1:2
Triodontophorus brevicauda	3	0	3	*
Triodontophous serratus	22	12	10	1:0.8
Cyathostomum catinatum	117	14	103	1:7.4
Cyathostomum pateratum	255	70	185	1:2.6
Coronocyclus coronatus	23	8	15	1:1.9
Coronocyclus labiatus	20	9	11	1:1.2
Coronocyclus labratus	6	1	5	1:5
Cylicocyclus asworthi	239	61	178	1:2.9
Cylicocyclus brevicapsulatus	10	4	6	1:1.5
Cylicocyclus elongatus	21	6	15	1:2.5
Cylicocyclus insigne	240	90	150	1:1.7
Cylicocyclus leptostomum	80	9	71	1:7.9
Cylicocyclus nassatus	898	207	691	1:3.3
Cylicocyclus radiatus	6	3	3	1:1
Parapoteriostomum mettami	1	0	1	*
Cylicostephanus bidentatus	1**	---	---	---
Cylicostephanus calicatus	31	5	26	1:5.2
Cylicostephanus goldi	33	7	26	1:3.7
Cylicostephanus longibursatus	380	145	235	1:1.6
Cylicostephanus minutus	54	13	41	1:3.2
Petrovinema poculatum	3	0	3	*
Poteriostomum imparidentatum	5	2	3	1:1.5
Cyathostomum spp. (L4/L5)	17**	---	---	---
Totals	2472	668	1786	

*No males collected; **Undetermined sex.

Most strongyles showed a ratio female:male greater than 1, with the exception of *Triodontophorus serratus* and *Cylicocyclus radiatus* (Table 6). However, this strongyle population showed a positive correlation between the most prevalent species and their absolute abundance in the studied samples (r=0.719, r^2= 0.517 and p <0.0001) and between the number of specimens and number of species collected per host (r=0.8713, r^2=0.759 and p <0.0001) (Figures 3 and 4).

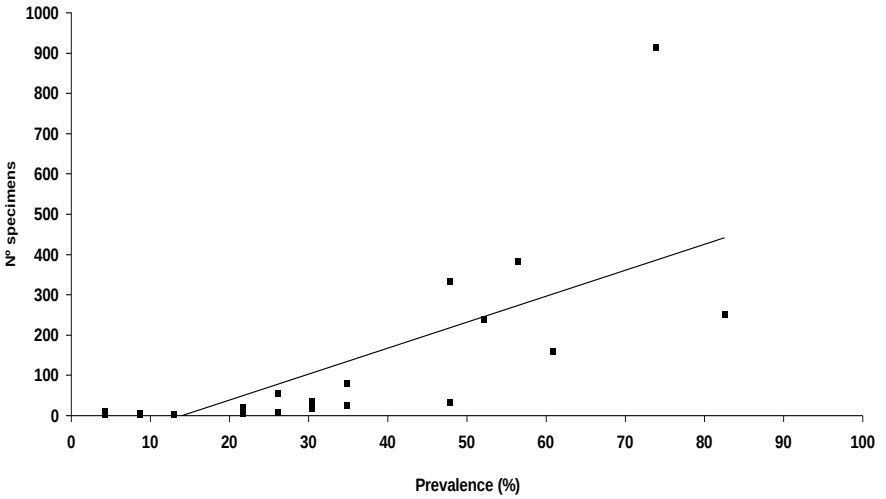


Figure 3. Correlation between strongyle species abundance and their prevalence.

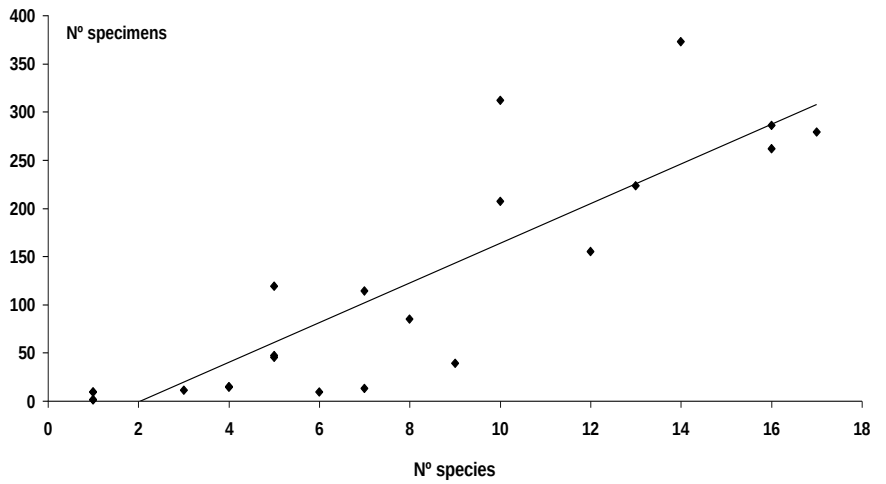


Figure 4. Correlation between strongyle abundance/horse and number of strongyle species/horse.

In Tables 7 and 8 are recorded the total collected species and their infection rates at the level of CL horse stud farm, according to the environment (barn or pasture), age (≤ 1 year and > 1year) and type of helminth shedding (after anthelmintic treatment or natural shedding).

Table 7. Infection rate, mean number of species and specimens identified by host, management, age and anthelmintic treatment

	Environment		Age		Shedding after anthelmintic treatment*		Natural shedding Totals	
	Barn	Pasture	≤ 1year	> 1year	IVM	DRM		
N	9	14	12	11	9	11	3	23
Number of positive	9	14	12	11	9	11	3	23
Nº species								
Total	18	23	22	17	18	22	11	25
Per horse								
Min-Max	1-14	1-17	1-17	1-14	1-14	1-16	1-10	1-17
Arithmetic mean ± SEM	6.8 ± 1.37	8.2 ±1.5	8.3 e 1,8	6.8 e 1.2	6.7 e 1.5	8.3 e 1.6	5.3 e 2.6	7.6 e 1.1
Nº specimens/ horse								
Min-Max	9-371	1-312	1-312	9-373	9-373	1-286	1-312	1-373
Arithmetic mean ± SEM	108.4 ± 40.5	158.3 ± 36.8	124.5 ± 38.6	103.1 ± 33.3	108.4 ± 40.5	110.9 ± 37.1	144 ± 90.8	114 ± 25.2

*No efficacy or comparative efficay studies were performed with the anthelmintics displayed on the table. IVM – Ivermectin - Eqvalan® Oral Paste, Merial; DRM – Doramectin - Dectomax, Injectable solution® SC/IM, Zoetis (ex-Pfizer). SEM – Standar Error Mean.

Table 8. Strongyle species identified at the level of CL horse stud farm according to the environment, age e and type of shedding (natural or after deworming)

	Species	Environment		Age		Dewormer*		Natural
		Pasture	Barn	≤ 1 year	> 1 year	IVM	DRM	shedding
Craterostomum acuticaudatum	+	+	-	+	-	-	-	+
Strongylus vulgaris	+	-	+	-	+	+	-	-
Triodontophorus brevicauda	+	+	-	+	-	-	+	-
Triodontophous serratus	+	+	-	+	-	-	+	-
Cyathostomum catinatum	+	+	+	+	+	+	+	+
Cyathostomum pateratum	+	+	+	+	+	+	+	+
Coronocyclus coronatus	+	+	+	+	+	+	+	-
Coronocyclus labiatus	+	+	+	+	+	+	+	-
Coronocyclus labratus	+	+	+	+	+	+	+	-
Cylicocyclus asworthi	+	+	+	+	+	+	+	-
Cylicocyclus brevicapsulatus	+	-	+	-	+	+	-	-
Cylicocyclus elongatus	+	+	+	+	+	+	+	-
Cylicocyclus insigne	+	+	+	+	+	+	+	+
Cylicocyclus leptostomum	+	+	+	+	+	+	+	-
Cylicocyclus nassatus	+	+	+	+	+	+	+	+
Cylicocyclus radiatus	+	+	+	+	+	+	+	+
Parapoteriostomum mettami	+	+	-	+	-	-	+	-
Cylicostephanus bidentatus	+	+	-	-	+	-	+	-
Cylicostephanus calicatus	+	+	+	+	+	+	+	+

Table 8. (Continued)

	Species	Environment		Age		Dewormer*		Natural
		Pasture	Barn	≤ 1 year	> 1 year	IVM	DRM	shedding
Cylicostephanus goldi	+	+	+	+	+	+	+	+
Cylicostephanus longibursatus	+	+	+	+	+	+	+	+
Cylicostephanus minutus	+	+	+	+	+	+	+	+
Petrovinema poculatum	+	+	-	+	-	-	+	-
Poteriostomum imparidentatum	+	+	-	+	-	-	+	-
Cyathostomum spp. (L4/L5)**	+	+	+	+	+	+	+	+
Totals	24	22	17	21	17	17	21	10

*No efficacy or comparative efficay studies were performed with the anthelmintics displayed on the table. IVM – Ivermectin - Eqvalan® Oral Paste, Merial; DRM – Doramectin - Dectomax, Injectable solution® SC/IM, Zoetis (ex-Pfizer). **These larval stages were not considered for the purpose of species identification, although they were present in every group.

Considering the two subfamilies involved, all nematodes of Strongylinae were found in animals on the pasture, except for *Strongylus vulgaris*, only recorded in animals in the barn. In the case of the Cyathostominae, all species identified in this work were found in grazing animals, except for *Cylicocyclus brevicapsulatus*. Horses in the barn did not show *Parapoteriostomum mettami*, *Cylicostephanus bidentatus*, *Petrovinema poculatum* and *Poteriostomum imparidentatum* (Table 8). The analysis by Fisher Exact test of strongyle populations, between grazing and housed horses, revealed no significant differences in the prevalence and intensity of infection of strongyle species ($p > 0.05$), with the exception of *Cylicostephanus calicatus*, *Cyathostomum catinatum and C. pateratum*, that showed higher intensity infection in housed horses than in grazing animals. Beyond this fact, we observed that horses on pasture presented 22 strongyle species, slightly above the 17 species found in animals kept in the barn (Table 8 and Figures 5 and 6).

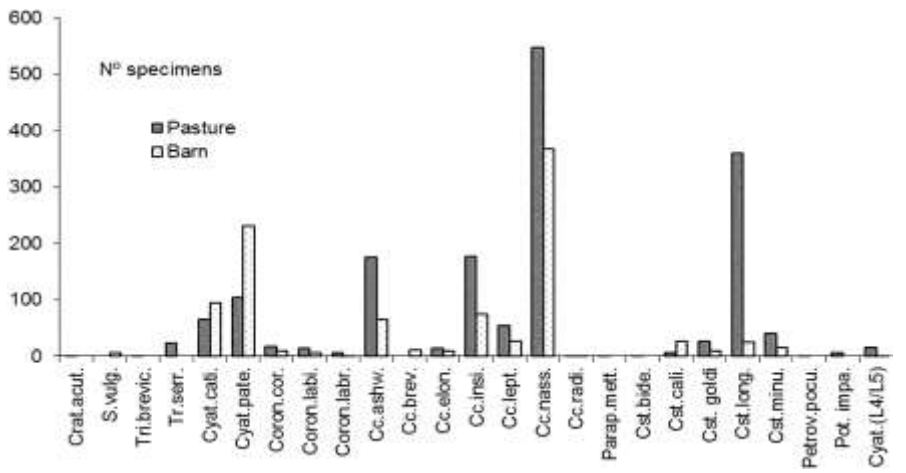


Figure 5. Abundance of horse strongyles in stabled (Barn, N=9) and grazing horses (Pasture, N=14) in CL.

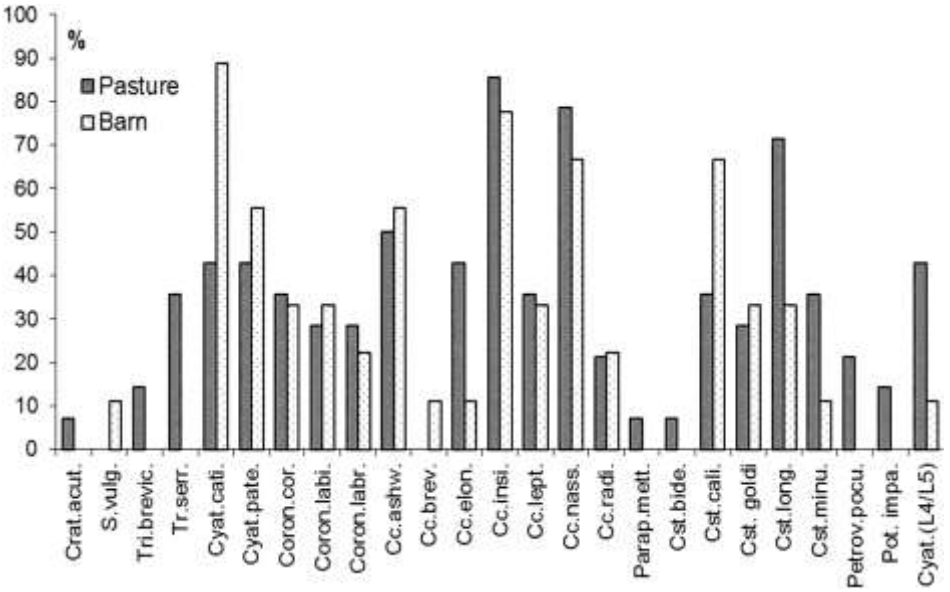


Figure 6. Prevalence of horse strongyles in penned (Barn, N=9) and grazing horses (Pasture, N=14) in CL.

Regarding age, there were no significant differences in the mean intensity of infection, except for species *C. calicatus* and *Cyathostomum catinatum* ($p<0.05$), whose presence was noticed mainly in horses older than 1 year of age (Table 8 and Figure 7).

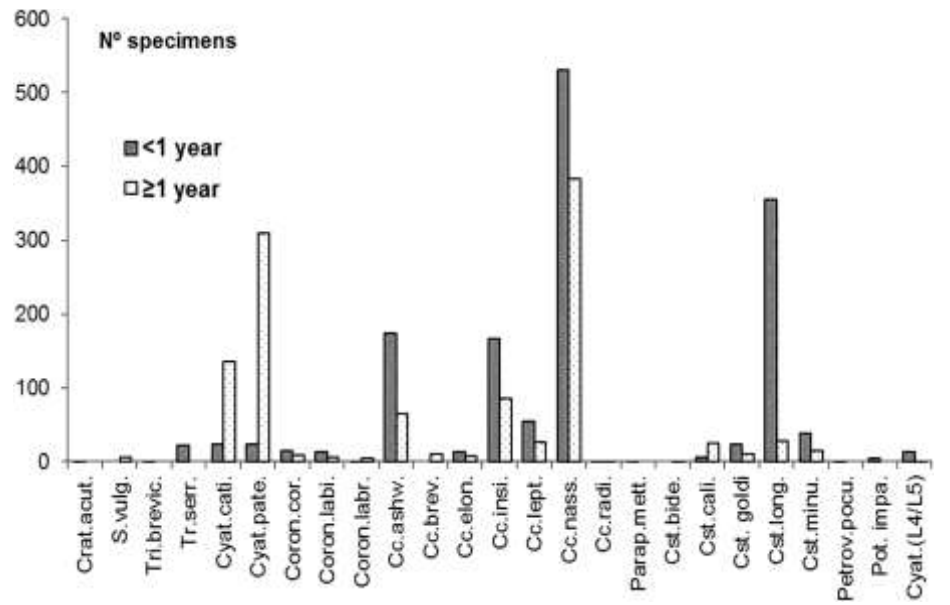


Figure 7. Abundance of horse strongyles in horses < 1year (N=12) and ≥ 1 year (N=11) in CL.

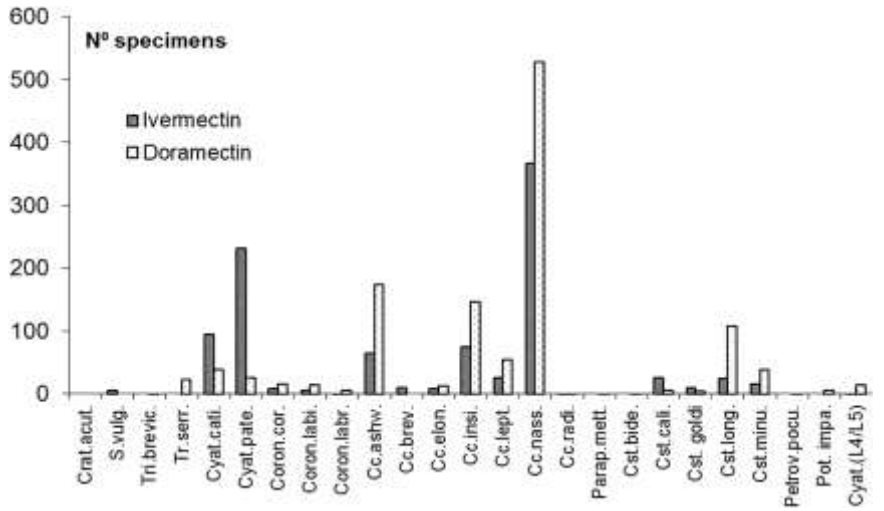


Figure 8. Abundance of horse strongyles in horses dewormed with Ivermectin (N=9) amd Doramectin (N=11) in CL.

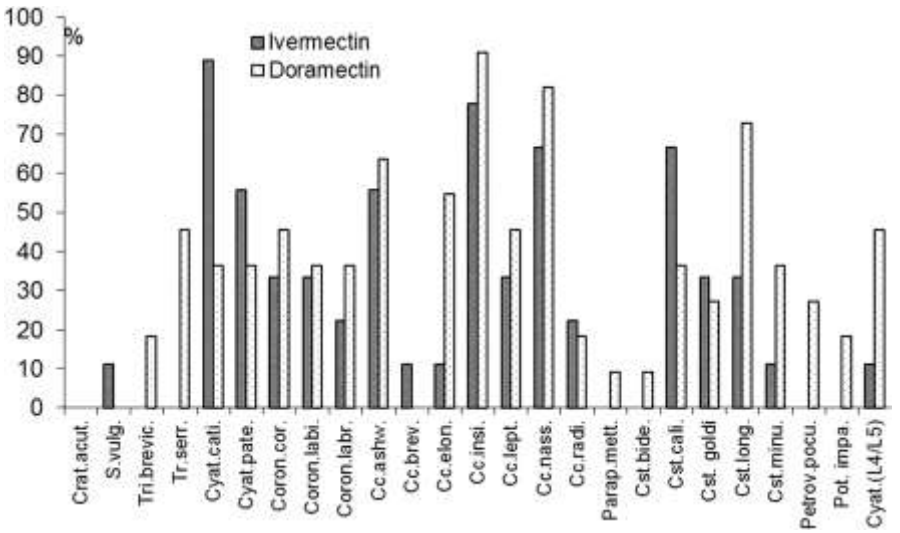


Figure 9. Prevalence of horse strongyles in horses dewormed with Ivermectin (N=9) and Doramectin (N=11) in CL.

We recorded 21 species of strongyles in animals up to 1 year of age, exceeding the 17 species marked in animals over one year old. Only 10 strongyle species were naturally shed, while in dewormed horses, 17 were spotted in animals treated with ivermectin and 21 in horses treated with doramectin.

Regarding strongyle subpopulations eliminated with different treatments, there was no significant difference ($p < 0.05$) (Table 8 and Figures 8 and 9).

DISCUSSION

This study based solely in equines eliminating strongyles in the faeces, allowed the identification of 24 species of strongyles, 4 belonging to subfamily Strongylinae and 20 to the subfamily Cyathostominae. Cyathostomins accounted for 99.3% of the total population of recovered strongyles, while nematodes od subfamily Strongylinae accounted only for 0.7% of the total.

Strongylus vulgaris was only found in one host, which seems to reveal some decrease in its importance since twenty years ago when a first study of this kind was conducted (Madeira de Carvalho, 1991).

After the analysis of Table 9 we can see some diversity in the prevalence values of these nematodes all over the world. However, if we look at the ranges and average numbers of species isolated (mostly in studies performed in abattoirs), Strongylinae range from 2-8 species (average 5.9) and Cyathostominae range form 20-32 (average 25.2). And in studies with strongyles shed in the faeces, Strongylinae range from 0-6 species (average 2.8) and Cyathostominae range from 16-20 (average 18) (Table 10). Therefore, our results are very close to the ones obtained in slaughterhouses and higher than the ones only performed with faecal specimens, since our total and partial number of species for Strongylinae and Cyathostominae were, respectively, 24, 4 and 20.

Concerning the *S. vulgaris* infection profile on horse stud farms, other authors showed values between 23 and 100% of animals infected with this nematode. In fact, this parasite that is recognized as highly pathogenic, can infect 84-100% (Tolliver *et al.*, 1987; Lyons *et al.*, 1994) or 95-100 % of the animals (Rodrigues *et al.*, 1994; Souto Maior *et al.*, 1995; Çirak *et al.*, 1996; Dorny *et al.*, 1999), mainly in farms where animals are not dewormed regularly with effective anthelmintics on L4/L5 larval stages of *S. vulgaris* or there is poor hygiene and sanitation regarding horse production.

However, parasitological surveys after necropsies, have shown a progressive reduction in *S. vulgaris* prevalence in horses belonging to farms where animals are dewormed regularly with macrocylic lactones, i.e., ivermectin/moxidectin, with prevalence rates ranging between 2.3-5.8 % of infected animals, meeting our results (Höglund *et al.*, 1997; Lyons *et al.*, 2000). Other Strongylinae like *Craterostomum acuticaudatum*, *Triodontophorus brevicauda* and *T. serratus* had also been reported previously in our country, but with higher prevalence (Madeira de Carvalho, 1991). The analysis of Table 9 shows that, prevalence of these 3 species ranges between 1.8-70%, 3-72.7% and 3.6-81%, respectively.

In other research conducted on the identification of strongyles eliminated in the faeces (Table 10), some authors show values close to ours (Anderson & Hasslinger, 1982; Osterman Lind *et al.*, 2003), or even higher for some species (like *S. vulgaris*, reaching a prevalence rate of 27.3% (Kuzmina *et al.*, 2005), while others do not indicate their presence (Gawor, 1997; Velazquez *et al.*, 1997; Kornas *et al.*, 2011).

This research only based in faecal shedding of horse strongyles confirmed the predominance of Cyathostominae, constituting 99.3 % of total observed strongyles, as originally reported in a survey based in animals from a slaughterhouse and whose expression level through infective larval stages (L3), had also been found in that farm area, and reported elsewhere (Madeira de Carvalho, *et al.*, 1997,1999, 2001; Madeira de Carvalho, 1991; 2010).

