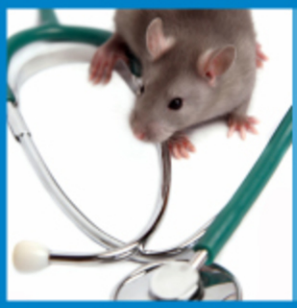
FOURTH EDITION

CLINICAL LABORATORY ANIMAL MEDICINE

An Introduction



Karen Hrapkiewicz,
Lesley Colby, and
Patricia Denison

WILEY Blackwell

with website

Clinical Laboratory Animal Medicine

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An Introduction

Fourth Edition

Karen Hrapkiewicz, DVM, MS, DACLAM
Lesley Colby, DVM, MS, DACLAM
Patricia Denison, LVT, LATG

WILEY Blackwell

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I dedicate this book in memory of my parents, Theodosia and Leopold, for their lifelong encouragement and love.

KH

I dedicate this book:

- To the memory of all the animals that have contributed both to my education and to the advancement of human and veterinary health. It has been a pleasure learning from you.
- To the animal technicians, veterinary technicians, and veterinarians who have devoted their lives to the care of laboratory animals and in support of animal welfare. It has been an honor working beside you.
- To my husband, Ben; our children, Nate and Tess; and our "special" family members (aka dogs), Troika, Ronal Danne, Tasha, and Bacca—for your incredible patience and support through all the nights, weekends, and holidays I spent studying or working over the years. Without you, I would not be who I am today.

LAC

I dedicate this work to the memory of my parents, Dr. Stefan and Shirley Foltan, who instilled a lifelong love of science and compassion for animals, and to my husband, Matt, for his endless patience and support. I also extend heartfelt gratitude to co-author Dr. Karen Hrapkiewicz, who has always been a mentor in all things laboratory animal.

PLD

NOTE

The dosages given in this text are derived from published literature, but as few drugs are specifically licensed for use in the species described, the application is often extra-label and may be empirical or based on clinical experience. The authors have made every attempt to verify all dosages and references; however, despite these efforts, errors in the original sources or in the preparation of this book may have occurred. Users of this text should exercise caution and evaluate all dosages prior to use to determine that they are reasonable.

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Preface

The purpose of this book is to provide basic information on unique anatomic and physiologic characteristics, care and maintenance, common diseases, and recommended treatments for rodents, rabbits, ferrets, and nonhuman primates. It has been prepared as a guide for practicing veterinarians, veterinary students, veterinary technicians, research scientists, and others interested in learning about smaller mammals and nonhuman primates. Knowledge is the key to a familiar dictum: *primum non nocere*—"first do no harm." To undertake medical care of a species with which one is unfamiliar can be dangerous. By gaining familiarity with the more significant, unique biologic features of laboratory animals, learning the most common disease processes, and applying knowledge and skills acquired during professional training, veterinarians should be capable of providing health care to these animals with a reasonable degree of competence.

This fourth edition has been significantly revamped and the material presented in a "user-friendly" format. The text has been updated and expanded with information on transgenic mice, drug dosages, techniques, and environmental enrichment, and it includes color photos and review questions for each chapter. A Web site with PowerPoint presentations and additional images corresponding to each chapter is available.

This book was made possible through the support and guidance of many people. Thanks to Dr. Donald Holmes, who wrote the first edition and gave us a stepping stone and place to start. The efforts of Beth Harries, LVT, and her creative suggestions and assistance in preparing many of the figures in this edition are gratefully acknowledged. We appreciate the University of Michigan Unit for Laboratory Animal Medicine, Providence–St. John Hospital Research Division, and Henry Ford Hospital Bioresources Department for permission to photograph equipment and animals in the facilities. Many thanks to the companies and vendors that provided us with images of and information about their products. We extend our thanks to Stephen Ramsey for his artistic talents. Special, heartfelt thanks to Leticia Medina (co-author of the third edition) and Marianne Tear, LVT, for her photography skills. Thanks to our colleagues for their encouragement when our energy levels were low. Finally, we thank our families and friends for their patience and support during the writing of this text.

Karen Hrapkiewicz
Lesley Colby
Patricia Denison

About the Companion Web Site

This book is accompanied by a companion Web site:

www.wiley.com/go/hrapkiewicz/laboratory

The Web site includes:

- Editable chapter review exercises and answers
- Teaching PowerPoint presentations
- Images in PowerPoint

Watch for throughout the book. These pinpoint materials that are also available on the Web site.

chapter 1

1 Introduction to Laboratory Animal Medicine

Laboratory animal science is the body of scientific and technical information, skills, and techniques that apply to laboratory animal care and use. This includes husbandry, nutrition, behavior, health, production, and management of laboratory animals. The field of laboratory animal medicine has grown rapidly because of a steady increase in biotechnology and genetically engineered rodent models and because good science and the public interest require that the best possible care be given to animals used in research.

Laboratory animal medicine is the specialty field within veterinary medicine that encompasses the diagnosis, treatment, and prevention of diseases in animals used in research, testing, and education. It includes methods to minimize and prevent pain, discomfort, and distress in research animals and ways to identify factors that may influence animal research. Veterinarians engaged in laboratory animal medicine may have a variety of responsibilities within an animal care and use program. They may be responsible for the provision of adequate veterinary care, the management of animal care and use facilities, the education of individuals who care for and use laboratory animals, assisting biomedical scientists in the selection of and humane use of animals, obtaining and interpreting quality data, and assuring compliance with all regulations and policies that affect research animals. Credentialed veterinary technicians work under the supervision of a veterinarian assisting them in carrying out these responsibilities. They often provide technical support in disease detection, including oversight of sentinel programs, treatment of ill animals, blood sampling, and necropsy and tissue collection. When engaged in research or drug study positions at a pharmaceutical firm or university, they administer test products and collect data. This type of employment

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normally requires the credentialed veterinary technician to have a bachelor's degree. Credentialed veterinary technicians may also work in research compliance or supervise other animal facility staff such as assistant laboratory animal technicians, animal caretakers, and cage-wash personnel.

ANIMALS USED IN RESEARCH, PRODUCT SAFETY TESTING, AND EDUCATION

Biomedical Research

Remarkable advances have been made in medicine and science in the past 60 years, such as the development of vaccines for polio and hepatitis B, antibiotics for infectious diseases, procedures for organ transplantation and open heart surgery, and drugs for chronic disorders such as diabetes and high blood pressure. Animals played a major role in these advances (Table 1.1). New treatment modalities for cancer, less invasive surgical approaches, and the development of equipment such as the laser and endoscope used in surgery would not have been possible without the use of animals. Sophisticated as *in vitro* methods and computer simulations may be, they often cannot generate sufficiently comprehensive data about how a substance affects a complex, living being. These *in vitro* methods are often used as early screens to avoid the use of animals for compounds that may be toxic or ineffective. Likewise, the *in vitro* methods are used to answer questions about compound–receptor interactions; this information can lead to a reduction in animal use. Currently, the best predictors of complex biomedical responses of humans are higher-order animals such as mice and rats.

Product Safety Testing

Several decades ago, consumers were subjected to products that were not adequately tested prior to use. Early treatments for syphilis included mercury and arsenic, which in themselves could cause death. An untested eyelash dye marketed in the early 1930s that caused blindness in a number of people and an untested elixir that caused the death of over 100 people led to the passage of the Food, Drug, and Cosmetic Act. The Food, Drug, and Cosmetic Act mandates that prescription drugs be tested first in preclinical animal studies and then in clinical human trials prior to marketing to the general public to ensure they are safe and effective products. Companies that sell beauty and cosmetic products to a largely uneducated group of consumers often misuse the term "cruelty-free" as a marketing tool. These companies claim that they conduct no animal testing. The reality is that their products are either tested by an outside laboratory or are made of compounds known to be safe through previous animal testing.

Education

Animals play a valuable role in education, starting from preschool and continuing to the college and graduate level. Through interactions with animals, youngsters can learn how to care for another living being. They also learn lessons in responsibility and respect. At the middle and high school levels, animal tissues may be used for hands-on experience with dissection. This often opens up the amazing world of biology and science to young people as they marvel at the complexity and specialization of the various organs. Although com-