Table 9. Prevalence of some species of equine strongyles in several countries - Strongylinae

	Nr Horses	Craterostomum acuticaudatum	Strongylus edentatus	Strongylus equinus	Strongylus vulgaris	Triodontophorus brevicauda	T. minor	T. nipponicus	T. serratus	T. tennuicollis	Nr species Strongy-linae
Present research	23	4.3	---	---	4.3	8.7	---	---	21,7	---	4
Portugal [1]	11	9.1	63.6	---	81.8	72.7	---	---	63,6	27,3	6
Poland [2]	50	70	22	28	78	14	---	12	36	---	7
Poland [3]	50	38	40	14	74	20	---	2	36	---	7
UK [4]	55	---	---	---	---	---	---	---	---	---	---
Germany [5]a	34	---	---	---	---	8.8	---	---	23,5	---	2
Germany [6]	16	25	44	---	100	56	19	---	81	19	7
Belgium [7]	11	64	100 b)	---	100 b)	55	---	---	55	---	5
Italy [8]	40	---	27.5	15	97.5	7,5	---	---	80	---	5
USA [9]	55	1.8	10.9	1.8	27	---	1.8	---	3.6	---	6
USA [10]	37	14	84	19	84	46	---	---	43	38	7
Panama [11]	86	6	76	80	100	15	80	---	42	15	8
Brazil [12]	14	---	---	---	---	---	---	---	---	---	---
Brazil [13]	36	---	---	---	---	---	---	---	---	---	---
Brazil [14]	33	---	12.1	12.1	84.8	---	---	---	18.2	15.1	5
S. Africa [15]	17	18	24	30	94	---	---	35	---	---	5

Present research	Nr Horses	Craterostomum acuticaudatum	Strongylus edentatus	Strongylus equinus	Strongylus vulgaris	Triodontophorus brevicauda	T. minor	T. nipponicus	T. serratus	T. tennuicollis	Nr species Strongy-linae
Australia [16]	57	---	22	22	28	11	15	13	30	---	7
a) Study based on strongyles eliminated in the faeces											
1] Madeira de Carvalho (1991); [2] Sobieszewski (1967); [3] Gawor (1995); [4] Ogbourne (1976); [5] Anderson & Hasslinger (1982); [6] Çirak et al. (1996); [7] Dorny et al. (1999);											
Ricci & Sabatini (1992); [9] Reinemeyer et al. (1984); [10] Torbert et al. (1986); [11] Foster & Ortiz (1937); [12] Carvalho et al. (1998); [13] Silva et al. (1999); [14] Souto-Maior et al. (1999);											
[15] Krecek et al. (1989); [16] Mfitilodze & Hutchinson (1990); [17] Bucknell et al. (1995)											

Table 10. Prevalence of some species of equine strongyles shed in faeces

	Present research	Germany [1]	Sweden [2]	Ukraine [3]	Poland [4]
Number of Horses	23	34	27	44	14
Cyathostomum alveatum	---	---	---	---	---
Cyathostomum catinatum	60.9	64.7	100	100	100
Cyathostomum pateratum	47.8	76.5	48	45.5	93
Cyathostomum sagittatum	---	---	---	---	---
Cyathostomum tetracanthum	---	---	---	---	---
Coronocyclus coronatus	34.8	32.4	57	72.7	43
Coronocyclus labiatus	30.4	67.7	57	50	57
Coronocyclus labratus	26.1	2.9	50	38.6	57
Cylicocyclus asworthi	52.2	---	40	93.2	64
Cylicocyclus auriculatus	---	---	---	---	---
Cylicocyclus brevicapsulatus	4.3	---	---	---	7
Cylicocyclus elongatus	30.4	20.6	---	25	21
Cylicocyclus insigne	82.6	5.9	38	36.4	43
Cylicocyclus leptostomum	34.8	82.4	85	84.1	64
Cylicocyclus nassatus	73.9	100	100	100	86
Cylicocyclus radiatus	21.7	88.2	---	4.5	29
Cylicocyclus triramosus	---	---	---	---	---
Cylicocyclus ultrajectinus	---	---	---	---	---
Cylicodontophorus bicoronatus	---	---	---	---	---
Parapoteriostomum euproctus	---	---	---	---	---
Parapoteriostomum mettami	4.3	---	15	---	21
Cylicostephanus asymetricus	---	---	---	---	---
Cylicostephanus bidentatus	4.3	---	---	---	---
Cylicostephanus calicatus	47.8	76.5	80	84.1	64
Cylicostephanus goldi	30.4	---	60	75	86
Cylicostephanus hybridus	---	---	---	11.4	---
Cylicostephanus longibursatus	56.5	58.8	95	93.2	93
Cylicostephanus minutus	26.1	14.7	80	81.8	50
Petrovinema poculatum	13	8.8	5	27.3	7
Poteriostomum imparidentatum	8.7	23.5	---	18.2	21
Poteriostomum ratzii	---	17.7	---	---	---
Gyalocephalus capitatus	---	---	<1	2.3	---
Cyathostomum spp.(L4/L5)	30.4	41.2	---	---	---
Nº species Cyathostominae	20	16	16	19	19
Craterostomum acuticaudatum	4.3	---	<1	---	---
Strongylus edentatus	---	---	---	13.6	---
S. equinus	---	---	---	15.9	---
S. vulgaris	4.3	---	---	27.3	---
Triodontophorus brevicauda	8.7	8.8	---	15.9	---
T. nipponicus	---	---	---	6.8	---
T. serratus	21.7	23.5	<1	15.9	---
Nº species Strongylinae	4	2	2	6	0
Nº total species	24	18	18	25	19
[1] Anderson & Hasslinger (1982); [2] Osterman Lind et al. (2003); [3] Kuzmina et al. (2005); [4] Kornas et al. (2011)					

Although based on nematodes shed in the faeces, this work allowed the identification of 20 species of cyathostomins. When compared to similar studies , we found a superior number

compared with Gawor (1997) in Poland (12 cyathostomin species), Hasslinger & Anderson (1982) in Germany (16), Osterman Lind *et al* (2003) in Sweden (16), Kuzmina *et al.* (2005) in Ukraine (19) and Kornas *et al.* (2011) in Poland (19), being identical to the one of Velazquez *et al.* (1997) in Argentina (20).

In a previous study of on horse strongyles conducted in Portugal, based on samples collected after necropsy in a slaughterhouse, 22 cyathostomin species were reported, of which *Cylicodontophorus bicoronatus*, *Parapoteriostomum euproctus*, *Poteriostomum ratzii* and *Gyalocephalus capitatus* were species not reported in the present research (Madeira de Carvalho, 1991). In contrast *Cylicocyclus brevicapsulatus* and *Cylicostephanus bidentatus* were recorded for the first time in Portugal, which we consider an important finding relative to the horse parasite fauna in Portugal, namely due to dificulties posed by the preservation of these nematodes.

According to Ogbourne (1978), the ten most prevalent and widely distributed species of cyathostomins worldwide, include: *Cylicostephanus longibursatus, Cyathostomum catinatum, Cylicostephanus goldi, Cylicocyclus nassatus, Coronocyclus coronatus, Cylicostephanus calicatus, Cylicostephanus minutus, Cylicocyclus leptostomum, Cyathostomum pateratum* and *Cylicocyclus insigne*. All these cyathostomin species have been reported in this work and in the ten most prevalent species group (except for *C. minutus*, although this was on the border). *Cylicocyclus insigne* showed the highest prevalence (82.6%) and *C. nassatus* was the most abundant (about 35 % of total) and the second most prevalent. The analysis of Tables 4 and 5, show that *C. insigne* had significant differences with respect to prevalence of 20 species of strongyles observed and *C. nassatus* showed significant differences with regard to the relative intensity of infection by other 17 species of cyathostomins.

When compared with results from previous research, we found slight differences in the prevalence and rank positions occupied by the ten most prevalent cyathostomin species (Madeira de Carvalho, 1991). In the present study *C. coronatus* and *C. pateratum* were considered in this group, over *C. elongatus* and *C. minutus*. However, 7 of the 10 species considered most important were in this group, with no significant differences in their prevalence (Fisher's Exact test) (Figure 10). After analysis of Table 11, we can see that this pattern of dominance concerning ten cyathostomin species has remained constant in various points of the globe, although some studies may show slight differences.

Our results are comparable to Hasslinger & Anderson (1982) (as regards the abundance of the species *C. nassatus*, *C. catinatum* and *C. longibursatus*), Çirak *et al.* (1996) and Velazquez *et al.* (1997) (concerning prevalence of *C. insigne*, *C. nassatus* and *C. catinatum*). The same pattern can be found in more recent surveys and nine of ten species more prevalent and abundant in our research are considered the core species, being found with an abundance > 2% (Kornas *et al.*, 2011).

The results are similar to previous studies concerning abundance and intensity of some cyathostomin species, even the ones based on nematodes collected from horses after necropsy, where a small number of cyathostomin species tends to dominate the population of small strongyles in a horse farm. Taken all together, the most prevalent cyathostomins constituted 93.3% of the observed specimens, which is in agreement with other studies. Foster (1936) recorded 15 species of small strongyles that formed 98% of the total observed. Reinemeyer *et al.* (1984) reported 10 species of cyathostomins responsible for 98.9 % of the total population and Mfitilodze & Huchinson (1990), reported 11 species which constituted 94% of the total.

Table 11. Prevalence of some species of equine strongyles in several countries - Cyathostominae

	Present research	Port. [1]	Pol. [2]	Pol. [3]	UK [4]	Ger. [5]a	Ger. [6]	Belg. [7]	Fra. [18]	Italy [8]	USA [9]	USA [10]	Pan. [11]	Brazil [12]	Brazil [13]	Brazil [14]	So. Af. [15]	Austra. [16]	Austra. [17]
Number of Horses	23	11	50	50	55	34	16	11	42	40	55	37	86	14	36	33	17	57	150
Cyathostomum alveatum	---	---	---	---	---	---	---	---	---	12,5	---	---	---	---	2,8	---	---	---	---
Cyathostomum catinatum	**60,9**	**90,9**	**32**	**80**	**94,2**	**64,7**	**94**	**82**	**50**	**72,5**	**93**	**97,3**	**89,5**	**100**	**94,4**	---	**88**	**76**	**68**
Cyathostomum pateratum	**47,8**	27,2	---	22	**43**	**76,5**	44	18	6	25	**24**	62,1	**65**	**85,7**	**66,7**	18,2	---	**33**	16
Cyathostomum sagittatum	---	---	---	---	---	---	---	---	---	**32,5**	---	---	---	---	---	---	---	---	---
Cyathostomum tetracanthum	---	---	---	---	---	---	---	---	19	2,5	---	---	---	---	---	**54,5**	---	---	---
Coronocyclus coronatus	**34,8**	**54,5**	**32**	**46**	**83,7**	**32,4**	**81**	**91**	**69**	**95**	**82**	**81,1**	**83,7**	50	**44,4**	**90,9**	**80**	**65**	**43**
Coronocyclus labiatus	30,4	18,2	10	16	25,6	**67,7**	---	45	**31**	20	14	59,5	60,5	**85,7**	41,7	21,2	---	30	13
Coronocyclus labratus	26,1	45,4	2	**34**	29,2	2,9	**75**	**100**	13	**35**	8	56,8	**66,3**	28,6	19,4	15,1	**60**	2	13
Cylicocyclus asworthi	**52,2**	**54,5**	---	---	---	---	---	36	---	---	---	---	---	---	**52,8**	---	---	---	---
Cylicocyclus auriculatus	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	0,7
Cylicocyclus brevicapsulatus	4,3	---	---	---	3,5	---	---	---	---	---	5	---	2,3	21,4	19,4	6	---	22	**26**
Cylicocyclus elongatus	30,4	**63,6**	**10**	10	2,3	20,6	25	---	3	**57,5**	---	29,7	9,3	---	---	**27,2**	5	9	4
Cylicocyclus insigne	**82,6**	**72,7**	8	32	**29,2**	5,9	**94**	64	**53**	7,5	**30**	**81,1**	**67,5**	50	30,6	---	39	**41**	**17**
Cylicocyclus leptostomus	**34,8**	**72,7**	2	**40**	**57**	**82,4**	50	**82**	6	**45**	**38**	**97,3**	51,2	**100**	**69,4**	**36,3**	**40**	**41**	**41**
Cylicocyclus nassatus	**73,9**	**90,9**	**32**	**72**	**93,1**	**100**	**88**	**73**	**69**	**62,5**	**84**	**94,6**	**87,2**	**92,9**	**97,2**	**63,6**	**94**	**67**	**54**

	Present research	Port. [1]	Pol. [2]	Pol. [3]	UK [4]	Ger. [5]a	Ger. [6]	Belg. [7]	Fra. [18]	Italy [8]	USA [9]	USA [10]	Pan. [11]	Brazil [12]	Brazil [13]	Brazil [14]	So. Af. [15]	Austra. [16]	Austra. [17]
Cylicocyclus radiatus	21,7	27,3	2	2	---	**88,2**	25	---	---	10	---	29,7	18,6	42,9	25	---	---	**33**	4
Cylicocyclus triramosus	---	---	---	**34**	---	---	**75**	---	---	---	---	**83,8**	---	---	---	---	---	---	---
Cylicocyclus ultrajectinus	---	---	---	---	4,7	---	---	---	**25**	---	3	16,2	10,5	28,6	11,1	---	---	9	0,7
Cylicodontophorus bicoronatus	---	36,4	12	22	10,5	---	38	27	9	2,5	4	35,1	13,9	42,9	27,8	---	---	4	3
Parapoteriostomum euproctus	---	18,2	---	---	11,6	---	---	---	3	---	3	16,2	11,6	50	27,8	---	**46**	15	0,7
Parapoteriostomum mettami	4,3	9,1	12	10	---	---	50	---	---	---	2	8,1	---	---	---	---	---	4	3
Cylicostephanus asymetricus	---	---	---	10	22,1	---	---	---	---	---	---	---	5,8	---	---	---	---	2	---
Cylicostephanus bidentatus	4,3	---	---	---	---	---	---	18	---	---	---	---	---	---	---	---	---	---	---
Cylicostephanus calicatus	**47,8**	**63,6**	**28**	**44**	**81,4**	**76,5**	44	**100**	19	**92,5**	**65**	**83,8**	**83,7**	**100**	**80,6**	**87,9**	**100**	**70**	**48**
Cylicostephanus goldi	**30,4**	**54,5**	**28**	**60**	**94,2**	---	**69**	**100**	**47**	20	**76**	**91,9**	**88,4**	**100**	**91,7**	12,1	**100**	**43**	**51**
Cylicostephanus hybridus	---	---	10	22	---	---	---	9	---	2,5	---	---	16,3	---	---	---	---	4	---
Cylicostephanus longibursatus	**56,5**	**72,7**	**20**	**54**	**98,9**	**58,8**	**88**	**100**	13	27,5	**84**	**100**	**94,2**	**100**	**100**	**39,4**	**100**	**67**	**76**
Cylicostephanus minutus	26,1	54,5	**18**	**40**	**72,1**	14,7	**88**	**100**	**19**	**55**	**56**	**100**	**83,7**	**100**	**88,9**	**36,4**	**88**	26	**36**
Petrovinema poculatum	13	18,2	**14**	8	11,6	8,8	**81**	**73**	---	**65**	7	48,6	8,2	14,3	13,9	**30,3**	5	9	2
Poteriostomum imparidentatum	8,7	36,4	8	20	---	**23,5**	38	73	**34**	---	2	27	22,1	---	2,8	---	---	24	3
Poteriostomum ratzii	---	9,1	4	2	9,3	17,7	---	---	3	---	8	18,9	29,1	---	8,3	3	18	11	0,7
Gyalocephalus capitatus	---	45,4	**38**	28	18,2	---	63	---	**19**	32,5	8	29,7	10,5	**64,3**	44,4	**33,3**	18	11	0,7

Table 11. (Continued)

	Present research	Port. [1]	Pol. [2]	Pol. [3]	UK [4]	Ger. [5]a	Ger. [6]	Belg. [7]	Fra. [18]	Italy [8]	USA [9]	USA [10]	Pan. [11]	Brazil [12]	Brazil [13]	Brazil [14]	So. Af. [15]	Austra. [16]	Austra. [17]
Cyathostomum spp.(L4/L5) Nº species	30,4	90,9	---	---	---	41,2	---	100	---	---	---	93	---	---	---	---	---	---	95
Cyathostominae	20	22	20	23	21	16	19	18	20	21	21	23	24	19	23	16	15	25	24
Nº total species	24	28	27	30	21	18	26	23	20	26	27	30	32	19	23	21	20	32	31

a) Study based on strongyles eliminated in the faeces

[1] MADEIRA DE CARVALHO (1991); [2] SOBIESZEWSKI (1967); [3] GAWOR (1995); [4] OGBOURNE (1976); [5] ANDERSON, I.G.; HASSLINGER, M.A. (1982); [6] ÇIRAK et al (1996); [7] DORNY et al (1999);
[8] RICCI & SABATINI (1992); [9] REINEMEYER et al (1984); [10] TORBERT et al (1986); [11] FOSTER & ORTIZ (1937); [12] CARVALHO et al (1998); [13] SILVA et al (1999); [14] SOUTO-MAIOR et al (1999);
[15] KRECEK et al (1989); [16] MFITILODZE & HUTCHINSON (1990); [17] BUCKNELL et al (1995) [18] COLLOBERT-LAUGIER et al (2002).

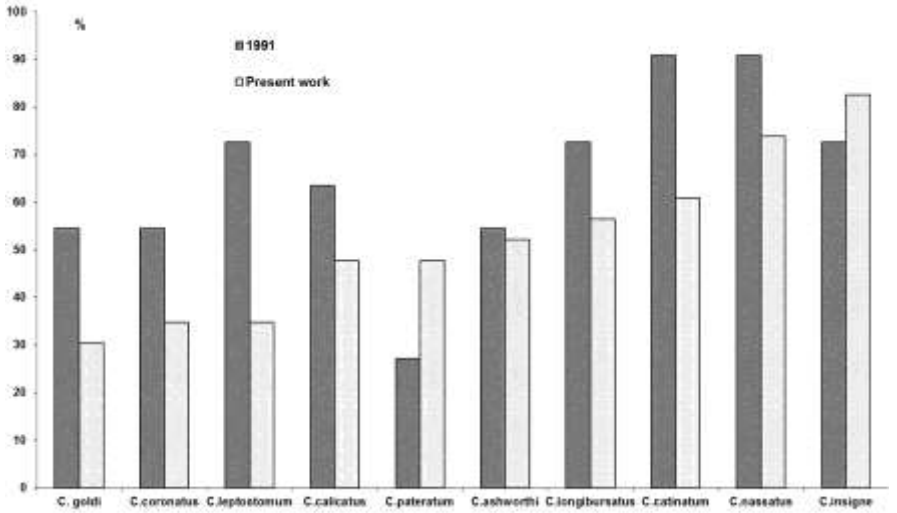


Figure 10. Ten most prevalent cyathostomin species: comparison between this research and the previous one in Portugal performed in 1991).

This phenomenon should be highlighted in view that the most abundant cyathostomin species are also considered the core of Cyathostominae resistant to anthelmintics (Reinemeyer, 1986; Tolliver, 2000).

The increase in reported cases of larval ciatostominosis has meant that these nematodes were no longer considered relatively benign, but potentially malignant, with the aggravating circumstance that they may become resistant to avermectin/milbemycin as originally happened to benzimidazoles (Lyons *et al.*, 2000a; Monahan, 2000; Lyons *et al*., 2008).

Madeira de Carvalho *et al.* (1990) cited previously the presence of L4 of *C. insigne* in animals with larval ciatostominosis examined in the Lisbon Slaughterhouse and its potential importance in diarrheal syndromes of horses. This species was responsible for serious cases of larval ciatostominosis in Argentina, with prevalence rates of 37-50%. This situation did not change despite the regular administration of IVM and it was proven that these nematodes were more pathogenic than other cyathostomin species (Velazquez *et al.*, 1997, 2001). In Argentina, another study also demonstrated that although the horses were regularly dewormed with IVM, *C. catinatum, C. pateratum, C. nassatus* and *C. longibursatus* were still prevalent (Rios-Centeno *et al.*, 2001). In terms of other species, we would like to highlight *C. ashworthi*, which maintained prevalence almost unchanged with respect to previous studies (Madeira de Carvalho, 1991). This species was long regarded as a synonym of *C. nassatus* (Lichtenfels, 1975; Hartwich, 1986), although others considered it a valid species (Lanfredi & Honer, 1984; Madeira de Carvalho, 1991). Indeed *C. ashworthi* presents many similarities with *C. nassatus*, but must be differentiated from this species by the shallow buccal capsule, short dorsal gutter and absence of a protrusion in the form of shelf inside the buccal capsule. From now on, this cyathostomin has been diagnosed in horse parasitological surveys in Belgium, Brazil, Sweden, Ukraine and Poland, with prevalence rates ranging 36-93.2%, approaching the value of 52.8 % recorded in this study (Dorny *et al.*, 1999; Silva *et al.*, 1999; Osterman Lind *et al.*, 2003; Kuzmina *et al.*, 2005; Kornas *et al.*, 2011).

Moreover, some authors draw attention to the fact that other species, like *Cylicocyclus triramosus*, may cause some confusion regarding the identification of *C. ashworthi*. However

C. triramosus is currently considered a specific parasite of zebras and many of the records with this cyathostomin species in Europe have been re-identified as *C. ashworthi*, being considered now a completely different *Cylicocyclus* species (Lichtenfels *et al.*, 1997; Kharchenko *et al.*, 1997; Lichtenfels *et al.*, 2008).

The remaining cyathostomin species have prevalence rates between 4.3 and 30.4%. Framed in this group are the species *C. brevicapsulatus* and *C. bidentatus*, first reported in Portugal. The comparison with data on Table 11 allows us to highlight their low or even episodic prevalence, belonging to the group of species that contribute less than 1-2% for the total population of cyathostomins (Tolliver, 2000; Kornas *et al.*, 2011).

Regarding the male:female ratio in strongyles from the horses at this stud farm, except for *T. serratus*, all species showed values ≤ 1, which is consistent with those recorded by Anderson & Hasslinger (1982), Ricci & Sabatini (1992) and Silva *et al.* (1999).

In the correlation studies carried out, we found that a high positive correlation exists between the prevalence of each species and the intensity of infection, as strongyles that had higher prevalence were also the most abundant, as suggested by Ogbourne (1976) and Reinemeyer (1986). Concerning the correlation between the total number of strongyle specimens and the number of species per host, we also noticed the existence of a high positive correlation, meaning that the greater the number and variety of species, the higher the intensity and abundance of infection status, already demonstrated after horse necropsies (Ricci & Sabatini, 1992). The number of species/host range registered in this study (1 to 17, with a mean of 7.6) is not very different from other authors: Ogbourne (1976) recorded 4-16, Reinemeyer *et al.* (1984) observed 2-11 with an average of 7, Mfitilodze & Hutchinson (1990), 2-21 with a mean of 10.3 and Gawor (1995), recorded 2-16, with an average of 7.6.

In the analysis of the strongyles infection according to the environment, age and dewormer, no significant differences were observed, except for *C. calicatus* (higher intensity of infection in housed horses and in those older than 1 year of age) and *C. catinatum* (higher intensity of infection in animals aged ≥ 1 year). We also observed a smaller number of species in housed horses (18), than in grazing animals (23), which agrees with other authors reporting this difference, even higher, for cyathostomin species of housed and grazing horses respectively 16 and 35 (Mfitilodze & Hutchinson, 1990). This difference can be explained by the less exposure to infection of animals in the barn, the largest number of anthelmintic treatments performed for these and also by the fact that the studied housed animals began their training over 1 year of age.

Animals older than 1 year had lower species diversity (17 species), when compared to horses aged under 1 year (22). Ricci & Sabatini (1992) and Bucknell *et al*. (1995) suggest that young animals compared to older, have higher prevalence, number of species/host and intensities of infection, meaning a higher resistance to infection with strongyles as animals become older.

To end this discussion, several L3 larval types of *Cyathostomum* spp. have been diagnosed in this horse stud farm, predominating A and C in this equine population, being these ones assigned with resitant cyathostomin population. This event may not be strange to the fact that cyathostomins *C. insigne, C. nassatus, C. minutus, C. catinatum, C. pateratum* and *C. longibursatus* yield L3 type A and *C. calicatus* and *C. longibursatus* are in the genesis of type C. As we saw earlier in this chapter, the adult stages of these cyathostomins were found to a great extension in this study and six of them belong to the group of 10 species with highest prevalence and abundance, being also associated with a resistant profile of the farm.

Therefore, de data gathered in this research point out that those species may have influenced the higher prevalence and abundance of *Cyathostomum* spp. types A and C reported in faecal cultures (Madeira de Carvalho *et al.*, 2008).

Finally, the premise that a study of a horse strongyle population through the analysis of the stages shed in faeces were found to be valid, meaning that faecal examination of shed strongyles produces results that mimic studies based on horse necropsies. Therefore, our methodology highlight the importance of using this approach at the farm level, since the data gathered in faecal samples rather than at necropsies, achieved similar results regarding the parasite scenario. With these results, we have shown that the horse strongyle population, and particularly the Cyathostominae subpopulation, are evolving the same way as found in other countries, using an easier sample collection technique, a less invasive and cheaper method, to overcome the difficulty of perfoming necropsies.

CONCLUSION AND FUTURE PERSPECTIVES

1. In horses from a stud farm, using a method of isolation and identification of strongyle adult stages shed in faeces, 24 species were identified: 20 of subfamily Cyathostominae (representing 99.66% of total) and 4 of subfamily Strongylinae (0.34% of total).
2. *Strongylus vulgaris* only accounted for 4.3% prevalence, being much reduced probably due to the intensive deworming system based on macrocyclic lactones.
3. The ten Cyathostominae species with higher prevalence rate in these horses, were in descending order: *Cylicocyclus insigne* (82.6%), *Cylicocyclus nassatus* (73.9%), *Cyathostomum catinatum* (60.9%), *Cylicostephanus longibursatus* (56.5%), *Cylicocyclus ashworthi* (52.2%), *Cylicostephanus calicatus* (47.8%), *Cyathostomum pateratum* (47.8%), *Cylicocyclus leptostomum* (34.8%), *Coronocyclus coronatus* (34.8%) and *Cylicostephanus goldi* (30.4%). In the present research, 7 of the 10 species considered most important in domestic horses in Portugal, remained in this group, with no significant differences in their prevalence.
4. The nematode species *C. nassatus* was the most abundant in this horse stud farm, constituting about 35% of studied strongyles. Globally, the 10 most prevalent species of cyathostomins in this horse stud farm accounted for 93.3 % of the total.
5. *Cylicocyclus brevicapsulatus* and *Cylicostephanus bidentatus*, two species of cyathostomins, were new records for the horse parasite fauna in Portugal.
6. The premises for a study of a parasite scenario in a stud farm, namely the horse strongyle population, can be achieved with this method based in the faecal examination of shed strongyles.

The assumption of L3 *Cyathostomum* spp. types regarding the most prevalent/abundant species of Cyathostomins should be the subject of future work for its better understanding.

Thus, we consider that a study of longer duration, aimed for regular identification of adults and larvae (L4/L5) of strongyles from fecal samples from horses with natural elimination or through deworming, allow a good evaluation of the panorama of species more

prevalent and better correlation of these data with those obtained through the simultaneous study of faecal cultures from the same host.

ACKNOWLEDGMENTS

To CIISA-FMV-UL and it's coordinator, Professor Luis Tavares, for funding this work and supporting our research in the field of horse parasites.

The Board of Companhia das Lezírias (CL), in particular to Eng. Francisco Perestrello, for the willingness and knowledge to provide us with the animals, locations and premises where the field works took place, as well as, with the health record of the animals used for this specific research. The technical and auxiliary Staff of CL for their invaluable input in the management and restraint of animals during the sample collections. We also thank the technical assistance by Dr. Lidia Gomes of the Parasitology and Parasitic Diseases sector, for her help and collaboration in the field trials and laboratory techniques.

We would like to acknowledge Professor Dwight Douglas Bowman, Full Professor of Parasitology and Parasitic Diseases, College of Veterinary Medicine, Cornell University, USA, for the thoughtful and critical reading of the manuscript.

A final word of appreciation to all the students of 3rd and 4th years of Veterinary Medicine, from 1997 to 2001, who helped us in the field and laboratory, allowing more rapid processes and a healthy environment of exchanging ideas related with this new type of parasite survey to be carried out at a horse stud farm level. To all of them our Big Thank You!

REFERENCES

Anderson, I.G., Hasslinger, M.A. (1982). *Cyathostominae* and other strongyles of horses in the Federal Republic of Germany. *J. S. Afr. Vet. Assoc.* 53 (3), 195-197.

Bowman, D.D. (1995). *Georgi's Parasitology for Veterinarians.* (*with a chapter on Antiparasitic Drugs* by LYNN, R.C.). 6th edition, W.B. Saunders, Philadelphia, USA, 430 pp.

Bucknell, D.G., Gasser, R.B., Beveridge, I. (1995). The prevalence and epidemiology of gastrointestinal parasites of horses in Victoria, Australia. *Int. J. Parasitol.* 25 (6), 711-724.

Bush, A.O.; Lafferty, K.D.; Lotz, J.M.; Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis et al revisited. *J. Parasitol.* 83 (4), 575-583.

Carvalho, R.O., Silva, A.V.M., Santos, H.A., Costa, H.M.A. (1998) Nematodes *Cyathostominae* parasites of *Equus caballus* in the state of Minas Gerais, Brazil. *Rev. Bras. Parasitol. Vet.* 7 (2), 165-168.

Chapman, M.R., Kearney, M.T., Klei, T.R. (2003). Equine cyathostome populations: accuracy of species composition estimations. *Vet. Parasitol.* 116, 15-21.

Çirak, V.Y., Hermosilla, C., Bauer, C. (1996) Study on the gastrointestinal parasite fauna of ponies in northern Germany. *Appl. Parasitol.* 37 (4), 239-244.

Collobert-Laugier, C., Hoste, H., Sevina, C. Dorchies, P. (2002). Prevalence, abundance and site distribution of equine small strongyles in Normandy, France. *Vet. Parasitol.* 110, 77-83.

Dorny, P., Meijer, I., Smets, K., Vercruysse, J. (2000). A survey of anthelmintic resistance on belgian horse farms. *Vlaams Dierg. Tijds.* 69, 334-337.

Duncan, J.L. (1982). Internal parasites of horses: Treatment and control. *In Practice* 4 (6), 183-188.

Duncan, J.L. (1985). *Parasitic Diseases*. 359-391 pp. *In Equine Surgery and Medicine, Vol.1*, Academic Press Inc., London, 1ª Ed., 850 pp.

Dvojnos, G.M., Kharchenko, V.A. (1994). [*Strongylids of domestic and wild horses*] (In russian). Ed. Izdatel'stvo 'Naukova Dumka', Kiev, Ukraine, 230 pp.

Foster, A.O. (1936). A quantitative study of the nematodes from a selected group of equines in Panama. *J.Parasitol.* 22, 479-510.

Foster, A.O., Ortiz, O.P. (1937). A further report on the parasites of a selected group of equines in Panama. *J. Parasitol.* 23, 360-364.

Gawor, J.J. (1995). The prevalence and abundance of internal parasites in working horses autopsied in Poland. *Vet. Parasitol*. 58 (1/2), 99-108.

Gawor, J.J. (1997). Evaluation of helminth infections in tarpans using the method of collection of worms after the treatment. *16th Conference of the World Association for the Advancement of Veterinary Parasitology Workshop, 10 – 15 August 1997, Sun City, South Africa*, pp.32, Abst.116.

Georgi, J.R. (1982). *Parasitologia Veterinária*. (com um capítulo sobre *Medicamentos Anti-parasitários*, de Vasiilios J. Theodorides). Trad. do inglês, 3ª Edição, Editora Interamericana, Rio de Janeiro, 353 pp.

Hartwich, G. (1986). Zum *Strongylus tetracanthus* – Problem und zur Systematik der Cyathostominea (*Nematoda: Strongyloidea*). *Mitt. Zool. Mus. Berl.* 62 (1), 61-102.

Herd, R.P. (1990). The changing world of worms: the rise of the cyathostomes and the decline of *Strongylus vulgaris*. *Comp. Cont. Educ. Prac.Vet.* 12 (5), 732-736.

Höglund, J., Ljungström, B.L., Nilsson, O., Lundquist, H., Osterman, E., Uggla, A (1997). Occurrence of *Gasterophilus intestinalis* and some parasitic nematodes of horses in Sweden. *Acta Vet.Scand.* 38 (2), 157-166.

Kester, W.O. (1975). *Strongylus vulgaris* – The Horse Killer. *Modern Vet. Prac.* 56 (8), 569-572.

Kharchenko, V.A., Dvojnos, G.M., Krecek, R.C., Lichtenfels, J.R. (1997). A Redescription of *Cylicocyclus triramosus (Nematoda: Strongyloidea*): a Parasite of the Zebra *Equus burchelli antiquorum*. *J. Parasitol.* 83 (5), 922-926.

Kornas, S., Basiaga, M., Kharchenko, V. (2011). Composition of the cyathostomin species in horses with a special focus on *Cylicocyclus brevicapsulatus*. *Medycyna Wet.* 67 (1), 48-50.

Krecek, R.C., Reinecke, R.K., Horak, I.G. (1989). Internal parasites of horses on mixed grassveld and bushveld in Transvaal, Republic of South Africa. *Vet. Parasitol.* 34 (1-2), 135-143.

Kuzmina, T.A., Kharchenko, V.A. (2008) Anthelmintic resistance in cyathostomins of brood horses in Ukraine and influence of anthelmintic treatments on strongylid community structure. *Vet. Parasitol*. 154, 277–288.

Kuzmina, T.A., Kharchenko, V.A., Starovir, A.I., Dvojnos, G.M. (2005) Analysis of the strongylid nematodes (Nematoda: Strongylidae) community after deworming of brood horses in Ukraine. *Vet. Parasitol*. 131, 283–290.

Lanfredi, R.M., Honer, M.R. (1984). Uma chave ilustrada para a identificação dos géneros e espécies dos pequenos estrongilídeos (subfamília *Cyathostominae*: *Nematoda*) em cavalos da baixada fluminense. *Pesq. Vet. Bras.* 4 (2), 67-72.

Lichtenfels, J.R. (1975). Helminths of Domestic Equids. Illustrated Keys to genera and species with emphasis on North American Forms. *Proc. Helm. Soc. Wash*. *Special Issue*, 1-92.

Lichtenfels, J.R., Kharchenko, V.A., Dvojnos, G.M. (2008), Illustrated identification keys to strongylid parasites (Strongylidae: Nematoda) of horses, zebras and asses (Equidae). *Vet. Parasitol*. 156, 4-161.

Lichtenfels, J.R., Kharchenko, V.A., Krecek, R.C., Gibbons, L.M. (1998). An annotated checklist by genus and species of 93 species level names for 51 recognized species of small strongyles (*Nematoda: Strongyloidea: Cyathostominea*) of horses, asses and zebras of the world. *Vet. Parasitol.* 79 (1), 65-79.

Lichtenfels, J.R., Kharchenko, V.A., Sommer, C., Ito, M. (1997). Key Characters for the Microscopical Identification of *Cylicocyclus nassatus* and *Cylicocyclus ashworthi* (*Nematoda: Cyathostominae*) of the Horse, *Equus caballus*. *J. Helminthol. Soc. Wash.* 64 (1), 120-127.

Love, S., Murphy, D., Mellor, D. (1999). Pathogenicity of cyathostome infection. *Vet Parasitol*. 85:113–122.

Love, S., Duncan, J.L. (1988). Parasitisme à "petits strongles" chez le cheval. *Le Point Vétérinaire* 20 (114), 5-11.

Lyons, E.T., Tolliver, S.C., Drudge, J.H. (1999). Historical perspective of cyathostomes: prevalence, treatment and control programs. pp. 97-111. *In* Little, S.A.; Moore, J.N.; Dipietro, J.A. (Eds.) *Special issue: Equine Cyathostome Conference. Proceedings of a Conference on Equine Cyathostomes held at the University of Georgia, Athens, GA, 7-8 November 1998. Vet. Parasitol*. 85 (2/3), 95-225 pp.

Lyons, E.T., Drudge, J.H., Tolliver, S.C. (2000a). Larval Cyathostomiasis. Pp. 501-513. *In* TIMONEY, P.J. (Ed.) V*et. Clin. North Amer., Equine Practice, Emerging Infectious Diseases* 16 (3), 387-628 + xii pp.

Lyons, E.T., Swerczek, T.W., Tolliver, S.C., Bair, H.D., Drudge, J.H., Ennis, L.E. (2000). Prevalence of selected species of internal parasites in equids at necropsy in central Kentucky. *Vet.Parasitol.* 52 (3-4), 257-269.

Lyons, E.T., Tolliver, S.C., Ionita, M., Lewellen, A., Collins, S.S. (2008). Field studies indicating reduced activity of ivermectin on small strongyles in horses on a farm in Central Kentucky. *Parasitol. Res* 103, 209–215.

Lyons, E.T., Tolliver, S.C., Stamper, S., Drudge, J.H., Granstrom, D.E., Collins, S.S. (1994). Transmission of some species of internal parasites in horses born in 1990, 1991, and 1992 in the same pasture on a farm in central Kentucky. *Vet.Parasitol.* 52 (3-4), 257-269.

Madeira de Carvalho, L.M. (1991) *Contribuição para o estudo dos Estrongilídeos (NEMATODA: STRONGYLOIDEA) do Cavalo em Portugal Continental*. Relatório da aula teórico-prática, elaborado para prestação de Provas de Aptidão Pedagógica e Capacidade Científica, Faculdade de Medicina Veterinária de Lisboa, 147 pp.

Madeira de Carvalho, L.M. (2001). *Epidemiologia e controlo da estrongilidose em diferentes sistemas de produção equina em Portugal*. Tese de Doutoramento, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, xxii + 445 pp.

Madeira de Carvalho, L.M. (2006). Os equídeos em Portugal: de animais de produção a animais de companhia. I – Impacte nas Doenças Parasitárias. *Medicina Veterinária* (Revta. Da AEFMV), Nº 62, 13-24.

Madeira de Carvalho, L.M. (2010). Epidemiologia da Estrongilidose dos Equídeos em Portugal – Especial referência à Ciatostominose. *Acta Parasitológica Portuguesa* 17 (1), 85-108.

Madeira De Carvalho, L.M., Afonso-Roque, M.M., Carvalho-Varela, M. (1990). Ciatostominose:Tiflocolite parasitária em cavalos por larvas de ciatostomíneos. *I Encontro Anual da Sociedade Portuguesa de Patologia Animal. LNIV, Lisboa, 15-16 Novembro1990.*

Madeira de Carvalho, L.M., Afonso-Roque, M.M., Carvalho-Varela, M. (1997). Aspectos epidemiológicos dos estrongilídeos nos cavalos em regime extensivo e dinâmica estacional das suas formas infectantes na pastagem. *"V Congresso Ibérico de Parasitologia, Universidade de Évora, 6-10 de Outubro de 1997". Resumo publicado na Acta Parasitol. Port.*, vol.4 (1/2), 41.

Madeira de Carvalho, L.M., Afonso-Roque, M.M., Carvalho-Varela, M. (1999). Seasonal pattern of a population of horse strongyles in Portugal. *17th International Conference of the World Association for the Advancement of Veterinary Parasitology, 15th-19th August 1999, Copenhaguen, Dinamarca*, 31.

Madeira de Carvalho, L.M., Afonso-Roque, M.M., Fazendeiro, M.I. (2001). Epidemiology of horse strongyle infections in Portugal: the rise of the cyathostomes! *1st International Symposium – Research in Veterinary Medicine, Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Lisbon, 24-25 May 2001*, 52.

Madeira De Carvalho, L.M., Cernea, M.S., Martins, S., Sousa, S., Gersão, S., Cernea, L.C. (2008). Comparative study of cyathostomin horse infection in Portugal and Romania based in L3 subpopulations of *Cyathostomum sensu latum*. *Revista Scientia Parasitologica*, vol. 9 (2), 48-56.

Matthews, J.B. (2011). Review Article: HBLB's advances in equine veterinary science and practice - Facing the threat of equine parasitic disease. *Equine Vet. J* 43 (2) 126-132.

Mfitilodze, M.W.; Hutchinson, G.W. (1990).Prevalence and abundance of equine strongyles (*Nematoda: Strongyloidea*) in tropical Australia. *J. Parasitol.* 76 (4), 487-494.

Monahan, C.M. (2000). Anthelminthic Control Strategies for Horses. 13 pp. *In* BOWMAN, D.D. (Ed.) *Companion and Exotic Animal Parasitology*, Publisher: International Veterinary Information Service (livro electrónico em www. ivis.org, documento Nº A0309.0500).

Monteiro, J. (1983). O Cavalo Lusitano - Contributo para o seu estudo. *Bolm .Pec*. 49, 1-205.

Nielsen, M.K. (2012). Sustainable equine parasite control: Perspectives and research needs. *Vet. Parasitol.* 185, 32–44.

Nielsen, M.K., Baptiste, K.E., Tolliver, S.C., Collins, S.S., Lyons, E.T. (2010). Analysis of multiyear studies in horses in Kentucky to ascertain whether counts of eggs and larvae per gram of feces are reliable indicators of numbers of strongyles and ascarids present. *Vet. Parasitol*. 174, 77–84.

Ogbourne, C.P. (1976). The prevalence, relative abundance and site distribution of nematodes of the subfamily *Cyathostominae* in horses killed in Britain. *J. Helminthol.* 50 (3), 203-214.

Ogbourne, C.P. (1978). *Pathogenesis of Cyathostome (Trichonema) infections of the horse. A review*. Commonwealth Agricultural Bureaux, Commonwealth Institute of Parasitology, Miscellaneous publication Nº 5, 25 pp.

Olsen, S.N., Schumann, T., Pedersen, A., Eriksen, L. (2003). Recovery of live immature cyathostome larvae from the faeces of horses by Baermann technique. *Vet. Parasitol.*, 116, 259–263.

Osterman Lind, E., Eysker, M., Nilsson, O., Uggla, A., Höglund, J. (2003). Expulsion of small strongyle nematodes (Cyathostomin spp.) following deworming of horses on a stud farm in Sweden. *Vet. Parasitol*. 115, 289–299.

Popova, T.I. (1955). *Strongyloids of Animals and Man. Strongylidae. Vol V. Essentials of Nematodology.* Academy of Sciences of USSR, Moscow, 236 pp. (Em russo, tradução inglesa, 1964, Israel Program for Scientific Translations, Jerusalem, OTS 64 – 1108, N.T.I.S., Springsfield, VA 22151, USA).

Popova, T.I. (1958). *Strongyloids of animals and man. Trichonematidae. Vol VII. Essentials of Nematodology.* Academy of Sciences of USSR, Moscow, 424 pp. (Em russo, tradução inglesa, 1965, Israel Program for Scientific Translations, Jerusalem, TT 65 – 50073, N.T.I.S., Springsfield, VA 22151, USA).

Reinemeyer, C.R. (1986). Small Strongyles. Recent Advances. pp. 281-312. *In* HERD, R.P. (Ed.) V*et. Clin. North Amer., Equine Practice, Parasitology* 2 (2), 263-463 pp.

Reinemeyer, C.R., Smith, S.A., Gabel, A.A., Herd, R.P. (1984). The prevalence and intensity of internal parasites of horses in the U.S.A. *Vet. Parasitol.* 15 (1), 75-83.

Ricci, M.; Sabatini, A. (1992). Notizie sugli elminti parassiti del cieco e del colon degli equidi in Italia. *Parassitologia* 34, 53-60.

Ríos-Centeno, A., Velazquez, S.M., Braun, M. (2001). Longitudinal study of cyathostome species in naturally infected horses. *18th Conference of the World Association for the Advancement of Veterinary Parasitology, Workshop, "Systematics of the Cyathostominae of Horses". 25-31 August 2001, Stresa, Milan, Italy*.

Rodrigues, M.L.A., Souto Maior, M.P., Rezende, A.M.L. (1994). Frequência e distribuição de lesões na artéria mesentérica cranial e seus ramos em equídeos naturalmente infectados por *Strongylus vulgaris*, no Estado do Rio de Janeiro, Brasil. *Parasitologia al Día* 18, 114-117.

Rózsa, L.; Reiczigel, J.; Majoros, G. (2000). Quantifying parasites in samples of hosts. *J. Parasitol.* 86 (2), 228-232.

Silva, A.V.M., Costa, H.M., Santos, H.A., Carvalho, R.O. (1999). *Cyathostominae* (*Nematoda*) parasites of *Equus caballus* in some Brazilian states. *Vet. Parasitol.* 86 (1), 15-21.

Sobieszewski, K. (1967). Parasitic nematodes of the alimentary tract of horses in the Lublin Palatinate. *Acta Parasitol. Polonica* 15 (14), 103-108.

Souto Maior, M.P., Rodrigues, M.L.A., Anjos, D.H.S., Andrade, A., Luque, J.L. (1999). Estrutura das infracomunidades de nematóides estrongilídeos (*Nematoda: Strongylidae*) do ceco de *Equus caballus* naturalmente infectados, provenientes da região metropolitana do Rio de Janeiro, Brasil. *Parasitología al Dia* 23, 24-32.

Souto Maior, M.P., Rodrigues, M.L.A., Rezende, A.M.L. (1995). Prevalência e intensidade de infecção de formas imaturas de *Strongylus vulgaris* (NEMATODA: STRONGYLIDAE) na região metropolitana do Rio de Janeiro – Brasil (Observações preliminares). *Rev. Bras. Med. Vet.* 17 (4), 179-182.

Tolliver, S.C. (2000). *A Practical Method of Identification of the North American Cyathostomes (Small strongyles) in Equids in Kentucky*. Kentucky Agric. Experim. Station, Univ. Kentucky, Coll. Agriculture, Dept. Vet. Science, Lexington, Kentucky 40546, USA, 1ª Ed., xii + 37 pp.

Tolliver, S.C., Lyons, E.T., Drudge, J.H. (1987). Prevalence of internal parasites in horses in critical tests of activity of parasiticides over a 28-year period (1956-1983) in Kentucky. *Vet. Parasitol.* 23 (3-4), 273-284.

Torbert, B.J., Klei, T.R., Lichtenfels, J.R., Chapman, M.R. (1986). A survey in Louisiana of intestinal helminths of ponies with little exposure to anthelmintics. *J. Parasitol.* 72 (6), 926-930.

Uhlinger, C. (1990). Effects of three anthelmintic schedules on the incidence of colic in horses. *Equine Vet. J.* 22 (4), 251-254.

Velazquez, S.M., Pietrobon, E.O., Raffo, F., Braun, M. (1997). Cyathostome species in horses of Buenos Aires, Argentina. Their relative distribution. *16th Conference of the World Association for the Advancement of Veterinary Parasitology Workshop, 1O – 15 August 1997, Sun City, South Africa*, 86.

Velazquez, S.M., Rey, M.C., Maure, P.F., Raffo, F., Braun, M. (2001). Pathological and parasitological studies of *Cyathostominae* infections. *18th Conference of the World Association for the Advancement of Veterinary Parasitology, Workshop, "Systematics of the Cyathostominae of Horses". 25-31 August 2001, Stresa, Milan, Italy*.

In: Horses
Editors: A. Paz-Silva, M. S. Arias Vázquez et al.
ISBN: 978-1-63117-560-2

Chapter 6

HORSE HANDLING CONDITIONS AND EMERGENCE OF NEGLECTED INFECTIONS: FASCIOLOSIS

J. Sanchís Polto[1], Luis M. Madeira de Carvalho[2], R. Bonilla[3], A. M. Duque de Araújo[2], F. Arroyo[4], J. Suárez[5], M. A. Solari[6], J. A. Romero[7] and R. Sánchez-Andrade[4,*]

[1] Parasitología, Universidad de la República (Regional Norte), Salto, Uruguay
[2] CIISA, Facultade de Medicina Veterinaria, Universidade Técnica de Lisboa, Lisboa, Portugal
[3] Laboratorios CARVAL, Colombia
[4] Equine Diseases Study Group – COPAR (GI-2120), Veterinary Faculty, Santiago de Compostela University, Lugo, Spain
[5] AOI Animal Oncology and Imaging Center, Hünenberg, Switzerland
[6] Departamento de Parasitología, División de Laboratorios Veterinarios (DILAVE), Uruguay
[7] Servicio de Industrias, Registro y Bienestar Animal, Dirección General de Ganadería, Gobierno de Canarias, Spain

ABSTRACT

In the last decades, loss of productivity has led to the closure of an elevated number of dairy bovine farms in many regions in Europe and the U.S. Accordingly, huge cultivated areas formerly employed for the nutrition of ruminants have been deserted. Cattle have been replaced by other livestock species such as sheep, horses or donkeys. Therefore it is frequent that horses feed on neglected pastures.

* Corresponding Author: Email: mariasol.arias@usc.es.

Horses can be kept under different management procedures, from extensive grazing on large unfenced plots to housing in box stalls. Fortunately, horses can easily adapt to the adverse atmospheric conditions in nature extensive environments. In the wild, they can spend most of the day in the meadows and fed on grass, so their diet is rich in fiber and low in starch.One of the greatest benefits created by exercise in grazing horses is in reduction of stress level. Another advantage is the decline in feed costs, although supplementation should be provided. Although grazing ensures the feeding of animals and vegetation control, it could also represent a risk for exposition to some parasitic disorders. There is lack of knowledge regarding the possibility of sensitization against *F. hepatica* in horses grazing in areas where bovines were firstly exploited. We carried out a chapter to investigate the presence of antibodies against *F. hepatica* in horses.Data were analyzed regarding several intrinsic (age, gender, breed) and extrinsic factors (housing) of animals. Antibodies against the liver trematode were demonstrated in nearly half of the horses in NW Spain and Uruguay, suggesting that pastures are contaminated by metacercariae (the infective stages). The finding of Pure Breed equines (Spanish Pure Breed and Anglo-Arabs) achieved the highest seroprevalence hints that our initial classification of the horses regarding their housing should be turned into only two categories: mixed and extensive. In the case of horses feeding on pastures previously grazed by ruminants, it is strongly recommended to apply some actions on the environment (grass) to avoid their infection.