Table 1.1. Animal roles in medical discoveries and advancements

Year	Scientist(s)	Animal(s) Used	Contribution
1901	von Behring	Guinea pig	Development of diphtheria antiserum
1904	Pavlov	Dog	Animal responses to various stimuli
1923	Banting, Macleod	Dog, rabbit, fish	Discovery of insulin and mechanism of diabetes
1924	Einthoven	Dog	Mechanism of the electrocardiogram
1945	Fleming, Chain, Florey	Mouse	Discovery of penicillin and its curative effect in various infectious diseases
1954	Enders, Weller, Robbins	Monkey, mouse	Culture of poliovirus that led to development of vaccine
1964	Block, Lynen	Rat	Regulation of cholesterol and fatty acid metabolism
1966	Rous	Rat, rabbit, hen	Discoveries concerning hormonal treatment of prostatic cancer
1970	Katz, von Euler, Axelrod	Cat, rat	Mechanism of storage and release of nerve transmitters
1979	Cormack, Hounsfield	Pig	Development of computer-assisted tomography (CAT scan)
1984	Milstein, Koehler, Jerne	Mouse	Techniques of monoclonal antibody formation
1990	Murray, Thomas	Dog	Organ transplant techniques
1997	Prusiner	Mouse, hamster	Discovery of prions, a new biological principle of infection
2003	Lauterbur, Mansfield	Clam, mouse, dog, rat, chimpanzee, pig, rabbit, frog	Discoveries concerning magnetic resonance imaging
2008	Barre-Sinoussi, Montagnier	Monkey, chimpanzee, mouse	Discovery of human immunodeficiency virus
2008	zur Hausen	Hamster, mouse, cow	Discovery of papilloma viruses causing cervical cancer
2011	Hoffman, Beutler	Fruit fly, mouse	Discoveries concerning the activation of innate immunity
2011	Steinman	Mouse	Discovery of the dendritic cell and its role in adaptive immunity

Sources: National Association of Biomedical Research (www.nabr.org) and Nobel Prize (www.nobelprize.org).

puter modeling and videos can replace some biology learning experiences, tissues and organs look remarkably different in real life. In college, animals are used in a variety of professional and graduate level courses in medical and health-related fields. Surgery courses provide young veterinary surgeons a chance to hone their skills before performing them on client-owned animals. Physicians use animals to practice robotic, endoscopic, and laser surgery prior to performing them in people. Animals are used in training courses for medical personnel so they may update their skills in placing endotracheal tubes and critical care monitoring

devices. Technique courses allow veterinary students and veterinary technician students to learn injection techniques and catheter placement on animals. Many of the animals used for educational purposes are subsequently adopted into loving homes.

Animal Usage Statistics

The majority of animals used in biomedical research are bred specifically for that purpose. According to the United States Government Statistics, 1.13 million animals were used in 2010 for research, product safety testing, and education. The U.S. Government Statistics figures do not include mice, rats, birds, and fish as these animals are not covered by the Animal Welfare Act. The precise number of mice and rats used is not available; however, it is estimated approximately 26 million are used every year. Mice and rats account for greater than 95% of the animals used. The number of dogs, cats, and primates together account for less than 1% of the animals used.

The use of nonrodent animals has been declining over the past two decades primarily due to the development of genetically engineered mice and rats. The number of dogs used in research currently is less than one-third of its numbers in the late 1970s. The number of primates used over the past decade has risen slightly, in part due to increasing emphasis of research into neurodegenerative diseases such as Alzheimer's.

To put the numbers in perspective, 25 million of the 26 million animals used in research in the United States are mice, rats, birds, or fish. According to Speaking of Research (http://www.speakingofresearch.com), "we consume over 1800 times the number of pigs than the number used in research. We eat over 340 chickens for each animal used in a research facility, and almost 9000 chickens for every animal used in research covered by the Animal Welfare Act. For every animal used in research, it is estimated that 14 more are killed on our roads."

Funding Sources

In the United States, the National Institutes of Health (NIH) and the National Science Foundation (NSF) are the primary public granting agencies for biomedical research, providing approximately two-thirds of the funds spent by universities and colleges. The NIH, a branch of the Public Health Service (PHS), provides competitive federal grants for investigators interested in the health-related advancement of humans and animals. The NSF encourages basic research in behavior, mathematics, physics, medicine, biology, and other sciences. In addition to the NIH and NSF, funding comes from universities and colleges, state governments, industry, and private foundations. Acquiring funds to conduct research is difficult because competition for grant money is high, with only 10%–20% of submitted proposals receiving funding. Typically, a grant provides money for the scientist's and research team's salaries, supplies, equipment, and purchase and maintenance of animals for a 3-year period. The scientist or principal investigator (PI) plans and coordinates all phases of the research study. The PI must conduct the research study, tabulate data, publish the results, and report findings to the funding agency. If the study has promise, the funding agency may renew the grant for an additional period of time.