Keywords: Equids, liver fluke, sensitization, endemic, pasturing

INTRODUCTION

For over 5000 years, horses have been domesticated and reared by humans with the aim to dedicate them for transport, work, competition, riding/driving, breeding, companion animal or industry (leather, meat). Therefore, horses can be kept under a variety of conditions, from extensive grazing in in natural environment to housing in single compartments (Keiper & Berger, 1982).

Horses can survive under adverse weather conditions (extreme temperatures, rain or wind), thus outdoor regimes do not represent a problem for them (FASS, 2010). It has been described that equines habitually stand with their backs to natural windbreaks of vegetation or terrain (McDonnell, 2003). Nevertheless, when horses are kept in paddocks, appropriate protection against sun and wind can be necessary, especially in those areas under extreme weather conditions.

In the last decades, loss of productivity has provoked the abandonment of an elevated number of dairy farms in many regions of Europe and the U.S. As a consequence, vast cultivated areas formerly employed for the nutrition of ruminants have been deserted, which implya loss of pastoral value, soil erosion, and reduction of biodiversity (González Bernáldez, 1991).

This new situation involves the replacement of cattle by other livestock species (sheep, horses or donkeys). Nowadays, it is frequent (in many regions) that horses resulting from crossing different breeds and even those of elevated economic value (Pure Bred specimens) have been brought to areas with extensive pastures (Arias *et al*., 2013). Autochthonous breeds of horse seem the most adequate ones in rangelands and forests due to their modesty in terms of nutrition, husbandry and care, as well as resistance to external environmental conditions

(Francisco *et al*., 2009). The risk of fire rises according to the increase of unwanted vegetation and shrubs. The control and reduction of weeds and bushes can be achieved without herbicides by maintaining herbivores in the grasslands and forests (Sharrow, 1999; Husak and Grado, 2002).

Grazing Horses

Horses may spend between 12 and 18 hours a day feeding on a high fiber and low starch diet (Harris, 2005; Budiansky 1997). They are also sometimes provided with nutritional regimes containing high levels of grains and supplements, which can lead to serious digestive problems. It has been stated that lactating mares spend 10 hours on feeding and their foals up to 8 hours (Crowell-Davis *et al*., 1985).

When managed properly, a small to medium paddock can significantly reduce nutrition charges, stable cleaning and other handling chores. Good quality pasture can provide much of the nutrition a horse needs. It should be considered that pasture also provides economical forage. The cost of pasture as a feed is estimated to be nearly one tenth the cost of hay.

Pasturing holds many other benefits such as feeding, exercise, playtime and the possibility to lie down on soft matter (Heleski *et al*., 2002). By allowing that horses can graze for 4-12 hours/day, a valuable effect on the bone mineral content has been pointed (Bell *et al*., 2001; Hiney *et al*., 2004; Spooner *et al*., 2008). Horses kept on pasture are in need of lower training time than stabled ones (McCall, 1990; Rivera *et al*., 2002). A possible explanation could rely on that the social environment teaches horses how to react to other individuals, so when it comes time for training, it is easier for horses to understand signals from the trainer (Waran *et al*., 2002; Søndergaard and Ladewig, 2004).

Other advantages in pasture-kept horses consist of unwanted behaviours like bucking, licking, chewing, kicking and jumping are less frequently performed (Heleski *et al*., 2002; McGreavy, 2004; Werhahn *et al*., 2011).

The dirt that horses usually ingest while grazing supplies some essential nutrients, particularly iron, which is also provided by forages and grains (Saastamoinen *et al*., 2012), so that iron deficiency is seldom observed. Anemia can be caused by a wide variety of illnesses, drugs, and other nutrient deficiencies. By opposite, excessive iron supplementation in horses, especially foals, can evolve to liver failure. Most anemic animals are anemic for reasons other than iron deficiency, especially if they have access to pasture (Brommer and Sloet, 2001).

Forage Species Composition

There are two components frequently described in forages, cell contents and cell walls (Pagan, 1998). Most of the protein and the starch, sugars, lipids, organic acids, and soluble ash are found in the cell contents, while the cell wall contains the fiber portion of the plant (Barry, 2001). Whereas horses can degrade the cell contents, cellulose, hemicellulose and lignin forming part of the cell wall are poorly digested (50% or less) (Rochon *et al*., 2004). This ability for digesting the forage components determines their nutritive value.

If we can properly manage grasslands, the total forage produced in a paddock and the period over which pasture is available can be extended (Smith *et al*., 2012). Grasses and

legumes are the two main types of forages (Vallentine, 2001). Legumes (their leaves) are much higher in protein than grasses, due to their ability for utilizing nitrogen from the air to synthesize plant protein (Marten *et al*., 1988).

Because of grasses and legumes are complementary, a pasture should contain at least one of each species (Dewhurst *et al*., 2009). There can be seed different varieties of grass for horse nutrition, as Perennial Rye Grass (*Lolium perenne*), Kentucky Blue Grass (*Poa pratensis*), Rough Stalked Meadow Grass (*Poa trivialis*), Orchardgrass (*Dactylis glomerata*), Timothy (*Phleum pratense*) or Tall fescue (*Festuca arundinacea*). The main species of legumes are White clover (*Trifolium pratense*), Alsike clover (*Trifolium hybridum*), Red clover (*Trifolium pratense*), Birdsfoot trefoil (*Lotus corniculatus*) and Alfalfa (*Medicago sativa*).

Neglected Disorders in Grazing Horses

Horses that graze have the advantage of lower feed costs, as well as more opportunities to exercise and interact with other horses, therefore they should be provided with as many chances to graze as possible (Houpt *et al*., 2001). It should be taken into consideration that not all grasslands ensure an adequate nutrition, thus supplementation must be provided.

When equines fed diets low in forage and high in concentrates (hard feeds such as cubes or grains), as stabled individuals, they should be given plenty of forage for avoiding the risk of digestive problems.

Although grazing ensures the feeding of the animals and the control of vegetation, it could also represent a risk for exposition to some ignored disorders.

Chronic Liver Disease and/or Photosensitization

There have been described certain plants containing harmful alkaloids (pyrrolizidine) for the horses, responsible for chronic liver disease and/or photosensitization (oversensitive to sunlight) (House & Elfenbein, 2011). On considering this, Alsike clover (*Trifolium hybridum*) or Red clover (*Trifolium pratense*) should be avoided in grasslands for horses. It is also required to limit the presence of other plant species as rattlepod (*Crotalaria* spp.), ragwort (*Senecio* spp.), fiddleneck (*Amsinckia* spp.), heliotrope (*Heliotropium europaeum*), Purple Viper's Bugloss (*Echium plantagineum*) or houndstongue (*Cynoglossum officinale*) (Mendel *et al*., 1988; Gava & Barros, 1997; Rivero *et al*., 2011).

Detection of affected horses becomes highly difficult, because of signs of liver dysfunction are not developed in all cases, and sunburned appearance of light-skinned areas (usually on the face, muzzle and legs) only looks associated to some equine breeds, as demonstrated in Arabian Pure Bred individuals from León (NW Spain) (Paz-Silva, 2012).

Reproductive Disorders

Ingestion of Tall Fescue infected with an endophyte (a fungus that lives inside the plant) can cause prolonged gestation in pregnant mares, stillborn foals, retained placenta, reduced or absent milk production and difficulty rebreeding. By opposite, there is no risk for non-pregnant mares, geldings, stallions or growing horses (Tapper and Latch, 1999).

Mud Ingestion

Horses should be fed only with good quality, mould and dust-free forage. Horses constantly ingest dirt when they graze. Excessive consumption of dirt, especially when pastures become short, can be responsible for sand accumulation in the large intestine and finally sand colic.

Mud represents an unhealthy environment due to the presence of bacteria and fungi can provoke lesions at the gut level as abscesses, scratches (also called mud fever), rain scald and thrush. Other problem relies on that mosquitoes and flies also breed in mud.

Parasitic Diseases

Sarcocystis spp. are coccidian parasites that can infect a wide range of mammals. Two hosts are needed to complete the life cycle, a definitive host (carnivore), and an intermediate host (herbivore) (Bonesi *et al*., 1999; Arias *et al*., 2012). Equine protozoal myeloencephalitis (EPM) is a neurologic disease caused by the protozoan parasites *Sarcocystis neurona* and *Neospora hughesi* (Hoane *et al*., 2006). Horses remain infected with *S. neurona* through ingestion of sporocysts passed in the feces of opossums, which are the definitive host for *S. neurona* (Fenger *et al*., 1995).

Infection by parasitic helminths as tapeworms or nematodes is frequently detected among horses, especially in grazing specimens. The possible involvement of liver parasites as *Fasciola hepatica* is seldom considered (Gajewska *et al*., 2005). This possibility should be taken into account when horses are feeding in areas where bovine shedding *F. hepatica* eggs were firstly exploited.

Infection by F. hepatica

Fasciolosis is an enzootic parasitosis in regions where high annual rainfall and large areas of poorly drained pastures provide suitable habitats for the intermediate host, Lymnaeidae amphibious snails (Sánchez-Andrade *et al*., 2002).

Infection occurs when metacercariae (the infective stages) are ingested with vegetation or water (Marcos *et al*., 2007; Sanchís *et al*., 2011; Rodríguez *et al*., 2012). After being excysted in the intestine, the immature worms migrate through the intestine, peritoneum and liver parenchyma. Clinical disease is caused by extensive damage to the hepatic parenchyma produced during 6-8 weeks by migration of juvenile flukes (Carnevale *et al*., 2001). Liver lesions predispose to infectious necrotic hepatitis and bacillary hemoglobinurie; also, host fertility can be decreased (López-Díaz *et al*., 1998).

Adult liver flukes reside in the bile ducts of host animals, and eggs are passed onto the pasture in the faeces. Egg can be released as early as 8 weeks after infection (Jemli *et al*., 1991), but most infections do not become patent until 11-12 weeks in ruminants (Rojo-Vázquez *et al*., 2012).

Nevertheless, after the experimental infection of horses with *F. hepatica* metacercariae only a small percentage of the infective stages could develop to adult flukes, and eggs were not observed in the equine stools after a period longer than 14 weeks post-infection (Grelck *et al*., 1977; Soulé *et al*., 1989). This indicates that copromicroscopical probes are not suitable, and thus immunological assays for detecting the presence of antibodies appear more adequate and helpful (Arias *et al*., 2012).

METHODS

Although fasciolosis has been widely analyzed in ruminants, there is lack of information regarding species as horses.. The possible risk of horses to become exposed to the liver fluke when feeding on pastures previously taken in advantage by ruminants should be investigated.

With the purpose to clarify these items, the investigation of the presence of antibodies against *F. hepatica* in horses from three areas was conducted. Equine sera were tested by using an immunoassay and a 2.9 kDa *F. hepatica*-recombinant surface antigen (Paz-Silva *et al*., 2005; Arias *et al*., 2007). Results were analyzed concerning some intrinsic (age, gender, breed) and extrinsic factors (housing).

Experimental Design

The current investigation was carried out with horses from three different countries: Portugal, Spain and Uruguay (Figure 1), where the most important agrarian economic activity is cattle breeding. The climate is different, Mediterranean (Portugal), Oceanic (NW Spain) and humid subtropical climate (Uruguay).

Figure 1. Areas where the horses have been sampled and analyzed for their exposure to the liver fluke *Fasciola hepatica*, Uruguay, Portugal and NW Spain.

A total of 345 serum samples were tested against a *F. hepatica* recombinant surface antigen (FhrAPS) for assessing the exposure to the liver trematode *F. hepatica*.

By considering the age, horses were divided into four groups: G1 (<1 yr), G2 (1-2.9), G3 (>3-5.9) and G4 (≥6).

According to the housing, three categories were established. Under the category of indoors all those specimens spending most of the day in the boxes, with limited or no access

to grasslands, were grouped. Equids kept outdoors are always under an extensive regime, with inappropriate care and nutrition. The last category, designated as mixed regime is formed by horses remaining stabled during the night and feeding on pastures during the day (unless unfavourable weather occur).

Areas of Study

Portugal

A total of 114 equids belonging to different breeds (Crossbred, CB; Lusitano, LU; Mule, MU) were sampled in Portugal (39º 30´ 00" N, 8º 00' 00" W) (Figure 2). There is a climate characterized by moderate temperatures and wet weather. Summers are hot and dry, except in the immediate coastal areas where milder summers are observed [18]. Distribution of samples is summarized in Table 1.

Figure 2. Grazing horses from Uruguay, Portugal and Spain were bled for assessing their exposure to the liver fluke *Fasciola hepatica*.

Table 1. Distribution of samples according to the breed and groups of horses from Portugal

	CB		LU		MU	
Age	Mare	Stallion	Mare	Stallion	Mare	Stallion
G1	0	3	0	0	0	0
G2	10	8	0	0	0	0
G3	7	4	0	0	0	0
G4	27	32	0	5	6	12

Crossbred, CB; Lusitano, LU; Mule, MU.

Spain

The current chapter was conducted in Northwest Spain (42º20'–43º45'N, 6º49'–8º00'W), a region under an oceanic climate. The presence of a slight annual range of temperatures throughout the year together with significant amounts of precipitation in summer favours the survival of *Galba truncatula*, the intermediate host for *F. hepatica* (Paz-Silva *et al*., 2010; Arias *et al*., 2010).

Blood samples were obtained from 121 equids (Anglo-Arab Breed, AAB; Spanish Sport Horse, SSH; Spanish Pure Breed, SPB; English Pure Breed, EPB; the autochthonous Pura Raza Galega, PRG, figure 3, and donkeys). Distribution was done as reflected in Table 2.

Figure 3. Autochthonous PRG horses grazing on forests from NW Spain.

Table 2. Distribution of samples according to the breed and groups of equids from Spain

	Age	G1	G2	G3	G4
AAB	Mare	0	0	0	1
	Stallion	0	2	1	0
Donkey	Mare	0	0	1	3
	Stallion	0	1	1	4
SSH	Mare	0	6	1	3
	Stallion	0	2	2	0
CB	Mare	0	27	0	0
	Stallion	0	17	0	2
SPB	Mare	0	6	1	1
	Stallion	3	3	2	5
PRG	Mare	11	1	0	0
	Stallion	12	0	1	1

Anglo-Arab Breed, AAB; Spanish Sport Horse, SSH; Spanish Pure Breed, SPB; English Pure Breed, EPB; Autochthonous Pura Raza Galega, PRG

Uruguay

This is a country located in the southern region of South America (30º 05' 00" - 34º 58' 00" S, 53º 12' 00" - 58º 43' 40" W), with moderate climate characterized by significant rainfall in all seasons and small annual variations of temperature (there are more than 4 months averaging above 10ºC). These conditions facilitate that forage can grow throughout the year, and herbivores are feeding on pasture (Sanchís *et al*., 2013). Specimens of *Lymnaea viatrix* and *L. columella* infected by *F. hepatica* have been previously detected (Cardozo and Nari, 1987; Heinzen *et al*. 1994).

Table 3. Distribution of samples according to the breed and groups of horses from Uruguay

	AAB		APB		UC		EPB	
Age	M	S	M	S	M	S	M	S
G1	0	0	0	0	0	0	0	0
G2	0	0	9	1	7	4	5	3
G3	0	1	1	2	22	8	10	7
G4	2	7	0	1	2	1	3	14

Anglo-Arab Breed, AAB; Uruguay Creole, UC; Arabian Pure Breed, APB; English Pure Breed, EPB.

A total of 110 horses representing the Anglo-Arab Breed (AAB), Uruguay Creole (UC) (figure 4), Arabian Pure Breed (APB) and English Pure Breed (EPB) were bled by veterinary clinicians during equestrian festivals as summarized in Table 3.

Figure 4. Uruguay Creole horses are utilized for extensively managed cattle.

Immunoassay

The presence of IgG antibodies against the liver trematode *F. hepatica* was evaluated by means of an ELISA and the FhrAPS surface recombinant protein (Arias *et al*., 2012). Briefly, wells of microtiter plates were added 1 µg mL^{-1} FhrAPS, sera (tested in duplicated) diluted at 1:100 in 10% PTL (PBS–0.3% Tween 20 and 10% skimmed milk) and horseradish peroxidase conjugated with rabbit anti-Horse IgG (Nordic Immunology Laboratories, The Netherlands) used at a 1:1000 dilution. Substrate consisting of 10 mg of ortho-phenylenediamine in 12 mL of citrate buffer and 10 µL of 30% H_2O_2 were then added to each well, and the absorbance was read using a spectrophotometer (Titertek Multiskan) at 492 nm. The sensitivity and specificity of the FhrAPS-ELISA were 83% and 86%, respectively (Arias *et al*., 2012).

Statistical Analysis

All tests were performed using SPSS for Windows (20.0; SPSS Inc., Chicago, IL, USA).

The percentages of seroprevalence were expressed as the value and the 95% confidence interval and were analyzed by χ^2 test. Differences were considered significant for $P< 0.05$.

RESULTS

1. Horse Exposure to *F. hepatica*

Antibodies against the liver trematode were demonstrated in nearly half of the horses from Spain and Uruguay, whereas we only recorded 12% in Portugal. The prevalence of exposure to *F. hepatica* in equids in Portugal ranged from 8% in the animals older than 6 years to 33% in those 1-3 years of age (Table 4). A significant increase in the percentage of exposition to the trematode was proven in relation to the age in the Spanish equids, opposite to that observed in Uruguay.

Table 4. Seroprevalence of exposition to *F. hepatica* regarding the horse age

Age (yr)	Portugal	NW Spain	Uruguay
G-1 (<1)	0%	4%	
G-2 (1-2.9	33%	48%	55%
G-3 (3-5.9)	9%	50%	60%
G-4 (≥6)	8%	65%	37%
Total	*12%*	*41%*	*52%*
Statistics	χ^2= 8.997 *P*= 0.029	χ^2= 21.083 *P*= 0.001	χ^2= 3.892 *P*= 0.143

As shown in Table 5, Crossbred horses achieved the highest percentages of seropositivity in Portugal, Spanish Pure Breed in NW Spain and the Anglo-Arabs in Uruguay. Significant differences were only demonstrated among the Spanish equids.

Table 5. Seroprevalence of exposition to *Fasciola* according to the horse breed

Breed	Portugal	NW Spain	Uruguay
Crossbred	14%	35%	
Lusitano	0%		
Mule	6%		
Donkey		50%	
AAB		50%	70%
SSH		64%	
SPB		67%	
PRG		15%	
APB			29%
UC			54%
EPB			52%
Statistics	χ^2= 1.795 *P*= 0.408	χ^2= 17.068 *P*= 0.004	χ^2= 4.491 *P*= 0.213

Anglo-Arab Breed, AAB; Spanish Sport Horse, SSH; Spanish Pure Breed, SPB; English Pure Breed, EPB; Autochthonous Pura Raza Galega, PRG; Uruguay Creole, UC; Arabian Pure Breed, APB

Finally, no gender influence on the risk of exposure to *F. hepatica* was recorded in any of the investigated areas as reflected in Table 6.

Table 6. Effect of the horse sex on the risk of exposure to *F. hepatica*

Sex	Portugal	NW Spain	Uruguay
Mare	12%	48%	54%
Stallion	12%	34%	49%
Statistics	χ^2= 0.007 *P*= 0.936	χ^2= 2.617 *P*= 0.106	χ^2= 0.285 *P*= 0.593

2. Influence of Horse Managing on the Risk of Exposure to *F. hepatica*

In order to assess the possible role of management of the horses in their exposure to the liver fluke, three categories were considered: indoors, , outdoors and mixed regime.

As drawn in Figure 5, the highest values were observed among the Spanish indoors (χ^2= 20.646, *P*= 0.001), and the lowest in the Portuguese indoors (χ^2= 10.244, *P*= 0.006). Similar percentages in the Uruguayan horses were achieved (χ^2= 0.617, *P*= 0.432).

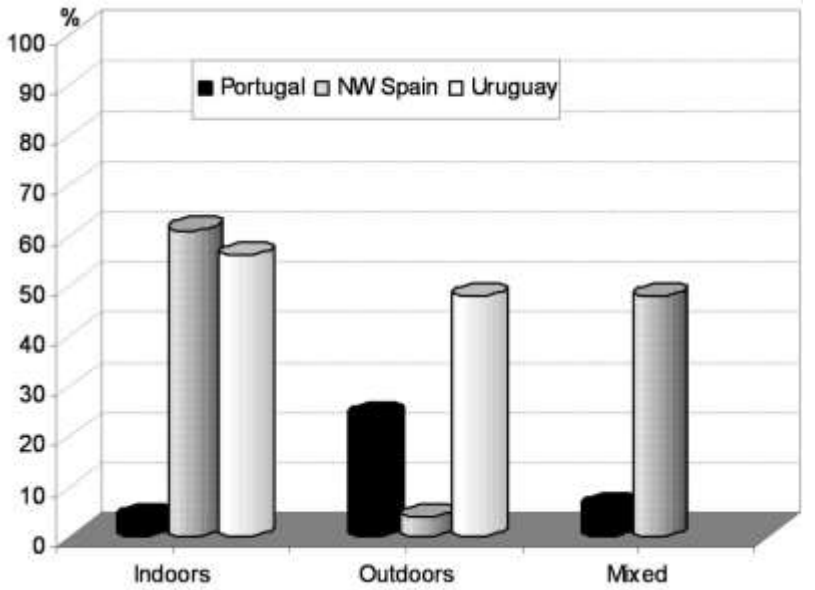


Figure 5. Distribution of horses sensitized against *F. hepatica* by considering their housing.

Figure 6. Spanish Pure Bred horses feeding on pastures formerly grazed by cattle.

There are some surprisingly results needing more discussion. Firstly, almost half of the horses in NW Spain and Uruguay have been exposed to the liver fluke, suggesting an elevated level of pastures contaminated by metacercariae (the infective stages). Previous investigations conducted among ruminants revealed the existence of two endemic areas of fasciolosis, and as a consequence the habitual presence of metacercariae in the grasslands seems very conceivable.

Secondly, the observation of the highest risk of exposure to *F. hepatica* among stabled Pure Bred Horses from NW Spain (Spanish Sport Horses and Spanish Pure Bred) (Figure 6) and Uruguay (Anglo-Arabs) does not match with these individuals remain housed all the day. In view of the results, it seems more plausible to believe that horses are spending time outdoors, and thus they have possibility for eating grass.

Data collected in the current investigation demonstrated a similar situation seems to take place in Uruguay and NW Spain. In both countries horses show an elevated chance of suffering fasciolosis. By opposite, our results showed that Portuguese equines exhibit a low risk of ingestion of metacercariae. This is in agreement with previous works conducted on cattle in the same areas (Sánchez-Andrade *et al*., 2000, 2002; Sanchís *et al*., 2011).

Horses sensitized against the trematode increased in relation to the age of the Spanish horses. The highest values of seropositivity were obtained in the oldest individuals. . This observation appears to denote that the length of grazing-period increases with age, and foals and yearlings spend less time pasturing than adult horses. Conversely, significant differences were not observed regarding the age in Uruguay, but we should note that we have not collected samples belonging to horses younger than 1-year.

CONCLUSION

By maintaining horses under pasturing regimes their nutrition, together with the possibility to exercise and interact with other individuals of the same species is improved. One of the few drawbacks of keeping animals in pastures is the increase in the chances of parasitic infection, horses could be exposed to certain pathogens such as the liver trematode *F. hepatica*.

Almost half of the horses in Uruguay and NW Spain (areas with important prevalence of cattle fasciolosis), had antibodies against the FhrAPS recombinant surface protein, which seems to indicate that these horses exhibit a very high risk of ingestion of *F. hepatica*-metacercariae. However, in non-endemic areas such as Portugal, equids showed low prevalence of sensitization. The observation of the highest percentages of positive horses in box stalls led us to consider more plausible to classify the horses regarding their housing only as mixed and extensive. This statement is supported by the finding of Pure Breed equines (Spanish Pure Breed and Anglo-Arabs) achieved the highest seroprevalence hints that our initial classification of the horses regarding their housing should be turned into only two categories: mixed and extensive. In view of these results, when horses are grazing grasslands which have been formerly pastured by ruminants, it is strongly recommended to apply some actions on the environment (grass) to avoid infection of horses grazing.

REFERENCES

Arias, M., Morrondo, P., Hillyer, G.V., Sánchez-Andrade, R., Suárez, J.L., Lomba, C., Pedreira, J., Díaz, P., Díez-Baños, P., Paz-Silva, A. (2007). Immunodiagnosis of current

fasciolosis in sheep naturally exposed to *Fasciola hepatica* by using a 2.9 kDa recombinant protein. *Vet. Parasitol.* 146, 46-49.

Arias, M., Piñeiro, P., Hillyer, G.V., Suárez, J.L., Francisco, I., Cortiñas, F.J., Díez-Baños, P., Morrondo, P., Sánchez-Andrade, R., Paz-Silva, A. (2010). An approach of the laboratory to the field: assessment of the influence of cattle management on the seroprevalence of fascioliasis by using polyclonal- and recombinant-based ELISA. *J. Parasitol*. 96, 626-631.

Arias, M., Yeargan, M., Francisco, I., Dangoudoubiyam, S., Becerra, P., Francisco, R., Sánchez-Andrade, R., Paz-Silva, A., Howe, D.K. (2012). Exposure to *Sarcocystis* spp. in horses from Spain determined by Western blot analysis using *Sarcocystis neurona* merozoites as heterologous antigen. *Vet. Parasitol.* 185, 301-304.

Arias, M.S., Piñeiro, P., Hillyer, G.V., Francisco, I., Cazapal-Monteiro, C.F., Suárez, J.L., Morrondo, P., Sánchez-Andrade, R. and Paz-Silva, A. (2012). Enzyme-linked immunosorbent assays for the detection of equine antibodies specific to a recombinant *Fasciola hepatica* surface antigen in an endemic area. *Parasitol. Res.*, 110, 1001-1007.

Arias, M.S., Sanchís, J., Cazapal-Monteiro, C.F., Hernández, J.A., Miguélez, S., Ortega, E., Bonilla, R., Miquel Femenias, S., Suárez, J.L., Sánchez-Andrade, R. (2013). Exposición a parásitos hepáticos en caballos en pastoreo. XIV Congreso Internacional de Medicina y Cirugía Equina, Sevilla, (Spain), 6-7 diciembre.

Barry, T.N., 2001. Forage feeding value: improving protein utilization by grazing livestock. In: Bell, R.A., Nielsen, B.D., Waite, K., Rosenstein, D., Orth, M. (2001). Daily access to pasture turnout prevents loss of mineral in the third metacarpus of Arabian weanlings. *J Animal Sci* 79, 142-1150.

Bonesi, G.L., Yamamura, M.H., Pereira, A.B.L. (1999). Distribution of *Sarcocystis* in equine muscular tissue. *Braz. J. Vet. Parasitol.* 8, 71-73.

Brommer, H., Sloet van Oldruitenborgh-Oosterbaan, M.M. (2001). Iron deficiency in stabled Dutch warmblood foals. *J. Vet. Intern. Med.* 15, 482-485.

Cardozo, H., Nari, A. (1987). *Fasciola hepatica* en ovinos. In: J. Bonino Morlán, A. Durán del Campo, J.J. Mari, Enfermedades de los lanares, (Ed. Hemisferio Sur, Montevideo, Uruguay), 71-111.

Carnevale, S., Rodríguez, M., Guarnera, E.A., Carmona, C., Tanos. T., Angel, S. (2001). Immunodiagnosis of fasciolosis using recombinant procathepsin L cystein proteinase. *Diagn. Microbiol. Infect. Dis*. 41, 43-49.

Crowell-Davis, S.L., Houpt, K.A., Carnevale, J. (1985). Feeding and drinking behavior of mares and foals with free access to pasture and water. *J. Anim. Sci.* 60, 883-889.

Dewhurst, R.J., Delaby, L., Moloney, A., Boland, T., Lewis, E. (2009). Nutritive value of forage legumes used for grazing and silage. *Irish J. Agr. Food Res.* 48, 167–187.

FASS (Federation of Animal Science Societies). (2010). Guide for the care and use of agricultural animals in research and teaching. Champagne, IL: FASS.

Fenger, C.K., Granstrom, D.E., Langemeier, J.L., Stamper, S., Donahue, J.M., Patterson, J.S., Gajadhar, A.A., Marteniuk, J.V., Xiaomin, Z., Dubey, J.P. (1995). Identification of opossums (*Didelphis virginiana*) as the putative definitive host of *Sarcocystis neurona*. *J. Parasitol*. 81, 916-919.

Francisco, I., Arias, M., Cortiñas, F.J., Francisco, R., Mochales, E., Sánchez, J.A., Uriarte, J., Suárez, J.L., Morrondo, P., Sánchez-Andrade, R., Díez-Baños, P., Paz-Silva, A. (2009).

Silvopastoralism and autochthonous equine livestock: analysis of the infection by endoparasites. *Vet Parasitol.* 164, 357-362.

Gajewska, A., Smaga-Kozłowska, K., Wiśniewski, M. (2005). Pathological changes of liver in infection of *Fasciola hepatica*. *Wiad Parazytol*. 51(2), 115-23.

Gava, A., Barros, C.S.L. (1997). Senecio spp. poisoning of horses in Southern Brazil. *Pesq. Vet. Bras*. 17(1), 36-40.

González Bernáldez, F. (1991). Ecological consequences of the abandonment of traditional land use systems in central Spain. In: J. Baudry and R.G.H. Bunce, (Eds), *Land abandonment and its role in conservation*. CIHEAM. *Options Méditerranéennes: Série A. Séminaires Méditerranéens* 15, 23-29.

Harris, P. (2005). Nutrition, behaviour and the role of supplements for calming horses: the veterinarian's dilemma. *Vet. J.* 170, 10-11.

Heinzen, T., Castro, O., Pepe, C. Ibarburu, A. (1994). *Lymnaea columella* como hospedero intermediario de *F. hepatica* en Uruguay. XXII Jornadas Uruguayas de Buiatria. Paysandú, Uruguay.

Heleski, C., R.., Shelle, A.C.., Nielsen, B.D., Zanella, A..J. (2002). Influence of housing on weaning horse behavior and subsequent welfare. *Appl. Anim. Behav. Sci.* 78, 291-302.

Hiney, K.M., Nielsen, B.D., Rosenstein, D. (2004). Short-duration exercise and confinement alters bone mineral content and shape in weanling horses. *J. Anim. Sci.* 82, 2313-2320.

Hoane, J.S., Gennari, S.M., Dubey, J.P., Ribeiro, M.G., Borges, A.S., Yai, L.E., Aguiar, D.M., Cavalcante, G.T., Bonesi, G.L., Howe, D.K. (2006). Prevalence of *Sarcocystis neurona* and *Neospora* spp. infection in horses from Brazil based on presence of serum antibodies to parasite surface antigen. *Vet. Parasitol.* 136, 155-159.

Houpt, K.A., Houpt, T.R., Johnson, J.L., Erb, H.N., Yeon, S.C. (2001). The effect of exercise deprivation on the behaviour and physiology of straight stall confined pregnant mares. *Anim. Welfare* 10, 257-267.

House, A.M., Elfenbein, J.R. (2011). Pasture-associated Liver Disease in Horses. VM180 series of the College of Veterinary Medicine-Large Animal Clinical Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Husak, A.L., Grado, S.C. (2002). Monetary benefits in a southern silvopastoral system. *South. J. Appl. Forestry* 26, 159-164.

Jemli, M.H., Rhimi, I., Jdidi, A., Mastouri, L., Kilani, M. (1991). La fasciolose ovine dans la région de Sejnane (nord de la Tunisie). *Rev. Med Vet-Toulouse* 142, 229-235.

Keiper, R.R., Berger, J. (1982). Refuge-seeking and pest avoidance by feral horses in desert and island environments. *Appl. Anim. Ethol.* 9, 111-120.

López-Díaz, M.C., Carro, M.C., Cadórniga, C., Díez-Baños, P., Mezo, M. (1998). Puberty and serum concentrations of ovari-an steroids during prepuberal period in Friesian heifers artificially infected with *Fasciola hepatica*. *Theriogenology* 50, 587-593.

Marcos, L.A., Terashima, A., Leguia, G., Canales, M., Espinoza, J.R., Gotuzzo, E. (2007). *Fasciola hepatica* infection in Peru: an emergent disease. *Rev. Gastroenterol. Perú* 27, 389-396.

Marten, G.C., Buxton, D.R., Barnes, R.F. (1988). Feeding value (forage quality). In Alfalfa and Alfalfa Improvement, Monograph no. 29. Madison, Wis.:ASSA/CSSA/SSSA.

McCall, C.A. (1990). A review of learning behavior in horses and its application in horse training. *J. Anim. Sci.* 68, 75-81.

McDonnell, S. (2003).A practical field guide to horse behavior. The equid ethogram. Hong Kong: The Blood-Horse Inc.

Mendel, V.E., Witt, M.R., Gitchell, B.S., Gribble, D.N., Rogers, Q.R., Segall, H.J., Knight, H.D. (1988). Pyrrolozidine alkaloid-induced liver disease in horses: an early diagnosis. *Am. J. Vet. Res*. 49, 572-578.

Pagan, J.D. (1998). Forages for horses: More than just filler. In: J.D. Pagan (Ed.) Advances in Equine Nutrition. pp. 13-28. Nottingham University Press. Nottingham, United Kingdom.

Paz-Silva, A. (2012). Fasciolosis in adult horses. Clinical case. Meeting of the European Veterinary Parasitology College. León, September 29.

Paz-Silva, A., Arias, M., Francisco, I., Cortiñas, J., Francisco, R., Mochales, E., Suárez, J.L., Díez-Baños, P., Morrondo, P., Sánchez-Andrade, R. (2010). Cross-immunity and interpretation of the diagnostics of parasitic trematodosis in ruminants by means of immunoenzymatic probes. In G.V. LaMann (Ed) Veterinary Parasitology. Novapublishers, NY, USA, pp 289-302.

Paz-Silva, A., Hillyer, G.V., Sánchez-Andrade, R., Rodríguez-Medina, J.R., Arias, M., Morrondo, P., Díez-Baños, P. (2005). Isolation, identification and expression of a *Fasciola hepatica* cDNA encoding a 2.9-kDa recombinant protein for the diagnosis of ovine fascioliasis. *Parasitol. Res*. 95, 129-135.

Rivera, E., Benjamin, S., Nielsen, B., Shelle, J., Zanella, A.J. (2002). Behavioral and physiological responses of horses to initial training: the comparison between pastured versus stalled horses. *Appl. Anim. Behav. Sci.* 78, 235-252.

Rivero, R., Matto, C., Adrien, M.L., Alvarez, V. (2011). Intoxicación por Senecio spp. (Asteraceae) en equinos en Uruguay. *Veterinaria*, (Montevideo) 47 (182), 29-32

Rochon, J.J., Doyle, C.J., Greef, J.M., Hopkinsm A., Molle, G., Sitzia, M., Scholefield, D., Smith, C.J. (2004). Grazing legumes in Europe: a review of their status, management, benefits, research needs and future prospects. *Grass Forage Sci.* 59, 197–214.

Rodríguez, D.C., Pino, N., Peñuela, G. (2012). Microbiological quality indicators in waters of dairy farms: detection of pathogens by PCR in real time. *Sci. Total Environ.* 427-428, 314-318.

Rojo-Vázquez, F.A., Meana, A., Valcárcel, F., Martínez-Valladares, M. (2012). Update on trematode infections in sheep. *Vet. Parasitol.* 189, 15-38.

Saastamoinen, M.T., Hellämäki, M. (2012). Forage analyses as a base of feeding of horses. In: Forages and grazing in horse nutrition. M.T. Saastamoinen, M.J. Fradinho, A.S. Santos, N. Miraglia, (Eds.). Series: European Association for Animal Production, 132, 305-314.

Sánchez-Andrade, R., Paz-Silva, A., Suárez, J., Panadero, R., Díez.-Baños, P., Morrondo, P. (2000). Use of a sandwich-enzyme-linked immunosorbent assay (SEA) for the diagnosis of natural *Fasciola hepatica* infection in cattle from Galicia (NW Spain). *Vet. Parasitol*. 93, 39-46.

Sánchez-Andrade, R., Paz-Silva, A., Suárez, J.L., Panadero, R., Pedreira, J., Lópezm C., Díez-Baños, P., Morrondo, P. (2002). Influence of age and breed on natural bovine fasciolosis in an endemic area (Galicia, NW Spain). *Vet. Res. Commun*. 26, 361-370.

Sanchís, J., Miguélez, S., Solari, M.A., Piñeiro, P., Macchi, M.I., Maldini, G., Venzal, J., Morrondo, P., Díez-Baños, P., Sánchez-Andrade, R., Paz-Silva, A., Arias, M.S. (2011). Seroprevalencia de la fasciolosis bovina en el departamento de Salto (Uruguay), *Rev. Ibero-Latin. Parasitol.* 70, 163-171.

Sharrow, S.H. (1999). Silvopastoralism: competition and facilitation between trees, livestock and improved grass-clover pastures on temperate rainfed lands. *In*: Agroforestry in Sustainable Agricultural Systems. L.E. Buck, J.P., Lassoie, E.C.M., Fernandes (Eds). CRC Press LLC. Boca Raton, Florida.

Smith, R., Cotton, K., Allman, R., Watson, R., Sena, K., Keene, T. (2012). Grazing and pasture management considerations from around the world. In Forages and grazing in horse nutrition. M.T. Saastamoinen, M.J. Fradinho, A.S. Santos, N. Miraglia, (Eds.). Series: European Association for Animal Production, 132, 197-208.

Søndergaard, E., Ladewig, J. (2004). Group housing exerts a positive effect on the behaviour of young horses during training. *Appl. Anim. Behav. Sci.* 87, 105-118.

Spooner, H.S., Nielsen, B.D., Woodward, A.D., Rosenstein, D.S., Harris, P.A. (2008). Endurance training has little impact on mineral content of the third metacarpus in two-year-old Arabian horses. *J. Equine Vet. Sci.* 28, 359-362.

Tapper, B.A., Latch, G.C.M. (1999). Selection against toxin production in endophyte-infected perennial ryegrass. In: D.R. Woodfield and C. Matthew (Eds.) Ryegrass Endophyte: An Essential New Zealand Symbiosis. Proc. NZ Grassland Assoc. Symposium, Napier, New Zealand.

Vallentine, J.F. (2001). Grazing Management, 2nd Edition. Academic Press.

Waran, N., McGreevy, P., Casey, R.A. (2002). Training methods and horse welfare. In: The Welfare of Horses N. Waran, N.D. Dordrecht, (Eds), Kluwer Publishers, pp. 151-180. Wageningen Academic Publishers.

Werhahn, J., Hessel, E.F., Schulze, H., Van den Weghe, H.F.A. (2011). Temporary turnout for free exercise in groups: effects on the behavior of competition horses housed in single stalls. *J. Equine Vet. Sci.* 31, 417-425.

In: Horses
Editors: A. Paz-Silva, M. S. Arias Vázquez et al.
ISBN: 978-1-63117-560-2

Chapter 7

AFRICAN HORSE SICKNESS, AN EQUINE DISEASE OF EMERGING GLOBAL SIGNIFICANCE

Liesel Stassen, Elaine Vermaak and Jacques Theron*

Department of Microbiology and Plant Pathology,
University of Pretoria,
Pretoria, South Africa

ABSTRACT

African horse sickness virus (AHSV) causes a non-contagious but infectious disease of equids and is transmitted by various species of *Culicoides* midges. Horses are the most severely affected by AHSV, whereas mules are less susceptible, and African donkeys and zebra appear to have some natural resistance to the development of acute disease. Although AHSV is endemic in most areas in sub-Saharan Africa, the virus periodically makes excursions beyond its endemic areas and outbreaks have been reported in North Africa, the Middle East and in the Mediterranean Basin. Due to the devastating effect that a widespread outbreak of African horse sickness (AHS) would have on the horse industry of affected countries, great emphasis has been placed on the control of disease incidence. Since there is currently no specific therapy or drug to cure individual horses affected by AHS, vector control and vaccination remains the most practical methods of preventing the disease. This chapter outlines the history and epidemiology of AHS, including information regarding virus structure, transmission, clinical disease, and present and future approaches for controlling the disease.

Keywords: African horse sickness, transmission, *Culicoides* spp., epidemiology, vaccines

* Corresponding author: Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, South Africa, Email: jacques.theron@up.ac.za.

INTRODUCTION

African horse sickness (AHS) is a non-contagious, infectious disease of equids caused by African horse sickness virus (AHSV). The disease is particularly devastating in horses, resulting in severe cardiovascular and pulmonary illness, and mortality rates in naïve horse populations may exceed 90% (Coetzer and Guthrie, 2004). The virus is transmitted primarily by female *Culicoides* midges during a blood meal, which they require for reproduction (Wilson et al., 2009). Due to the economic impact of disease outbreaks, severity of disease in horses, and its capacity for sudden and rapid expansion, AHS is listed by the Office International des Epizooties (OIE) as a notifiable equine disease.

There have been numerous documented prior extensions of AHSV from its enzootic domain within sub-Saharan Africa into North Africa, Europe, and the Middle and Near East. The incursion of AHSV into countries of Europe or the Middle East that are extensively involved in the international trade and movement of horses would be economically devastating, thus there is substantial concern regarding potential spread of AHS from Africa into adjacent regions (Dufour et al., 2008; Gale et al., 2009). AHS can be prevented by vaccination, vector insect control measures and strict control of animal movement in endemic areas (Sánchez-Vizcaíno, 2004). However, use of the current commercial vaccine, comprising a cocktail of cell-cultured live attenuated strains of the different AHSV serotypes, is associated with several risks and is not acceptable for use in non-endemic regions (Mellor and Hamblin, 2004). Moreover, there is little information on which to base a rational strategy for controlling *Culicoides* biting midges or for predicting the likely impact of such interventions (Carpenter et al., 2008). This paucity of control options could therefore be expected to not only impact negatively on the protection of horses against AHSV, but may also have severe economic consequences for the equine industry.

AFRICAN HORSE SICKNESS VIRUS (AHSV)

Aetiology

AHSV is a member of the genus *Orbivirus* in the family *Reoviridae* (Mertens et al., 2005). Orbiviruses can be distinguished from other members of the *Reoviridae* in that they replicate in both insects and vertebrates, show greater sensitivity to lipid solvents and detergents, and virus infectivity is lost in mild acidic conditions (Spence et al., 1984). The prefix "orbi" in "orbiviruses" is derived from the Latin word "orbis", meaning ring or circle, and describes the characteristic capsomers (rings) on the orbiviral core-surface (Borden et al., 1971).

The AHSV virion (Figure 1), which is approximately 70 nm in diameter, is a non-enveloped particle and consists of two concentric protein layers that enclose the viral genome (Manole et al., 2012). The AHSV genome comprises 10 double-stranded ribonucleic acid (dsRNA) segments (Bremer, 1976), each of which encodes at least one polypeptide (Grubman and Lewis, 1992). The outer capsid of the virion is composed of the two major structural proteins VP2 and VP5 and contains the neutralization determinants of AHSV. Although the major neutralization determinants of AHSV are expressed on VP2, the VP5 protein affects

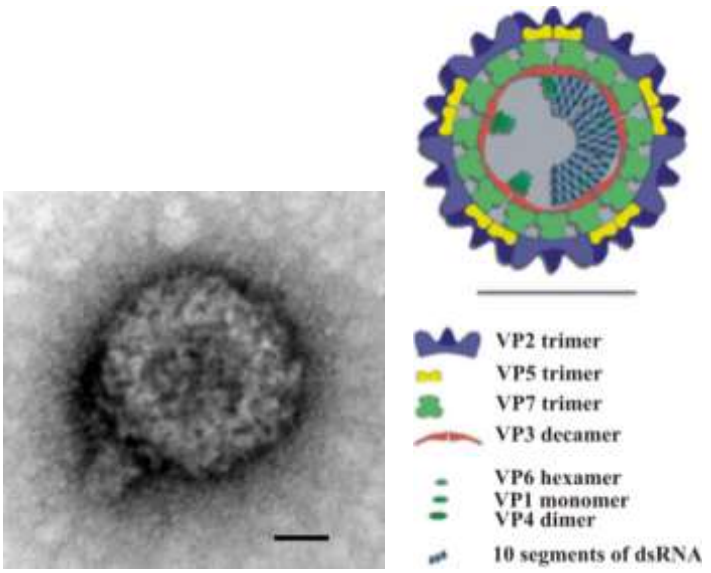
neutralization through its conformational interaction with VP2 (Martínez-Torrecuadrada and Casal, 1995; Martínez-Torrecuadrada et al., 1999). The AHSV outer capsid proteins also mediate cell attachment and penetration during the early stages of infection in mammalian cells (Stassen et al., 2011) and they determine the range of host cell types that the virus is able to infect, thereby influencing the sites of virus replication and tissues in which the virus is concentrated (Huismans et al., 2004). The inner capsid or core is composed of the structural proteins VP3 and VP7, which contain group-specific antigenic determinants (Roy et al., 1991; Roy et al., 1994). The core encloses the dsRNA genome of the virus, as well as the three minor proteins VP1, VP4 and VP6 (Manole et al., 2012). These viral proteins play a fundamental role in virus replication and appear to be largely concerned with the processes of transcription, capping and replication of the viral RNAs (Roy et al., 1994; Vreede and Huismans, 1998; de Waal and Huismans, 2005). In addition to these structural proteins, at least three non-structural proteins (NS1, NS2 and NS3/3A) have also been identified in infected cells (Huismans and Els, 1979; Uitenweerde et al., 1995; Stoltz et al., 1996; Maree and Huismans, 1997; Martin et al., 1998; Van Staden et al., 1998; Meiring et al., 2009). The non-structural proteins are believed to facilitate replication, morphogenesis and exit of progeny virions from infected cells.

To date, nine antigenically distinct serotypes of AHSV have been identified. Serotypes 1 to 7 were identified between 1908 and 1921 (Theiler, 1921), and the last two serotypes, 8 and 9, were identified later (McIntosh, 1958; Howell, 1962). Of the nine AHSV serotypes, serotypes 1 to 8 are found in restricted areas of sub-Saharan Africa, while serotype 9 is more widespread and has been responsible for most epidemics outside Africa (Mellor, 1993).

Susceptible Species

AHSV affects mainly equine species (horses, zebras, mules and donkeys), with horses being the most susceptible. The disease is usually peracute to acute and in naïve animals more than 90% of those affected die (Coetzer and Guthrie, 2004). Compared to horses, mules are more resistant and the mortality rate is lower (50-70%) (MacLachlan and Guthrie, 2010). Zebras are considered the natural vertebrate host and reservoir of AHSV, since they rarely exhibit clinical signs of infection (Barnard, 1998). Interestingly, in contrast to donkeys in the Middle East (Asian donkeys), donkeys in South Africa appear to be naturally resistant to AHSV (mortality rate less than 10%) and animals that become infected rarely display clinical symptoms. Therefore, donkeys, in addition to zebras, may act as reservoir hosts in South Africa (Alexander, 1948; Hamblin et al., 1998).

In addition to the above equines, dogs (Piercy, 1951; Haig et al., 1956; van Rensburg et al., 1981) and camels (Awad et al., 1981; Salama et al., 1980) are the only other domestic animals known to occasionally contract AHS naturally through consumption of infected horse meat. Among wildlife other than zebra, AHSV antibodies have been detected in buffalo (Coetzer and Guthrie, 2004), elephants (Barnard et al., 1995), as well as black and white rhinoceroses (Fischer-Tenhagen et al., 2000). However, it is unlikely that these domestic animals and wildlife play a significant role in the spread or maintenance of AHSV as the *Culicoides* spp. vector insects do not readily feed on them.



(a) (b)

Figure 1. The African horse sickness virus particle. (a) Electron micrograph of a negatively stained, purified AHSV-4 particle. Bar = 25nm. (b) Schematic diagram illustrating the structure of the orbivirus particle. Figure published in Mertens, P. P., Diprose, J., Maan, S., Singh, K.P., Attoui, H. & Samuel, A.R. (2004). Veterinaria Italiana, 40 (4), 426-437.