Regulatory Oversight and Accreditation

Multiple levels of regulation (e.g., federal, state, and voluntary) exist to provide oversight of animal care and use. Federal and state regulations mandate standards for animal care and

use. Many institutions choose to obtain the "gold standard" of voluntary accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Chapter 2 provides additional information regarding the oversight provided by governmental and voluntary organizations.

Institutional Animal Care and Use Committee

Prior to the initiation of a research study, a product safety test, or an educational program that uses animals, a protocol must be submitted to and approved by the institution's Institutional Animal Care and Use Committee (IACUC). The protocol is a detailed, written description of the proposed animal care and use. It justifies the use of vertebrate animals to accomplish the study's aims, details the procedures that will be performed on the animals, and describes how the animals will be housed and cared for throughout the project. Additionally, the PI must give several assurances, including that the study does not unnecessarily duplicate previous studies, that the staff working with the animals have adequate training to accomplish the study tasks in a humane manner, and that alternatives to animal use and painful or distressful techniques have been carefully considered. Animal use protocols are usually approved for 3 years but must undergo an annual review by the IACUC and be resubmitted for full, *de novo* (anew) review every 3 years. Protocols must also be amended each time a study technique or any activity involving an animal changes. Semiannual inspections by the IACUC members are mandated by the United States Department of Agriculture (USDA) and described in the *Guide for the Care and Use of Laboratory Animals* (the *Guide*; ILAR, 2011) to help ensure that all laboratory animals are observed for appropriate care and for adherence to regulations and use guidelines. Additional information about IACUCs can be found at www.iacuc.org.

ETHICAL CONSIDERATIONS

The 3Rs: Replacement, Refinement, and Reduction

Two English scientists, Russell and Burch, coined the term "the 3Rs." In 1959, they carried out a systematic study of the ethical aspects and "the development and progress of humane techniques in the laboratory." The 3Rs represent three ethical tenets of responsible animal use: replacement, refinement, and reduction. Research institutions and regulatory authorities have developed methods to make sure the principles of the 3Rs are followed to ensure animals are used in an ethical manner. There is an ethical imperative that scientists use animals only when they have provided assurance to the IACUC that no nonanimal methods will allow them to achieve their scientific aim. This search for alternatives is mandated for species covered by the Animal Welfare Act (AWA; see, e.g., APHIS, 2010a) regulations. For species not covered by the AWA, both the Public Health Service Policy and the *Guide* refer to the "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training," which includes language about consideration of alternatives to animals (see Chapter 2, Table 2.1). The U.S. Government Principles mandate using the minimum number of animals necessary to obtain valid results. This is synonymous with reduction, one of the 3Rs.

Replacement refers to replacing animals with a nonanimal alternative, such as *in vitro* screens with cell culture or computer modeling or by using the least sentient animal that will enable good data collection (rat versus dog or fish versus mouse). The ever-increasing sophistication of testing methods, such as molecular diagnostics, has allowed development of alternative tests. Any alternative test, however, must be validated before it can be used to replace a product safety test currently using animals. The development and use of special rodents, such as nude and transgenic mice, has made it possible to reduce the number of more highly evolved species such as dogs and cats. Environmental toxicity studies often use zebrafish rather than mice and other mammals. Alternate tests for ophthalmic safety testing have been developed using eyes from slaughter animals as well as cell and tissue culture systems.

Refinement refers to methods that incorporate modification of a procedure to lessen animal pain and distress or enhance animal well-being. Use of less invasive procedures, provision of pain relief, provision of environmental enrichment, and decreased restraint time are examples of refinements. For example, a study reported a refinement of the urine concentration test used in behavior and physiology studies. During the test, animals had historically been deprived of water for 24 hours or longer. This was found to be unnecessary because the same results were obtained after only 16 hours of water deprivation. Investigators must constantly review the way animal studies are conducted to ensure that the methods used are the most humane or refined so as to minimize pain and distress. Investigators work closely with laboratory animal veterinarians and the IACUC to assure that humane endpoints are in place to minimize pain and distress to the greatest degree possible. The IACUC often develops humane endpoint guidelines that help investigators determine when an animal should be euthanized or removed from a study. Examples of humane experimental endpoints include a defined percentage of weight loss or tumor size, presence of labored breathing, and an inability to ambulate. There is a delicate balance between collecting the necessary scientific data from a study and ensuring that animal welfare is upheld through the use of euthanasia before an animal becomes extremely ill. When appropriate, less invasive methods should be used, and anesthesia or analgesia must be administered to minimize unnecessary pain and distress of research animals.