AFRICAN HORSE SICKNESS (AHS)

History and Epidemiology

The first recorded reference to a disease resembling AHS concerns an epidemic that occurred in 1327 in Yemen (Moule, 1896), but it is most likely that the virus originated on the African continent where it could have been transmitted in the natural zebra population (Mellor and Hamblin, 2004). The disease was first recognized on the African continent after the introduction of horses from India in the 16th century for purposes of exploring central and east Africa. The first outbreak of AHS in southern Africa was recorded in 1719 when over 1 700 animals died of the disease in the Cape of Good Hope (Henning, 1956). Subsequently, over the next 217 years, at least 10 major outbreaks and several minor outbreaks of AHS have been recorded in southern Africa. These outbreaks typically coincided with warm-phase El Niño oscillation (ENSO) events (Baylis et al., 1999) and they have had devastating effects on the African horse population, most notably, the 1854-1855 outbreak in South Africa when more than 70 000 horses died (Coetzer and Guthrie, 2004). The frequency and severity of the outbreaks have, however, declined over the last century. This is most likely due to the major decrease in zebra populations due to hunting, as well as improved surveillance, strict zoning measures and the introduction of AHS vaccines.

At present AHS is endemic in tropical and sub-tropical areas of Africa south of the Sahara, occupying a broad band stretching from Senegal in the west to Ethiopia and Somalia in the east, and extending as far south as northern South Africa (Mellor and Hamblin, 2004). All 9 serotypes of AHSV have been documented in eastern and southern Africa, while

serotype 9 is more widespread and appears to predominate in the northern parts of sub-Saharan Africa (Mellor, 1993). Although AHS was thought to be confined to sub-Saharan Africa, repeated outbreaks in countries beyond its traditional enzootic range have been recorded, including Egypt (1928, 1943, 1953, 1958 and 1971), Yemen (1930, 1997), the Middle East (Palestine, Syria, Lebanon and Jordan) in 1944 and Saudi Arabia (1989) (MacLachlan and Guthrie, 2010). In the period between 1959 and 1961, an outbreak of AHSV serotype 9 (AHSV-9) occurred in Asia (throughout Afghanistan, Cyprus, India, Iraq, Jordan, Lebanon, Pakistan, Saudi Arabia and Syria) that resulted in the death of over 300 000 equids (Howell, 1960; Rafyi, 1961; Mirchamsy and Hazrati, 1973). The inability of the virus to persist in these regions was likely due to a combination of vaccination and vector control campaigns, as well as the high mortality rate that resulted in much of the area being depopulated of susceptible equids (Mellor, 1993). During 1965, an outbreak of AHSV-9 first occurred in Morocco before spreading to Algeria and Tunisia and subsequently spread to Spain in 1966 (Diaz Montilla and Panos Marti, 1967; Hazrati, 1967). The disease was eliminated from Spain within three weeks, following a vigorous vaccination and slaughter policy.

In 1987, an outbreak of AHSV-4, a serotype that had not previously been seen outside southern Africa, was reported in central Spain. This outbreak is believed to have been caused by the importation of sub-clinically infected zebra from Namibia to a safari park near Madrid (Lubroth, 1988). The epidemic continued for four months and ended with the onset of colder climatic conditions, suggesting that the virus was incapable of overwintering in Europe. Nevertheless, throughout the years that followed more severe outbreaks of AHSV-4 occurred in Spain (1988, 1989 and 1990), Portugal (1989) and Morocco (1989, 1990 and 1991) (Zientara et al., 1998). In total, the outbreak in Spain resulted in the deaths of over 400 equids, and a further 900 were destroyed in attempts to control the virus spread (Rodriguez et al., 1992). Portugal reported a total of 206 cases, but the infection was removed quickly following a mass vaccination policy, eradication of animals on infected farms and strict animal movement controls (Portas et al., 1999). Not only have these outbreaks dispelled the long-held belief that AHSV could not survive European winter conditions, but recent expansions in the distribution of competent *Culicoides* insect vectors in Europe due to changes in the climate has raised concerns that AHSV may emerge throughout increasingly extensive portions of Europe (Purse et al., 2008; Wilson et al., 2009).

Vectors and Transmission

AHS is not contagious and AHSV is transmitted primarily through the bites of adult female midges belonging to the genus *Culicoides* (Diptera: Ceratopognidae) (du Toit, 1944; Wetzel et al., 1970; Mellor et al., 1975). An adult *Culicoides* becomes infective eight days after feeding on a viraemic animal and stays infected until death. The virus replicates in the gut and salivary glands of the insect vector, and is subsequently transmitted from the *Culicoides* vector to a susceptible equine host when the midge takes a blood meal (Mellor, 2000).

The major vector of AHSV in Africa is *C. imicola*, which is common throughout Africa and much of Southeast Asia (Wilson et al., 2009). However, a second African species, *C. bolitinos*, has also been implicated as a potential field vector of AHSV (Meiswinkel and

Paweska, 2003). *C. bolitinos* has a wide distribution in southern Africa and is common in cooler highland areas where *C. imicola* is rare (Venter and Meiswinkel, 1994; Venter et al., 2000). Although C. *imicola* was first recorded in parts of southern Spain in 1982, it is now known to be widespread across southern Europe having been recorded throughout much of Portugal, much of Spain and Italy, large areas of mainland Greece, many Mediterranean islands including Corsica, Sardinia, Sicily, Malta and much of the Greek archipelago (Mellor and Hamblin, 2004). The presence of *C. imicola* in these areas means that they may be vulnerable to incursions of AHSV. Indeed, during the 1987-1990 outbreaks of AHS in Spain and Portugal, most isolations of AHSV were made from *C. imicola* (Mellor et al., 1990). Moreover, isolations of AHSV were also made from mixed pools of *Culicoides* species that consisted of *C. obsoletus* and *C. pulicaris* but excluded *C. imicola*, suggesting that one or both of these species may also be involved in the transmission of AHSV in Europe (Mellor et al., 1990). Since these midges are the most common *Culicoides* species across the whole of central and northern Europe, future excursions of AHSV could therefore potentially extend well beyond the regions where *C. imicola* is distributed. In addition to the aforementioned Afro-Asiatic and European *Culicoides* species, *C. variipennis* (=*sonorensis*), which is dominant in North America, has been shown to transmit AHSV under laboratory conditions (Boorman et al., 1975; Mellor et al., 1975). This suggests that should viraemic equids gain entry to parts of North America where *C. sonorensis* occurs (most of the southern and western United States) transmission of AHSV would be likely (Mellor and Hamblin, 2004).

Epidemic outbreaks of AHS have been well correlated with climate conditions favoured by the *Culicoides* species, with early heavy rainfall followed by warm, dry spells generally considered as optimal breeding conditions (Venter and Meiswinkel, 1994; Baylis et al., 1999). However, in some regions, conditions are not suitable for year-round vector activity or virus replication and a break in the transmission cycle occurs. In this regard, it is important to note that AHSV infection rates of *Culicoides* species and virogenesis within them are temperature-dependent (Wellby et al., 1996). Replication of AHSV does not appear to occur within the vector at temperatures below 15°C, and at temperatures below this level the apparent infection rate rapidly falls to zero (Wellby et al., 1996). However, adults of *C. imicola*, the major vector of AHSV, are active at temperatures as low as 12°C and, at these temperatures, the life span of some *Culicoides* species can also be extended up to 90 days (Sellers and Mellor, 1993; Mellor and Wellby, 1998). Notably, when midges are maintained for extended periods at these cooler temperatures and then transferred to temperatures within the virus permissive range, "latent" virus that has presumably persisted at very low levels in some individuals commences replication and rapidly reaches sufficiently high titres for transmission to occur (Wellby et al., 1996). This finding suggests that AHSV transmission is not only possible over the warmer parts of a vector's range, but also provides a possible mechanism for AHSV to overwinter within the adult vector population, provided that some infected individuals survive through the winter period. This mechanism was implicated in the successful overwintering of AHSV in Spain, Portugal and Morocco (Bouayoune et al., 1998; Capela et al., 2003).

Although *Culicoides* species are widely considered to be the principal vector for the transmission of AHSV, several laboratory experiments have shown that the mosquito species *Aedes aegypti*, *Anopheles stephensi* and *Culex pipiens* are all capable of AHSV infection and transmission (Ozawa et al., 1966; Ozawa et al., 1970; Braverman and Boorman, 1978). However, the AHSV titre recovered from these insects were not significantly higher than the

amount of virus ingested and the virus underwent replication in only a limited number of mosquitoes. Furthermore, AHSV has also been isolated from the dog tick *Rhipicephalus sanguineus sanguineus* and the camel tick *Hyalomma dromadarii* (Salama et al., 1980). These arthropod species are, however, considered to play an insignificant role in AHSV transmission (Wilson et al., 2009).

PATHOGENESIS

Despite distinct differences in the clinical severity of AHSV infection in different equids, the typical pattern of pathogenesis is similar. Upon infection, as result of blood feeding by an infected vector insect, AHSV is transported to the regional lymph nodes of the animal, where initial virus replication takes place. The virus is then disseminated throughout the body via the circulatory system, resulting in primary viraemia. Subsequent virus replication in target organs, namely the lungs, spleen and other lymphoid tissues, as well as certain endothelial cells gives rise to secondary viraemia (Coetzer and Guthrie, 2004). During both primary and secondary viraemia, AHSV is often associated with the red blood cells of the infected animal. Oedema of the lungs, pleura and subcutaneous tissues, effusions into body cavities, and haemorrhages in various organs and tissues develop as a result of impaired function of the circulatory and respiratory systems (Erasmus, 1973). The duration of this process from initial infection to secondary viraemia may vary between 2-21 days, although it frequently occurs in less than 9 days (Mellor, 1994). In horses, the period of viraemia typically lasts between 4-8 days (Coetzer and Guthrie, 2004), while in donkeys it may persist for up to 28 days (Hamblin et al., 1998). In zebra, the reservoir host of AHSV, this period may be extended to approximately 40 days post-infection (Barnard et al., 1994).

Clinical Signs

According to the extent and severity of clinical symptoms caused by AHSV infection, the disease is classified into four distinct forms: pulmonary (acute), cardiac (subacute), mixed (cardio-pulmonary) and horse sickness fever (Erasmus, 1973).

The most severe form of AHS is the pulmonary form, which results in mortality rates often exceeding 95%. This form of the disease develops rapidly (within 4-5 days) and usually begins with an acute fever, followed by the onset of severe respiratory distress and dyspnoea. Infected animals often stand with the forelegs spread, head extended and nostrils fully dilated. Other clinical symptoms include pulmonary oedema and pleural effusion, spasmodic coughing, profuse sweating and a frothy serofibrinuous nasal exudate. Death of the animal usually occurs a few hours after the onset of dyspnoea (Spickler et al., 2010).

The cardiac form of AHS has an incubation period of 7-14 days and usually begins with a fever that lasts for 3-6 days. Shortly before the fever starts to subside, oedematous swelling appear in the supraorbital fossae that later spreads to involve the head, neck and chest. Other clinical signs, usually seen in the terminal stages of the disease, include severe depression, colic, petechial haemorrhages in the eyes and ecchymotic haemorrhages on the ventral

surface of the tongue. Death often occurs from cardiac failure and mortality rates exceeding 50% have been reported (Spickler et al., 2010).

The most common form of AHS is the mixed form and develops after an incubation period of 5-7 days. In the mixed form of the disease, symptoms of both the pulmonary and cardiac forms are seen. In most cases, the cardiac form is subclinical and is followed by severe respiratory distress. Occasionally, mild respiratory signs may be followed by oedema and death from cardiac failure. The mortality rate is approximately 70%, with death occurring within 3-6 days after the onset of fever (Spickler et al., 2010).

The horse sickness fever form of AHS develops after an incubation period of 5-14 days and the clinical signs are mild. The animal displays a mild to moderate fever, which lasts for 3-8 days. Other symptoms include mild anorexia or depression, oedema of the supraorbital fossae, congested mucous membranes and an increased heart rate. The animals make a complete recovery and there is no mortality associated with this form of the disease (Spickler et al., 2010). Horse sickness fever frequently occurs in horses with some degree of existing immunity or following infection with less virulent AHSV strains, and it is also the form of disease exhibited by the African donkey and zebra (Howell, 1963).

Post-Mortem Lesions

Macroscopical lesions vary in accordance with the form of AHS (Mirchamsy and Hazrati, 1973; Mellor and Hamblin, 2004; Spickler et al., 2010). In the pulmonary form of AHS, interlobular oedema of the lungs and hydrothorax are the characteristic lesions. The sub-pleural and interlobular tissues are infiltrated with a yellowish gelatinous exudate, and the whole bronchial tree may be filled with a stabilized froth. In more prolonged cases, there may be extensive interstitial and sub-pleural oedema and hyperaemia may be less apparent. Fluid accumulation can occur in the abdominal and thoracic cavities. The lymph nodes, particularly the nodes in the thoracic and abdominal cavities, are usually enlarged and oedematous. The stomach mucosa may be hyperaemic and oedematous. Hyperaemia and petechial haemorrhages may also be apparent in the small and large intestines, and the pericardium may contain petechiae. In the cardiac form, the most conspicuous lesions are gelatinous exudate in the subcutaneous, sub-fascial and intramuscular tissues and lymph nodes. Hydropericardium is common, and the epicardium and endocardium often contain peretechial and ecchymotic haemorrhages. In addition, petechial haemorrhages and/or cyanosis may also be noted on the serosal surfaces of the caecum and large colon. Similar to the pulmonary form, ascites can also be seen, but the lungs are usually normal or slightly enlarged, and the thoracic cavity rarely contains excess fluids. In the mixed form of AHS, the post-mortem lesions are a mixture of typical findings from both the pulmonary and cardiac forms.

DIAGNOSIS

Presumptive diagnosis of AHS may be made from clinical signs and lesions, in association with previous epidemiological information, although definitive diagnosis and

serotype determination are important for control measures to be implemented (Rodríguez-Sánchez et al., 2008).

Isolation and Propagation of AHSV

Traditionally, identification of AHSV has relied on isolation of infectious virus from whole-blood collected in anticoagulant during the febrile stage of infection (Hazrati et al., 1973). Given that the mortality rate in susceptible horses infected with AHSV exceeds 90%, virus can also be readily isolated from tissue specimens collected from dead animals such as the lungs, spleen, lymph nodes and salivary glands (Erasmus, 1973; Hamblin et al., 1992). Virus from whole-blood cells or infected tissues can be propagated in cultured mammalian cells, mice brains or embryonated hen's eggs (Boorman et al., 1975). The preferred method for primary isolation, however, is intracerebral inoculation of 2 to 4 day-old suckling mice. Suitable mammalian cell lines for the propagation of AHSV include baby hamster kidney (BHK), African green monkey (Vero) and monkey kidney (MS) cells, all of which usually show cytopathic effects (CPE) within seven days (Erasmus, 1964; Mirchamsy and Hazrati, 1973; Stassen et al., 2012). Although AHSV can be propagated in insect cell lines such as mosquito (*Aedes albopictus*) C6/36 cells (Mirchamsy et al., 1970) and *Culicoides* (KC) cells (Martin et al., 1998; Stassen et al., 2012), these cell lines do not display CPE post-infection and are usually used only as a sensitive intermediary to amplify virus prior to virus isolation in mammalian cells.

Detection of AHSV Antigens

AHSV isolates can be identified by group-specific serological assays such as agar gel immunodiffusion (Verwoerd et al., 1979), complement fixation (McIntosh, 1956), direct or indirect immunofluorescence assays (Davies and Lund, 1974) and enzyme-linked immunosorbent assays (ELISA) (Laviada et al., 1992; Rubio et al., 1998). Serotyping of AHSV isolates is typically performed with the virus neutralization test (VNT), using type-specific antisera, and takes five or more days before results are obtained (McIntosh, 1958; Verwoerd et al., 1979). Since this test relies on the presence of live replicating virus, either mammalian cells (Hazrati and Ozawa, 1965) or suckling mice (McIntosh, 1958) can be used as indicator of virus neutralization. Immunohistochemical staining methods have also been used for the detection and determination of the location of AHS antigens within infected tissues (Wohlsein et al., 1998; Clift and Penrith, 2010).

Detection of AHSV-Specific Antibodies

Several assays have been developed for the detection of group- and serotype-specific antibody against AHSV. Group-specific antibody detection assays are primarily directed to the major AHSV core protein VP7, and include complement fixation (Blackburn and Swanepoel, 1988), agar gel immunodiffusion (Verwoerd et al., 1979), immunofluorescence (Davies and Lund, 1974) and ELISA assays (Hamblin et al., 1992; Laviada et al., 1992;

Maree and Paweska, 2005). Serotype-specific antibody detection can be accomplished with the serum neutralization test (SNT) (Hopkins et al., 1966; House et al., 1990). Although primary laboratory diagnosis of AHS is performed with group-specific diagnostic assays, the SNT is nevertheless an important tool for identification of the AHSV serotype circulating during an outbreak and thus important in epidemiological surveillance.

Detection of AHSV Nucleic Acids

The reverse transcription polymerase chain reaction (RT-PCR) is frequently used for routine diagnosis of AHSV since it allows for the rapid, sensitive and specific detection of AHSV nucleic acid. RT-PCR diagnostic assays have been described based on the use of serogroup-specific or nested primers specific for genome segments encoding the structural proteins VP3 (Aradaib, 2009) and VP7 (Ferández-Pinero et al., 2009), as well as for genome segments encoding the non-structural proteins NS1 (Mizukoshi et al., 1994), NS2 (Stone-Marschat et al., 1994; Rodríguez-Sánchez et al., 2008) and NS3 (Zientara et al., 1995). Serotype-specific RT-PCR assays that enable identification of the AHSV serotype within 24 hours have also been described (Sailleau et al., 2000; Ferández-Pinero et al., 2009; Quan et al., 2010). In addition to the rapid identification of the serotype, these methods also have the advantage that they are effective in typing very low levels of AHSV in samples that do not contain live replicating virus.

PREVENTION AND CONTROL OF AHSV

Although there is currently no specific treatment for animals suffering from AHS, the virus is non-contagious and can only be spread via the bites of infected vector species of *Culicoides*. Consequently, the control of AHS may be effected by the introduction of animal movement restrictions to prevent infected animals from initiating new foci of infection and viraemic animals may be slaughtered very early in an epidemic to minimize them acting as a source of virus for vector insects (Mellor and Hamblin, 2004; Sánchez-Vizcaíno, 2004). Other approaches aimed at preventing and controlling AHS that warrant further discussion include husbandry modification, vector control and vaccination.

Husbandry Modification

This measure is aimed at denying or reducing vector insects access to susceptible animals. Protective housing can be used as a means of shielding horses from biting *Culicoides* and thus potential AHSV transmission (Barnard, 1997; Meiswinkel et al., 2000). Its success, however, depends on at least two factors: firstly, how well the housing is ‘midge-proofed’ to minimize entry of vector *Culicoides*, and, secondly, the degree of exophilic/endophilic behaviour exhibited by the local vector species of *Culicoides*. Since *C. imicola* is exophilic, stabling of susceptible equids during the night when the vector insects are most active may therefore reduce biting rates and the likelihood of infection (Meiswinkel et al., 2000). In

contrast, *C. bolitinos* is less reluctant to enter animal housing and thus potentially reduces the effectiveness of stabling as a means of limiting transmission in regions where it is a significant vector (Meiswinkel et al., 2000).

Vector Control

It is rarely possible to completely eliminate populations of vector *Culicoides*. The aim is therefore rather to reduce the number of potentially infecting bites that susceptible animals may receive. Different approaches, as indicated below, have been investigated as a means to control vector species of *Culicoides*, but it should be kept in mind that a combination of these approaches it likely to yield the best results.

Spraying with Insecticides

Although broad-scale spraying of insecticides against adult *Culicoides* species is unlikely to be environmentally acceptable, various synthetic pyrethroid insecticides, which have low mammalian toxicity, have been used to reduce biting levels. Application of a synthetic pyrethroid with a long residual life, such as deltamethrin, to external resting surfaces (houses, fences and vegetation) can reduce *Culicoides* numbers in close proximity to animals and thus help to reduce the probability of virus transmission (Floore, 1985; Standfast et al., 2003). Insecticide-treated screens can be a useful strategy to reduce the risk of biting *Culicoides* from entering the animal housing through windows and doors. Laboratory studies showed that propoxur- or malathion-treated aluminium mesh can provide rapid (less than 1 h) knockdown and greater than 97% mortality for between 21-35 days post-treatment, even when the insecticide-treated mesh was exposed to weathering (Jamnback, 1961; Jamnback, 1963; Dukes and Axtell, 1976; Kline and Roberts, 1981).

Application of Repellents

Several candidate and established repellents have been tested on *Culicoides* (Schreck et al., 1979; Trigg, 1996; Braverman and Chizov-Ginsburg, 1997; Carpenter et al., 2005), but none appear to be completely effective and their deterrent effect rarely persists for more than a few hours. In addition, some of the active ingredients may be absorbed into the skin of the animals, thereby reducing not only the period of efficacy but also potentially increasing the likelihood of adverse reactions. However, di-ethyl toluamide (DEET), a commercially available repellent, has been shown to have a significant deterrent effect against *Culicoides* for periods of up to four hours (Braverman and Chizov-Ginsburg, 1997). Since *C. imicola* attacks apparently peak during the first four hours of the night, if applied nightly to susceptible animals, DEET may have a significant but temporary effect in reducing the biting rate of this species (Mellor and Hamblin, 2004).

Application of Larval Control Measures to Breeding Sites

Successful control of the immature stages of *Culicoides* depends largely on correct identification and subsequent destruction of their breeding sites, and the use of an agent known to be effective against the species of *Culicoides* concerned. With regards to the former, good farm management practices can prevent the creation of breeding sites, such as

overflowing horse troughs, leaky irrigation pipes, dripping taps and standing pools of water (Mellor and Wittmann, 2002). With the exception of some laboratory studies conducted on *C. variipennis* (=*sonorensis*) (Wall and Marganian, 1971; Holbrook and Agun, 1984; Woodward et al., 1985), the majority of trials to assess the efficacy of larvacides against *Culicoides* have been concerned with controlling species responsible for biting nuisance rather than virus transmission. Initial attempts at control utilized organochlorine insecticides until the emergence of cross-resistance in larval populations (Clements and Rogers, 1968). Application of a larvacide such as Abate (5% temephos granulated with gypsum) to *Culicoides* breeding sites provides slow release of the insecticide over a period of 30 days and was shown to be effective for *Culicoides* control (Braverman, 1989). Hormonal and biological control agents have also been considered for use against *Culicoides*, albeit in a relatively small number of studies. Insect growth regulators that disrupt metamorphosis have been assessed in the laboratory using field-collected larvae of *C. variipennis* (=*sonorensis*) (Apperson and Yows, 1976). The results showed that dimilin and methoprene reduced emergence by 90%. Initial trials of standard biocontrol agents, such as the bacterium *Bacillus thuringiensis*, were disappointing when applied to field-collected larvae of *Culicoides* (Kelson et al., 1980). Although other natural pathogens of *Culicoides* have been investigated as biopesticides (Carpenter et al., 2008), the issues of mass production and effective release of these parasites, together with initial field trials, have yet to be addressed.

Vaccination

Control of AHS in endemic areas may be effected by vaccination of susceptible equines, which remains the most practical and effective measure (van Dijk, 1998; Sánchez-Vizcaíno, 2004; Patel and Heldens, 2009). For these vaccines to be efficacious, they must be stable, elicit protective immunity in different breeds of a species, induce no or minimal side-effects, and should ideally be effective with a single dose.

Attenuated Vaccines

The first AHS vaccination strategy involved injection of horses with a virulent AHSV strain and hyperimmune horse sera against the same strain (Theiler, 1908; Theiler, 1909). This approach was applied until 1933, but was discontinued due to the occurrence of vaccine-related deaths (2-10%), limitations in the amounts of hyperimmune sera that could be produced, and the requirement for multiple inoculations. During the 1930s, attenuated viruses of several AHSV serotypes were prepared through serial intracerebral passage in mice (Nieschulz, 1932; Alexander and du Toit, 1934; Alexander et al., 1936). These neurotropic vaccines were used for several decades in South Africa, as well as during outbreaks in the Middle East, Egypt and Israel. Although these vaccines induced a solid immunity, they often resulted in fatal cases of post-vaccination encephalitis, characterized by blindness and neurological disorders in horses and donkeys (Nobel and Neumann, 1961; Shah et al., 1964). Furthermore, these neurotropic strains were infectious to humans and non-fatal encephalitis and chorioretinitis were reported in laboratory workers (Swanepoel et al., 1992; van der Meyden et al., 1992).

The above problems were minimized by attenuation of the vaccine virus strains through passage in Vero cell cultures (Erasmus, 1963). These cell culture-adapted viruses still form

the basis of the current AHS vaccine commercially available from Onderstepoort Biological Products (OBP), Onderstepoort, South Africa, and have been used for the control of AHSV in and out of Africa (Erasmus, 1976; Sánchez-Vizcaíno, 2004). Horses are sequentially immunized, three weeks apart, with cocktails of different AHSV serotypes contained in two vials, i.e. serotypes 1, 3 and 4 (vial 1) and serotypes 2, 6, 7 and 8 (vial 2). Serotypes 5 and 9 are excluded from the current vaccine formulation because of putative cross-protection afforded by immunization with serotypes 8 and 6, respectively (von Teichman et al., 2010). In southern Africa, susceptible animals are vaccinated routinely, twice in the first and second years of life and annually thereafter.

Although this polyvalent live attenuated vaccine has reduced the impact of AHS, outbreaks of the disease continue to occur, even in well-vaccinated horses. Furthermore, the current AHS vaccine is limited by its potential for variable attenuation and weak immunogenicity of some vaccine strains (Laegreid, 1996; van Dijk, 1998). There is also a concern that the live virus vaccines may reassort individual genome segments with those of field strains of the virus to create novel virus strains, and that vector *Culicoides* insects might acquire and disseminate the vaccine virus strains in nature (MacLachlan et al., 2007). In addition, replication of live attenuated strains *in vivo* complicates the distinction between vaccinated and infected animals (DIVA) for import/export purposes (Bhanuprakash et al., 2009). These drawbacks are considered to make the live attenuated vaccines unsuitable for use in the naïve host populations in non-endemic geographic regions such as Europe.

Inactivated Vaccines

Although inactivated vaccines should not contain infectious virus, it is often difficult to ensure complete virus inactivation (Laegreid, 1996). Such vaccines are furthermore expensive to produce and require multiple inoculations to elicit and maintain high levels of protective immunity (House et al., 1994). An inactivated monovalent (serotype 4) AHS vaccine, based on virus purification and inactivation with formalin, was produced commercially in the early 1990s (House et al., 1992). A similar formalin-inactivated vaccine, Equipest®, was used for the eradication of AHSV from Spain, Portugal and Morocco during the 1987-1991 epidemic (Dudourget et al., 1992). Although this vaccine was reasonably efficacious, it is not commercially available at the present time.

Subunit Vaccines

With the advent of new technologies and a greater understanding of the molecular and structural biology of orbiviruses, vaccine development has taken new directions that avoid the difficulties and the risks associated with live virus, or preparation of killed virus vaccines. Attention has been focused on the identification of relevant proteins and sequences involved in protective immune responses to infection, and on systems to produce and present these proteins or sequences to elicit the required protection. Several vaccines, including subunit vaccines, have been evaluated experimentally. Subunit vaccines based on the AHSV-4 outer capsid protein VP2 either expressed from recombinant baculovirus (Martinez-Torrecuadrada et al., 1996; Roy et al., 1996; Scanlen et al., 2002) or a DNA vaccine (Romito et al., 1999) have been described. In addition, AHSV VP7, a major serogroup-specific antigen, was shown to protect mice against lethal, heterologous serotype challenge (Wade-Evans et al., 1997).

Recombinant virus vectors have more recently been investigated as vaccine delivery vehicles. Horses were immunized with a vaccinia vector expressing AHSV-4 VP2 and

afforded the same level of protection as the AHS live virus vaccine (Stone-Marschat et al., 1996). Recombinant Venezualen equine encephalitis (VEE) replicon vectors, individually expressing the VP2 and VP5 genes of AHSV-4, have been described, but failed to induce neutralizing antibodies in immunized horses (MacLachlan et al., 2007). The development and preliminary characterization of a canarypox virus vectored (ALVAC) vaccine that co-expresses both outer capsid proteins of AHSV-4, has also been described (Guthrie et al., 2009). Horses immunized with the ALVAC vaccine developed variable titres of virus-specific neutralizing antibodies and were completely resistant to challenge infection with a virulent strain of AHSV-4. Furthermore, a modified vaccinia Ankara (MVA) strain, which is replication deficient, was used for expression of different AHSV antigens (VP2, VP7 and NS3 of AHSV-4) and presented in ponies (Chiam et al., 2009). Analysis of the antibody responses indicated that only the VP2 vaccine was capable of inducing a neutralizing antibody response.

While the above studies have confirmed that a subunit vaccine can induce protective immunity in horses against lethal challenge, they have not been commercialized, which may be a reflection of their cost and/or difficulties associated with their large-scale production.

The Use of Reverse Genetics to Develop a New Generation of AHS Vaccines

One major option in generating a new generation of AHS vaccines and specifically the development of designer attenuated live vaccine virus strains is the so-called reverse genetics approach. The development of reverse genetics systems for major groups of DNA- and RNA-containing viruses has been one of the major technological advances in modern virology. Despite variations in their molecular design and methodology, the various reverse genetics systems all share a common feature, which is the availability of cloned cDNA encoding viral genomes that can be genetically modified and manipulated to generate live viruses containing engineered changes in their genomic nucleic acids. This technology has supported the rapid generation of vaccines against a variety of infectious agents (Zevenhoven-Dobbe et al., 2004; van Gennip et al., 2012; Engelhardt, 2013).

Over the last five years, the development of powerful reverse genetics techniques for several *Reoviridae* viruses has been described (Kobayashi et al., 2007; Boyce et al., 2008; Komoto and Taniguchi, 2013). We and others have shown the recovery of infectious AHSV following transfection of permissive cells with the complete set of 10 purified viral mRNAs derived *in vitro* from transcribing viral core particles (Matsuo et al., 2010; Paterson, 2011). It was subsequently demonstrated that infectious AHSV could also be recovered using a mixture of authentic core-derived viral mRNAs and *in vitro*-synthesized transcripts derived from a cDNA clone. Indeed, this methodology has been used successfully not only for the targeted replacement of genome segment 10 of AHSV-3 with that of AHSV-4, but also to rescue mutant viruses in which the wild-type AHSV-4 S10 genome segment was replaced with a mutated AHSV-4 S10 genome segment (Paterson, 2011). The recovery of directed recombinant and mutated AHSV containing a plasmid cDNA-derived genome segment represents a valuable milestone toward the development of a reverse genetics system for AHSV. As opposed to random attenuation by serial passage of a virus, a reverse genetics approach may enable the rational design of attenuated vaccines via directed mutagenesis. The risk of reversion to virulence in vaccinated animals can be reduced by introducing multiple attenuating mutations and/or by engineering of disabled infectious single cycle (DISC) vaccines, which can be designed to combine the safety and advantages of inactivated vaccines

with the immunogenic activity of live virus vaccines (Zevenhoven-Dobbe et al., 2004; Roy et al., 2009; Matsuo et al., 2011). The DISC viruses replicate only once in normal cells due to the lack of an essential gene product, but can still trigger both a neutralizing antibody response and an innate immune response through replication in the natural target cells. Moreover, DISC vaccines will make it possible to differentiate between animals that have been vaccinated and animals that have been infected with replicating virus (DIVA principle), which could have important consequences for disease control efforts, animal welfare and export of animals.

CONCLUSION

AHS is one of the most lethal of equid diseases and, on the basis of previous outbreaks of AHSV worldwide, it is clear that this disease can have serious consequences for animal health and mortality. Several aspects of the epidemiology of AHSV indicate that it represents a significant risk to especially Europe. Despite being largely restricted to sub-Saharan Africa, AHSV has a history of rapid expansion, without warning, into countries beyond these areas, and it has persisted for several years in these regions. This suggests that the geographical area that is potentially suitable for AHSV transmission is considerably greater than that in which it is currently found. To a great extent, the distribution of AHS is controlled by the abundance, prevalence and seasonal incidence of its *Culicoides* insect vectors. In this regard, it is important to note that climate change has resulted in the major vector species, *C. imicola*, expanding northwards into many areas of Europe previously considered to be AHS risk-free, thereby raising concerns that AHS may spread more globally (Purse et al., 2008; Wilson et al., 2009; Thompson et al., 2012). At present, *Culicoides* vector control measures are poorly developed. Treatment of animal housing with pyrethroids, the use of midge-proofed stabling for viraemic or high-value animals and the promotion of good farm practices to at least partially eliminate local breeding sites are the best options currently available (Carpenter et al., 2008), but a combination of these approaches is likely to yield the best results. In light of these developments, studies aimed at improved understanding of environmental, virus and vector insect determinants that can influence the distribution and spread of AHSV, as well as research to assess and improve the efficacy of vector control methods is needed.

The availability of safe efficacious vaccines is critical to the control of AHS worldwide and to the continued safe international movement of horses. Control of AHS is based on the use of a cocktail of cell-cultured live attenuated strains of the nine different AHSV serotypes. Although these vaccines have proven useful, their use is associated with a number of risks and drawbacks, and they are not registered for use in Europe. Although much effort has been put into developing subunit vaccines, realization of the promise shown by these approaches has been a slow process and none of them have been commercialized as yet. The development of efficacious inherently safe vaccines is therefore a priority, and necessitates the development of alternative strategies to AHS vaccine development. One major option in generating a new generation of AHS vaccines is through a reverse genetics approach. The development and establishment of a reverse genetics system for AHSV may provide several benefits. On the one hand, such a reverse genetics system could facilitate greatly the generation of defined attenuated vaccine strains, such as DISC viruses, thereby expanding the

scope for the development of improved AHS vaccines considerably. On the other hand, the ability to directly and precisely engineer the AHSV genome through a reverse genetics approach will significantly improve our capacity to study the molecular biology, replication mechanism, pathogenesis and transmission of this virus. Through these types of studies, it can be envisaged that the knowledge gained of different aspects of the virus life cycle will aid the development of new approaches to diagnosis, control and prevention of AHS.

REFERENCES

Alexander, R. A. (1948). The 1944 epizootic of horsesickness in the Middle East. *Onderstepoort J. Vet. Sci. Anim. Ind.*, *23*, 77-92.

Alexander, R. A. & du Toit, P. J. (1934). The immunisation of horses and mules against horsesickness by means of the neurotropic virus of mice and guinea pigs. *Onderstepoort J. Vet. Sci. Anim. Ind.*, *2*, 375-391.

Alexander, R. A., Neitz, W. O. & du Toit, P. J. (1936). Horse sickness. Immunisation of horses and mules in the field during 1934-1935 with a description of the technique of preparation of polyvalent mouse neurotropic vaccine. *Onderstepoort J. Vet. Sci. Anim. Ind.*, *7*, 17-30.

Apperson, C. S. & Yows, D. G. (1976). Laboratory evaluation of activity of insect growth-regulators against *Culicoides variipennis* (Diptera, Ceratopogonidae). *Mosq. News*, *36*, 203-204.

Aradaib, I. E. (2009). PCR detection of African horse sickness virus serogroup based on genome segment three sequence analysis. *J. Virol. Methods*, *159*, 1-5.

Awad, F. I., Amin, M. M., Salama, S. A. & Kinde, S. (1981). The role played by *Hylomma dromedarii* in transmission of African horse sickness virus in Egypt. *Bull. Anim. Hlth. Prod. Africa*, *29E*, 337-340.

Barnard, B. J. (1998). Epidemiology of African horse sickness and the role of the zebra in South Africa. *Arch. Virol.*, *14*, 13-19.

Barnard, B. J., Bengis, R. G., Keet, D. & Dekker, E. H. (1995). Epidemiology of African horsesickness, antibodies in free-living elephants (*Loxodonta africans*) and their response to experimental infection. *Onderstepoort J. Vet. Res.*, *62*, 271-275.

Barnard, B. J. H. (1997). Some factors governing the entry of *Culicoides* spp. (Diptera: Ceratopogonidae) into stables. *Onderstepoort J. Vet. Res.*, *64*, 227-233.

Barnard, B. J. H., Bengis, R., Keet, D. & Dekker, E. H. (1994). Epidemiology of African horsesickness - Duration of viremia in zebra (*Equus burchelli*). *Onderstepoort J. Vet. Res.*, *61*, 391-393.

Baylis, M., Mellor, P. S. & Meiswinkel, R. (1999). Horse sickness and ENSO in South Africa. *Nature*, *397*, 574.

Bhanuprakash, V., Indrani, B. K., Hosamani, M., Balamurugan, V. & Singh, R. K. (2009). Bluetongue vaccines: The past, present and future. *Expert Rev. Vaccines*, *8*, 191-204.

Blackburn, N. K. & Swanepoel, R. (1988). African horse sickness in Zimbabwe: 1972 to 1981. *Trop. Anim. Hlth. Prod.*, *20*, 169-176.

Boorman, J., Mellor, P. S., Penn, M. & Jennings, M. (1975). The growth of African horse sickness virus in embryonated hen eggs and the transmission of virus by *Culicoides variipennis* Coquillett (Diptera: Ceratopogonidae). *Arch. Virol.*, *47*, 343-349.

Borden, E. C., Shope, R. E. & Murphy, F. A. (1971). Physicochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus - a new taxonomic group. Electron microscopic studies. *J. Gen. Virol.*, *13*, 273-283.

Bouayoune, H., Touti, J., el Hasnaoui, H., Baylis, M. & Mellor, P. S. (1998). The *Culicoides* vectors of African horse sickness virus in Morocco: Distribution and epidemiology implications. *Arch. Virol.*, *14*, 113-125.

Boyce, M., Celma, C. C. P. & Roy, P. (2008). Development of reverse genetics systems for bluetongue virus: Recovery of infectious virus from synthetic RNA transcripts. *J. Virol.*, *82*, 8339-8348.

Braverman, Y. & Chizov-Ginsburg, A. (1997). Repellency of synthetic and plant-derived preparations for *Culicoides imicola*. *Med. Vet. Entomol.*, *11*, 355-360.

Braverman, Y. & Boorman, J. (1978). Rates of infection in, and transmission of, African horsesickness virus by *Aedes-aegypti* mosquitoes. *Acta Virol.*, *22*, 329-332.

Braverman, Y. (1989). Control of biting midges *Culicoides* (Diptera: Ceratopogonidae), vectors of bluetongue and inducers of sweet itch: A review. *Isr. J. Vet. Med.*, *45*, 124-129.

Bremer, C. W. (1976). A gel electrophoretic study of the protein and nucleic acid components of African horsesickness virus. *Onderstepoort J. Vet. Res.*, *43*, 193-199.

Capela, R., Purse, B. V., Pena, I., Wittmann, E. J., Margarita, Y., Capela, M., Mellor, P. S. & Baylis, M. (2003). Spatial distribution of *Culicoides* species in Portugal in relation to the transmission of African horse sickness and bluetongue viruses. *Med. Vet. Entomol.*, *17*, 165-177.

Carpenter, S., Eyres, K., McEndrick, I., Smith, L., Turner, J., Mordue, W. & Mordue, A. J. (2005). Repellent efficiency of BayRepel® against *Culicoides impunctatus* (Diptera: Ceratopogonidae). *Parasitol. Res.*, *95*, 427-429.

Carpenter, S., Mellor, P. S. & Torr, S. J. (2008). Control techniques for *Culicoides* biting midges and their application in the U.K. and northwestern Palaearctic. *Med. Vet. Entomol.*, *22*, 175-187.

Chiam, R., Sharp, E., Maan, S., Rao, S., Mertens, P., Blacklaws, B., Davis-Poynter, N., Wood, J. & Castillo-Olivares, J. (2009). Induction of antibody responses to African horse sickness virus (AHSV) in ponies after vaccination with recombinant modified vaccinia Ankara (MVA). *PLoS One*, *4*, e5997.

Clements, B. W. & Rogers, A. J. (1968). Tests of larvicides for control of salt-marsh sandflies (*Culicoides*), 1967. *Mosq. News*, *28*, 529-534.

Clift, S. J. & Penrith, M. -L. (2010). Tissue and cell tropism of African horse sickness virus demonstrated by immunoperoxidase labeling in natural and experimental infection in horses in South Africa. *Vet. Pathol.*, *47*, 690-697.

Coetzer, J. A. W. & Guthrie, A. J. (2004). African horse sickness. In J.A.W. Coetzer, & R. C. Tustin (Eds.), Infectious Diseases of Livestock (Second edition, 1231-1246). Cape Town, Southern Africa: Oxford University Press.

Davies, F. G. & Lund, L. J. (1974). The application of fluorescent antibody techniques to the virus of African horse sickness. *Res. Vet. Sci.*, *17*, 128-130.

de Waal, P. J. & Huismans, H. (2005). Characterization of the nucleic acid binding activity of inner core protein VP6 of African horse sickness virus. *Arch. Virol., 150*, 2037-2050.

Diaz Montilla, R. & Panos Marti, P. (1967). Epizootologia de la peste equina en Espana. *Bull. Off. Int. Epizoot., 86*, 705-714.

du Toit, R. M. (1944). The transmission of bluetongue and horse sickness by *Culicoides*. *Onderstepoort J. Vet. Sci. Anim. Ind., 19*, 7-16.

Dudourget, P., Preaud, J. M., Detraz, F., Lacoste, A. C., Erasmus, B. J. & Lombard, M. (1992). Development, production and quality control of an industrial inactivated vaccine against African horse sickness virus serotype 4. In T.W. Walton, & B.I. Osburn (Eds.), Bluetongue, African horse sickness and Related Orbiviruses (874-886). Boca Raton, FL: CRC Press.

Dufour, B., Moutou, F., Hattenberger, A. M. & Rodhain, F. (2008). Global change: Impact, management risk approach and health measures - the case of Europe. *Rev. Sci. Tech., 27*, 529-550.

Dukes, J. C. & Axtell, R. C. (1976). Residual effectiveness of insecticide-treated screens for control of biting midges, *Culicoides furens* (Poey) (Diptera, Ceratopogonidae). *Mosq. News, 36*, 488-491.

Engelhardt, O. G. (2013). Many ways to make an influenza virus: Review of influenza virus reverse genetics methods. *Influenza Other Respir. Viruses, 7*, 249-256.

Erasmus, B. (1973). *The pathogenesis of African horse sickness*. In J.T. Bryans, & H. Gerber (Eds.), Proceeding of the Third International Conference on Equine Infectious Diseases (1-11). Basel, Switzerland: Karger Publishers.

Erasmus, B. J. (1963). Cultivation of horsesickness virus in tissue culture. *Nature, 16*, 716-719.

Erasmus, B. J. (1964). Some observations on the propagation of horse sickness virus in tissue culture. *Bull. Off. Int. Epizoot., 62*, 923-928.

Erasmus, B. J. (1978). *A new approach to polyvalent immunization against African horse sickness*. In J.T. Bryans, & H. Gerber (Eds.), Proceeding of the Fourth International Conference on Equine Infectious Diseases (401-403). Princeton, NJ: Veterinary Publishers International.

Fernández-Pinero, J., Fernández-Pacheco, P., Rodríguez, B., Sotelo, E., Robles, A., Arias, M. & Sánchez-Vizcaíno, J. M. (2009). Rapid and sensitive detection of African horse sickness virus by real-time PCR. *Res. Vet. Sci., 86*, 353-358.

Fischer-Tenhagen, C., Hamblin, C., Quandt, S. & Fröhlich, K. (2000). Serosurvey for selected infectious disease agents in free-ranging black and white rhinoceros in Africa. *J. Wildlife Dis., 36*, 316-323.

Floore, T. G. (1985). Laboratory wind tunnel tests of nine insecticides against adult *Culicoides* species. *Fla. Entomol., 68*, 678-681.

Gale, P., Brouwer, A., Ramnial, V., Kelly, L., Kosmider, R., Fooks, A. R. & Snary, E. L. (2009). Assessing the impact of climate change on vector-borne viruses in the EU through the elicitation of expert opinion. *Epidemiol. Infect., 7*, 1-12.

Grubman, M. J. & Lewis, S. A. (1992). Identification and characterisation of the structural and nonstructural proteins of African horsesickness virus and determination of the genome coding assignments. *Virology, 186*, 444-451.

Guthrie, A. J., Quan, M., Lourens, C. W., Audonnet, J. -C., Minke, J. M., Yao, J., He, L., Nordgren, R., Gardner, I. A. & MacLachlan, N. J. (2009). *Vaccine, 27*, 4434-4438.

Haig, D. A. M., Mcintosh, B. M., Cumming, R. B. & Hempstead, J. F. D. (1956). An outbreak of horsesickness, complicated by distemper in a pack of foxhounds. *J. SA Vet. Med. Assoc.*, *27*, 245-249.

Hamblin, C., Anderson, E. C., Mellor, P. S., Graham, S. D., Mertens, P. P. C. & Burroughs, J. N. (1992). The detection of African horse sickness virus antigens and antibodies in young *Equidae*. *Epidemiol. Infect.*, *108*, 193-201.

Hamblin, C., Salt, J. S., Mellor, P. S., Graham, S. D., Smith, P. R. & Wohlsein, P. (1998). Donkeys as reservoirs of African horse sickness virus. *Arch. Virol.*, *14*, 37-47.

Hazrati, A. (1967). Identification and typing of horsesickness virus strains isolated in the recent epizootic of the disease in Morocco, Tunisia and Algeria. *Arch. Inst. Razi*, *19*, 131-143.

Hazrati, A. & Ozawa, Y. (1965). Serologic studies of African horse sickness virus with emphasis on neutralisation test in tissue culture. *Can. J. Comp. Med.*, *29*, 173-178.

Hazrati, A., Mirchamsy, H. & Bahrami, S. (1973). Comparative studies on the serological responses of horses to African horse sickness virus. In J.T. Bryans, & H. Gerber (Eds.), *Proceeding of the Third International Conference on Equine Infectious Diseases* (69-80). Basel, Switzerland: Karger Publishers.

Henning, M. M. (1956). Animal Diseases of South Africa: African horse sickness, perdesiekte, Pestis equorum (Third edition, 785-808). South Africa: Central News Agency Ltd.

Holbrook, F. R. & Agun, S. J. (1984). Field trials of pesticides to control larval *Culicoides variipennis* (Ceratopogonidae). *Mosq. News*, *44*, 233-235.

Hopkins, I. G., Hazarati, X. & Ozawa, Y. (1966). Development of plaque techniques for the titration and neutralisation tests with African horsesickness virus. *Am. J. Vet. Res.*, *27*, 96-105.

House, C., Mikiciuk, P. E. & Berninger, M. L. (1990). Laboratory diagnosis of African horse sickness, comparison of serological techniques and evaluation of storage methods of samples for virus isolation. *J. Vet. Diagn. Invest.*, *2*, 44-50.

House, J. A., Lombard, M., Dubourget, P., House, C. & Mebus, C. A. (1994). Further studies on the efficacy of an inactivated African horse sickness serotype 4 vaccine. *Vaccine*, *12*, 142-144.

House, J. A., Mikiciuk, P. E. & Berringer, M. L. (1992). *Efficacy of an inactivated vaccine for African horse sickness serotype-4*. In T.W. Walton, & B.I. Osburn (Eds.), Bluetongue, African horse sickness and Related Orbiviruses (874-886). Boca Raton, FL: CRC Press.

Howell, P. G. (1962). The isolation and identification of further antigenic types of African horse sickness virus. *Onderstepoort J. Vet. Res.*, *29*, 139-149.

Howell, P. G. (1960). The 1960 epizootic in the Middle East and SW Asia. *J. SA Vet. Med. Assoc.*, *31*, 329-334.

Howell, P. G. (1963). *African horse sickness. In Emerging Disease of Animals*, FAO Agricultural Studies no. 61 (71-108). Rome, FAO.

Huismans, H. & Els, H. J. (1979). Characterization of tubules associated with the replication of three different orbiviruses. *Virology*, *92*, 397-406.