Reduction refers to using the minimal number of animals in a study that is consistent with sound scientific and statistical standards. Investigators must constantly strive to find ways to reduce animal numbers. Using a combination of computer-based simulators with the animal portion of the study, employing better statistical methods, or using one control group with multiple study groups are potential methods used to reduce animal numbers. The number of animals used in product safety testing has been significantly reduced through validation of alternative testing methods. Experiments can be designed using multiple sections with the results derived from earlier sections used to refine the number of animals or experimental groups used in later sections. For example, the staircase design is often used in acute toxicology testing. This method involves administration of a limited number of drug dosages (high and low) to then determine a more precise dose range for further testing. Used with sophisticated computer-assisted computational methods, the staircase design can determine a point estimate of the lethal dose, approximate confidence intervals, and determine toxic signs for the substance tested, yet use fewer animals.

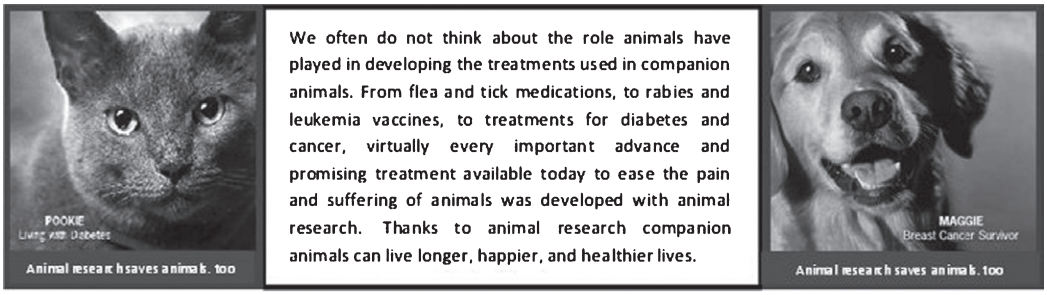

Fig 1.1. Animal research saves animals, too. (Source: Foundation for Biomedical Research.)

Overall, the research community must continually challenge itself to consider whether the animal research being performed is ethical and justifiable. Only in that way will we be assured of continued public support for our use of animals for scientific research that benefits so much of society, including animals! (See Figure 1.1.)

Animal Rights and Animal Welfare

The terms "animal rights" and "animal welfare" are not synonymous. Animal rights is a philosophical belief that gives animals the same equality and protection as humans. In other words, a field mouse has the same right to life as a human. Animal rights purports that animals should not be regarded as property. No matter how humane, animal use is viewed as exploitation and should be banned. This includes keeping dogs and cats as pets; displaying animals in zoos and aquariums; using chickens, cattle, or swine for food; and using animals in research, testing, and education. Their dogmas are not recognized as mainstream ideology and can include abstaining from medication including vaccines or medical treatments that were developed through animal research.

The term animal welfare represents a philosophical belief that it is morally acceptable for humans to use animals provided they are treated humanely and their physical and psychological well-being is met. This creed is based on a belief that animals can contribute to human welfare. Animals provide companionship, entertainment, labor, food, fiber, and advancement of knowledge when used in research and education. When animals are used, it is paramount that responsible practices of animal welfare are adhered to, including provision of appropriate housing, handling, management, disease prevention and treatment, and, when necessary, euthanasia.

Random Source versus Purpose Bred Animals

Dogs and cats used in research and education can come from a number of sources. Random source animals are procured from USDA licensed Class B dealers who obtain them from random sources such as individual owners, breeders, pounds, and animal shelters. Random source dogs may have particularly desirable attributes such as genetic diversity and size. They may be more sociable and tractable, making them easier to handle in a research environment. Purpose bred animals are procured from USDA-licensed Class A dealers or from

privately owned research colonies. These animals are bred and raised specifically for use in research. The most common breeds of purpose bred dogs are beagles and hound crosses. Purpose bred animals are of genetically similar backgrounds, often having known pedigrees and being of a desired age. They tend to have fewer health problems than random source dogs and are accustomed to cage life, but they may be shy and difficult to handle unless previously socialized.