Huismans, H., Van Staden, V., Fick, W. C., van Niekerk, M. & Meiring, T. L. (2004). A comparison of different orbivirus proteins that could affect virulence and pathogenesis. *Vet. Ital.*, *40*, 417-425.

Jamnback, H. (1961). The effectiveness of chemically treated screens in killing annoying punkies, *Culicoides obsoletus*. *J. Econ. Entomol.*, *54*, 578-580.

Jamnback, H. (1963). Further observations on the effectiveness of chemically treated screens in killing biting midges, *Culicoides sanguisuga* (Diptera: Ceratopogonidae). *J. Econ. Entomol.*, *56*, 719-720.

Kelson, R. V., Colwell, A. E. & McKluskey, D. K. (1980). Studies of *Culicoides occidentalis* at Borax Lake, California. In C.D. Grant (Ed.), Proceedings and Papers of the Forty-eighth Annual Conference of the California Mosquito and Vector Control Association (130-135). Visalia CA: CMVCA Press.

Kline, D. L. & Roberts, R. H. (1981). Effectiveness of chlorpyrifos, fenthion, malathion and propoxur as screen treatments for the control of *Culicoides mississippiensis* (Diptera, Ceratopogonidae). *J. Econ. Entomol.*, *74*, 331-339.

Kobayashi, T., Antar, A. A. R., Boehme, K. W., Danthi, P., Eby, E. A., Guglielmi, K. M., Holm, G. H., Johnson, E. M., Maginnis, M. S., Naik, S., Skelton, W. B., Wetzel, J. D., Wilson, G. J., Chappell, J. D. & Dermody, T. S. (2007). A plasmid-based reverse genetics system for animal double-stranded RNA viruses. *Cell Host and Microbe, 1*, 147-157.

Komoto, S. & Taniguchi, K. (2013). Genetic engineering of rotaviruses by reverse genetics. *Microbiol. Immunol.*, *57*, 479-486.

Laegreid, W. W. (1996). *African horse sickness: Virus infections of equines*. In M.J. Studdert (Ed.), Virus Infections of Vertebrates (First edition, 101-123). Amsterdam, The Netherlands: Elsevier Press.

Laviada, M. D., Babin, M., Dominguez, J. & Sánchez-Vizcaíno, J. M. (1992). Detection of African horsesickness virus in infected spleens by a sandwich ELISA using two monoclonal antibodies specific for VP7. *J. Virol. Methods*, *38*, 229-242.

Lubroth, J. (1988). African horse sickness and the epizootic in Spain 1987. *Equine Prac.*, *10*, 26-33.

MacLachlan, N. J. & Guthrie, A. (2010). Re-emergence of bluetongue, African horse sickness, and other Orbivirus diseases. *Vet. Res.*, *41*, 35.

MacLachlan, N. J., Balasuriya, U. B., Davis, N. L., Collier, M., Johnston, R. E., Ferraro, G. L. & Guthrie, A. J. (2007). Experiences with new generation vaccines against equine viral arteritis, West Nile disease and African horse sickness. *Vaccine*, *25*, 5577-5582.

Manole, V., Laurinmäki, P., van Wyngaardt, W., Potgieter, C. A., Wright, I. M., Venter, G. J., van Dijk, A. A., Sewell, B. T., Butcher, S. J. (2012). Structural insight into African horsesickness virus infection. *J. Virol.*, *86*, 7858-7866.

Maree, F. F. & Huismans, H. (1997). Characterisation of tubular structures composed of nonstructural protein NS1 of African horsesickness virus expressed in insect cells. *J. Gen. Virol.*, *78*, 1077-1082.

Maree, S. & Paweska, J. T. (2005). Preparation of African horse sickness virus VP7 antigen via a simple method and validation of a VP7-based indirect ELISA for the detection of group-specific IgG antibodies in horse sera. *J. Virol. Methods*, *125*, 55-65.

Martin, L. A., Meyer, A. J., O'Hara, R. S., Fu, H., Mellor, P. S., Knowles, N. J. & Mertens, P. P. (1998). Phylogenetic analysis of African horsesickness virus segment 10: Sequence variation, virulence characteristics and cell exit. *Arch. Virol.*, *14*, 281-293.

Martínez-Torrecuadrada, J. L., Diaz-Laviada, M., Roy, P., Sanchez, C., Vela, C., Sánchez-Vizcaíno, J. M. & Casal, J. I. (1996). Full protection against African horsesickness (AHS)

in horses induced by baculovirus-derived AHS virus serotype 4 VP2, VP5 and VP7. *J. Gen. Virol.*, *77*, 1211-1221.

Martínez-Torrecuadrada, J. L. & Casal, J. I. (1995). Identification of a linear neutralisation domain in the protein VP2 of African horse sickness virus. *Virology*, *210*, 391-399.

Martínez-Torrecuadrada, J. L., Langeveld, J. P. M., Venteo, A., Sanz, A., Dalsgaards, K., Hamilton, W. D. O., Meloen, R. H. & Casal, J. I. (1999). Antigenic profile of African horsesickness serotype 4 VP5 and identification of a neutralising epitope shared with bluetongue virus and epizootic hemorrhagic disease virus. *Virology*, *257*, 449-459.

Matsuo, E., Celma, C. C. & Roy, P. (2010). A reverse genetics system of African horse sickness virus reveals existence of primary replication. *FEBS Lett.*, *584*, 3386-3391.

Matsuo, E., Celma, C. C. P., Boyce, M., Viarouge, C., Sailleau, C., Dubois, E., Breard, E., Thiery, R., Zientara, S. & Roy, P. (2011). Generation of replication-defective virus-based vaccines that confer full protection in sheep against virulent bluetongue virus challenge. *J. Virol.*, *85*, 10213-10221.

McIntosh, B. M. (1956). Complement fixation with horsesickness virus. *Onderstepoort J. Vet. Res.*, *27*, 165-169.

McIntosh, B. M. (1958). Immunological types of horse sickness virus and their significance in immunization. *Onderstepoort J. Vet. Res.*, *27*, 465-538.

Meiring, T. L., Huismans, H. & Van Staden V. (2009). Genome segment reassortment identifies non-structural protein NS3 as a key protein in African horsesickness virus release and alteration of membrane permeability. *Arch. Virol.*, *154*, 263-271.

Meiswinkel, R. & Paweska, J. T. (2003). Evidence for a new field *Culicoides* vector of African horse sickness in South Africa. *Prev. Vet. Med.*, *60*, 243-253.

Meiswinkel, R., Baylis, M. & Labuschagne, K. (2000). Stabling and the protection of horses from *Culicoides bolitinos* (Diptera: Ceratopogonidae), a recently identified vector of African horse sickness. *Bull. Entomol. Res.*, *90*, 509-515.

Mellor, P. S. & Wellby, M. P. (1998). Effect of temperature on African horse sickness virus infection of and transmission by vector species of *Culicoides* (Diptera: Ceratopogonidae). In U. Werney, J.F. Wade, J.A. Mumford, & O.R. Kaaden (Eds.), Proceedings of the Eighth International Conference on Equine Infectious Diseases (246-251). East Aurora, NY: R & W Publications.

Mellor, P. S. (1993). African horse sickness: Transmission and epidemiology. *Vet. Res.*, *24*, 199-212.

Mellor, P. S. (2000). Replication of arboviruses in insect vectors. *J. Comp. Pathol.*, *124*, 231-247.

Mellor, P. S. (1994). Epizootiology and vectors of African horse sickness virus. *Comp. Immunol. Microbiol. Infect. Dis.*, *17*, 287-296.

Mellor, P. S. & Hamblin, C. (2004). African horse sickness. *Vet. Res.*, *35*, 445-466.

Mellor, P. S. & Wittmann, E. J. (2002). Bluetongue virus in the Mediterranean basin, 1998-2001. *Vet. J.*, *164*, 20-37.

Mellor, P. S., Boned, J., Hamblin, C. & Graham, S. D. (1990). Isolations of African horse sickness from vector insects made during the 1988 epizootic in Spain. *Epidemiol. Infect.*, *105*, 447-454.

Mellor, P. S., Boorman, J. & Jennings, M. (1975). Multiplication of African horse-sickness virus in 2 species of *Culicoides* (Diptera, Ceratopogonidae). *Arch. Virol.*, *47*, 351-356.

Mertens, P. P. C., Maan, S., Samual, A. & Attoui, A. (2005). The double-stranded RNA viruses: Genus Orbivirus. In C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, & L.A. Ball (Eds.), Virus Taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses (466-483). London, U.K.: Elsevier Academic Press.

Mirchamsy, H. & Hazrati, A. (1973). A review of the aetiology and pathology of African horse sickness. *Arch. Inst. Razi*, *25*, 23-46.

Mirchamsy, H., Hazrati, A., Bahrami, S. & Shafyi, A. (1970). Growth and persistent infection of African horse sickness in a mosquito cell line. *Am. J. Vet. Res., 31*, 1755-1761.

Mizukoshi, N., Sakamoto, K., Iwata, A., Ueda, S., Kamada, M. & Fukusho, A. (1994). Detection of African horsesickness virus by reverse transcriptase polymerase chain reaction (RT-PCR) using primers for segment 5 (NS1 gene). *J. Vet. Med. Sci., 56*, 347-352.

Moulé, L. (1896). *Histoire de la Médecine Vétérinaire*. Paris, France: Imprerie Maulde.

Nieschulz, O. (1932). Over die infectie van muizen met het virus der Zuid-Afrikaansche paardenziekte. *Tydsch. Voor Diergeneesk., 19*, 1433-1445.

Nobel, T. A. & Neumann, F. (1961). Vaccination against African horse sickness and postvaccination reactions in Israel. *Refuah Vet., 18*, 168-173.

Ozawa, Y., Nakata, G., Shad-del, F. & Navai, S. (1966). Transmission of African horse-sickness by a species of mosquito, *Aedes aegypti* Linnaeus. *Am. J. Vet. Res., 27*, 695-697.

Ozawa, Y., Shad-del, F., Nakata, G. & Navai, S. (1970). Transmission of African horse sickness by means of mosquito bites and replication of the virus in *Aedes-aegypti*. *Arch. Inst. Razi*, *22*, 113-122.

Patel, J. R. & Heldens, J. G. M. (2009). Immunoprophylaxis against important virus disease of horses, farm animals and birds. *Vaccine, 13*, 1797-1810.

Paterson, D. J. (2011). Towards the development of a reverse genetics system for African horse sickness virus, MSc dissertation, University of Pretoria, South Africa.

Piercy, S. E. (1951). Some observations on African horsesickness including an account of an outbreak among dogs. *East Afr. Agric. For. J., 17*, 62-64.

Portas, M., Boinas, F. S., Oliveira, J., Sousa, E. & Rawlings, P. (1999). African horse sickness in Portugal: A successful eradication programme. *Epidemiol. Infect., 123*, 337-346.

Purse, B. V., Brown, H. E., Harrup, L., Mertens, P. P. C. & Rogers, D. J. (2008). Invasion of bluetongue and other orbivirus infections into Europe: The role of biological and climatic processes. *Rev. Sci. Tech., 27*, 427-442.

Quan, M., Lourensa, C. W., MacLachlan, N. J., Gardner, I. A. & Guthrie, A. J. (2010). Development and optimisation of a duplex real-time reverse transcription quantitative PCR assay targeting the VP7 and NS2 genes of African horse sickness virus. *J. Virol. Methods*, *167*, 45-52.

Rafyi, A. (1961). Horse sickness. *Bull. Off. Int. Epizoot., 56*, 216-250.

Rodriguez, M., Hooghuis, H. & Castano, M. (1992). African horse sickness in Spain. *Vet. Microbiol., 33*, 129-142.

Rodríguez-Sánchez, B., Fernandez-Pinero, J., Sailleau, C., Zientara, S., Belak, S., Arias, M. & Sánchez-Vizcaíno, J. M. (2008). Novel gel-based and real-time PCR assays for the improved detection of African horse sickness virus. *J. Virol. Methods*, *151*, 87-94.

Romito, M., Du Plessis, D. H. & Viljoen, G. J. (1999). Immune response in a horse inoculated with the VP2 gene of African horse sickness virus. *Onderstepoort J. Vet. Res.*, *66*, 139-144.

Roy, P., Bishop, D. H. L., Howard, S., Aitchison, H. & Erasmus, B. (1996). Recombinant baculovirus-synthesized African horsesickness virus (AHSV) outer capsid protein VP2 provides protection against lethal AHSV challenge. *J. Gen. Virol.*, *77*, 2053-2057.

Roy, P., Boyce, M. & Noad, R. (2009). Prospects for improved bluetongue vaccines. *Nature Rev.*, *7*, 120-128.

Roy, P., Hirasawa, T., Fernandez, M., Blinov, V. M. & Sanchez-Vixcain Rodrique, J. M. (1991). The complete sequence of the group-specific antigen, VP7, of African horsesickness disease virus serotype 4 reveals a close relationship to bluetongue virus. *J. Gen. Virol.*, *72*, 1237-1241.

Roy, P., Mertens, P. P. & Casal, I. (1994). African horse sickness virus structure. *Comp. Immunol. Microbiol. Infect. Dis.*, *17*, 243-273.

Rubio, C., Cubillo, M. A., Hooghuis, H., Sánchez-Vizcaíno, J. M., Diaz-Laviada, M., Plateau, E., Zientara, S., Crucière, C. & Hamblin, C. (1998). Validation of ELISA for the detection of African horse sickness virus antigens and antibodies. *Arch. Virol.*, *14*, 311-315.

Sailleau, C., Seignot, J., Davoust, B., Cardinale, E., Fall, B., Hamblin, C. & Zientara, S. (2000). African horse sickness in Senegal: Serotype identification and nucleotide sequence determination of segment S10 by RT-PCR. *Vet. Rec.*, *146*, 107-108.

Salama, S. A., El-Husseine, M. M. & Abdulla, S. K. (1980). Isolation and identification of African horse sickness virus in the camel tick. 4th Annual Report, US AHS project, *Cairo*, *169*, 91-98.

Sánchez-Vizcaíno, J. M. (2004). Control and eradication of African horse sickness with vaccine. *Dev. Biol. (Basel, Switz.) 119*, 255-258.

Scanlen, M., Paweska, J. T., Verschoor, J. A. & van Dijk, A. A. (2002). The protective efficacy of a recombinant VP2-based African horsesickness subunit vaccine candidate is determined by adjuvant. *Vaccine*, *20*, 1079-1088.

Schreck, C. E., Smith, N. & McGovern, T. P. (1979). Repellency of selected compounds against two species of biting midges (Ceratopogonidae: *Culicoides*). *J. Med. Entomol.*, *16*, 524-527.

Sellers, R. F. & Mellor, P. S. (1993). Temperature and the persistence of viruses in *Culicoides* spp. during adverse conditions. *Rev. Sci. Tech.*, *12*, 733-755.

Shah, K. V., Chinoy, D. N. & Gokhale, T. B. (1964). Investigation of African horse sickness in India. II. Reaction in non-immune horses after vaccination with the polyvalent African horse sickness vaccine. *Indian J. Vet. Sci.*, *34*, 75.

Spence, R. P., Moore, N. F. & Nuttall, P. A. (1984). The biochemistry of Orbiviruses. *Arch. Virol.*, *82*, 1-18.

Spickler, A. R., Roth, J. A., Galyon, J. & Lofstedt, J. (2010). African horse sickness. In A.R. Spickler, & J.A. Roth (Eds.), Emerging and exotic diseases of Animals (Fourth edition, 81-83). Ames, IA: The Centre for Food Security & Public Health, College of Veterinary Medicine, Iowa State University.

Standfast, H., Fanning, I., Maloney, L., Purdie, D. & Brown, M. (2003). Field evaluation of Bistar 80SC as an effective insecticide harbourage treatment for biting midges

(*Culicoides*) and mosquitoes infesting peridomestic situations in an urban environment. *Bull. Mosq. Control Assoc. Aust.*, *15*, 19-33.

Stassen, L., Huismans, H. & Theron, J. (2011). Membrane permeabilization of the African horse sickness virus VP5 protein is mediated by two N-terminal amphipathic α-helices. *Arch. Virol.*, *156*, 711-715.

Stassen, L., Huismans, H. & Theron, J. (2012). African horse sickness virus induces apoptosis in cultured mammalian cells. *Virus Res.*, *163*, 385-389.

Stoltz, M. A., van der Merwe, C. F., Coetzee, J. & Huismans, H. (1996). Subcellular localization of the nonstructural protein NS3 of African horsesickness virus. *Onderstepoort J. Vet. Res.*, *63*, 57-61.

Stone-Marschat, M. A., Moss, S. R., Burrage, T. G., Barber, M. L., Roy, P. & Laegreid, W. W. (1996). Immunisation with VP2 is sufficient for protection against lethal challenge with African horsesickness virus Type 4. *Virology*, *220*, 219-222.

Stone-Marschat, M., Carville, A., Skowronek, A. & Laegreid, W. W. (1994). Detection of African horse sickness virus by reverse transcription PCR. *J. Clin. Microbiol.*, *32*, 697-700.

Swanepoel, R., Erasmus, B. J., Williams, R. & Taylor, M. B. (1992). Encephalitis and chorioretinitis associated with neurotropic African horsesickness virus infection in laboratory workers. Part III. Virological and serological investigations. *S. Afr. Med. J.*, *81*, 458-461.

Theiler, A. (1908). The immunisation of mules with polyvalent serum and virus. *Rep. Gov. Vet. Bact.*, 1906-1907, 192-213.

Theiler, A. (1909). Investigations into South African diseases. The inoculation of mules with polyvalent virus. *Rep. Gov. Vet. Bact.*, 1907-1908, 24-50.

Theiler, A. (1921). African horsesickness (pestis equorum). *SA Dept Agri Sci Bull*, *19*, 1-29.

Thompson, G. M., Jess, S. & Murchie, A. K. (2012). A review of African horse sickness and its implications for Ireland. *Irish Vet. J.*, *65*, 9.

Trigg, J. K. (1996). Evaluation of a eucalyptus-based repellent against *Culicoides impunctatus* (Diptera: Ceratopogonidae) in Scotland. *J. Am. Mosq. Control Assoc.*, *12*, 329-330.

Uitenweerde, J. M., Theron, J., Stoltz, M. A. & Huismans, H. (1995). The multimeric nonstructural NS2 proteins of bluetongue virus, African horsesickness virus, and epizootic hemorrhagic disease virus differ in their single-stranded RNA-binding ability. *Virology*, *209*, 624-632.

van der Meyden, C. H., Erasmus, B. J., Swanepoel, R. & Prozesky, O. W. (1992). Encephalitis and chorioretinitis associated with neurotropic African horsesickness virus infection in laboratory workers. Part I. Clinical and neurological observations. *S. Afr. Med. J.*, *81*, 451-454.

van Dijk, A. A. (1998). *African horse sickness vaccine development.* In U. Werney, J.F. Wade, J.A. Mumford, & O.R. Kaaden (Eds.), Proceedings of the Eighth International Conference on Equine Infectious Diseases (261-265). East Aurora, NY: R & W Publications.

van Gennip, H. G. P., van de Water, S. G. P., Veldhuis, M. & van Rijn, P. A. (2012). Bluetongue viruses based on modified-live vaccine serotype 6 with exchanged outer shell proteins confer full protection in sheep against virulent BTV8. *PLoS One*, *7*, e44619.

van Rensburg, L. B. J., De Clerk, J., Groenewald, H. B. & Botha, W. S. (1981). An outbreak of African horsesickness in dogs. *J. SA Vet. Assoc., 52*, 323-325.

Van Staden, V., Smith, C. C., Stoltz, M. A., Maree, F. F. & Huismans, H. (1998). Characterization of two African horse sickness virus nonstructural proteins, NS1 and NS3. *Arch. Virol., 14*, 251-258.

Venter, G. J. & Meiswinkel, R. (1994). The virtual absence of *Culicoides imicola* (Diptera: Ceratopogonidae) in a light-trap survey of the colder, high-lying area of the eastern Orange Free State, South Africa, and implications for the transmission of arboviruses. *Onderstepoort J. Vet. Res., 61*, 327-340.

Venter, G. J., Graham, S. D. & Hamblin, C. (2000). African horse sickness epidemiology: Vector competence of South African *Culicoides* species for virus serotypes 3, 5 and 8. *Med. Vet. Entomol., 14*, 245-250.

Verwoerd, D. W., Huismans, H. & Erasmus, B. J. (1979). Orbiviruses. In H. Fraenkel-Conrat, & R.R. Wagner (Eds.), *Comprehensive Virology*, (Volume *14*, 285-345). New York, NY: Plenum Press.

von Teichman, B. F., Dungu, B. & Smit, T. K. (2010). *In vivo* cross-protection to African horse sickness serotypes 5 and 9 after vaccination with serotypes 8 and 6. *Vaccine, 28*, 6505-6517.

Vreede, F. T. & Huismans, H. (1998). Sequence analysis of the RNA polymerase gene of African horse sickness virus. *Arch. Virol., 143*, 413-419.

Wade-Evans, A. M., Pullen, L., Hamblin, C., O'Hara, R., Burroughs, J. N. & Mertens, P. P. (1997). African horsesickness virus VP7 sub-unit vaccine protects mice against a lethal, heterologous serotype challenge. *J. Gen. Virol., 78*, 1611-1616.

Wall, W. J. & Marganian, W. M. (1971). Control of *Culicoides melleus* (Coq.) (Diptera: Ceratopogonidae) with granular organophosphorus pesticides, and the direct effect on other fauna. *Mosq. News, 31*, 209-214.

Wellby, M. P., Baylis, M., Rawlings, P. & Mellor, P. S. (1996). Effects of temperature on the rate of virogenesis of African horse sickness virus in *Culicoides* (Diptera: Ceratopogonidae) and its significance in relation to the epidemiology of the disease. *Med. Vet. Entomol., 86*, 715-720.

Wetzel, H., Nevill, E. M. & Erasmus, B. J. (1970). Studies on the transmission of African horsesickness. *Onderstepoort J. Vet. Res., 37*, 165-168.

Wilson, A., Mellor, P. S., Szmaragd, C. & Mertens, P. P. C. (2009). Adaptive strategies of African horse sickness virus to facilitate vector transmission. *Vet. Res., 40*, 16.

Wohlsein, P., Pohlenz, J. F., Salt, J. S. & Hamblin, C. (1998). Immunohistochemical demonstration of African horse sickness viral antigen in tissues of experimentally infected equines. *Arch. Virol., 14*, 57-65.

Woodward, D. L., Colwell, A. E. & Anderson, N. L. (1985). Use of a pyrethrin larvicide to control *Culicoides variipennis* (Diptera, Ceratopogonidae) in an alkaline lake. *J. Am. Mosq. Control Assoc., 1*, 363-368.

Zevenhoven-Dobbe, J. C., Greve, S., van Tol, H., Spaan, W. J. M. & Snijder, E. J. (2004). Rescue of disabled infectious single-cycle (DISC) equine arteritis virus by using complementing cell lines that express minor structural glycoproteins. *J. Gen Virol., 85*, 3709-3714.

Zientara, S., Sailleau, C., Plateau, E., Moulay, S., Mertens, P. P. & Cruciere, C. (1998). Molecular epidemiology of African horse sickness virus based on analyses and comparisons of genome segments 7 and 10. *Arch. Virol.*, *14*, 221-234.

Zientara, S., Sailleau, C., Moulay, S. & Cruciere, C. (1995). Differentiation of African horse sickness viruses by polymerase chain reaction and segments 10 restriction patterns. *Vet. Microbiol.*, *47*, 365-375.

EDITORS' CONTACT INFORMATION

Dr. Adolfo Paz Silva,
Senior Lecturer, Parasitology,
Zooonoses and Epidemiology
Faculty of Veterinary
University of Santiago de Compostela
27002-Lugo Spain
Email: adolfo.paz@usc.es

Dr. María Sol Arias Vázquez
Faculty of Veterinary
University of Santiago de Compostela
27002-Lugo Spain
Email: mariasol.arias@usc.es

Dr. Rita Sánchez-Andrade Fernández
Faculty of Veterinary
University of Santiago de Compostela
27002-Lugo Spain
Email: rita.sanchez-andrade@usc.es

INDEX

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

Y

Z