The majority of dogs used in biomedical research are purpose bred. Of approximately 64,000 used in 2010, roughly 1000 were obtained from the nine existing Class B dealers in the United States. Although only a small number of random source dogs are used, there has been growing concern over their use (GAO, 2010). Concerns often cited are the use of prior pets in research or their unsanctioned use after owner surrender at a pound or shelter. Animals must be held at a pound or shelter for a minimum of 5 days, not including the day of acquisition or disposition, to allow an owner adequate time to reclaim a lost pet. Owners who relinquish a pet to a pound or shelter must sign a form that acknowledges the potential release of the animal for research. USDA conducts tracebacks to insure random source animals were obtained through legitimate channels. Three to four million dogs and cats are put to death each year in pounds across our nation. The question becomes, "Should research be permitted access to a small percentage of random source dogs or should all dogs be purpose bred?"

In May 2009, the National Academy of Science delivered a study report, *Scientific and Humane Issues in the Use of Random Source Dogs and Cats in Research*, to the NIH that made a number of recommendations. The report concluded that animals should be made available with random source characteristics but that their availability can be accomplished other than through Class B dealers. The NIH is currently implementing a plan prohibiting the procurement of dogs from USDA Class B dealers using NIH grant funds (NIH, 2011a). NIH is anticipating the new policy will be fully implemented by 2015.

Nonhuman Primate Use

Nonhuman primates account for less than 1% of the USDA-regulated animals used in the United States. The vast majority of primates used are rhesus and cynomolgus macaques; only a very small percentage used are chimpanzees. Nonhuman primates share anatomic and physiologic proximity to humans and as such should be used only when another animal model cannot be used. Chimpanzees, our closest relatives in the animal kingdom, have served an important role in advancing human health, especially in the development of vaccines for polio and hepatitis B. It is their evolutionary closeness that brings with it moral and ethical implications for their use. There is debate among scientists as to the further necessity of using chimps. The biomedical community has developed new methods and technologies that have provided alternative models to the use of nonhuman primates. Scientists agree that chimps should be used judiciously although newly emerging, or re-emerging, diseases may require their future use. Studies using chimps in the United States have undergone rigorous review and demonstrated justification for using the species. Many countries, however, currently prohibit invasive research on chimps thus prompting the United States to review its policies. In December 2011, after the Institute of Medicine addressed the issue and reported its findings, the NIH announced it will not fund any new projects for research involving chimps (NIH, 2011b). Proposed federally funded projects utilizing chimps will be evaluated

using three principles and criteria: (1) the knowledge gained must be necessary to advance the public's health; (2) there must be no other research model by which the knowledge could be obtained, and the research cannot be ethically performed on human subjects; and (3) the animals used in the proposed research must be maintained either in ethologically appropriate physical and social environments or in natural habitats.

ORGANIZATIONS

The need for more systematic and specific information on laboratory animal husbandry, medical care, and management of animal facilities led to the development of several organizations that support the laboratory animal science community in a variety of ways. The following is an introduction to some of the most important organizations and a brief description of their purpose.

American Association for Laboratory Animal Science

In 1950, the Animal Care Panel (ACP), a national organization professionally concerned with the care, production, and study of laboratory animals, was established. In 1967, the ACP became the American Association for Laboratory Animal Science (AALAS). AALAS is a nonprofit, professional association that serves as the principal means of communication between individuals and organizations within the field of laboratory animal science. AALAS currently has over 12,000 individual and institutional members and more than 48 local branches. AALAS produces two scientific journals, *Comparative Medicine* and *Journal of the American Association for Laboratory Animal Science*, and several technician-targeted publications; certifies trained technicians; promotes education through publications; hosts an annual national meeting; and supports the AALAS Learning Library, an extensive Web-based continuing education site. Scientists, veterinarians, technicians, managers, and suppliers share information through presentations, discussions, and exhibits at the annual meeting. For further information, contact the American Association for Laboratory Animal Science, 9190 Crestwyn Hills Drive, Memphis, TN 38125; phone, 901-754-8620; fax, 901-753-0046; email, info@aalas.org; Web site, www.aalas.org.

Training programs are available through AALAS with certification at three levels: Assistant Laboratory Animal Technician (ALAT), Laboratory Animal Technician (LAT), and Laboratory Animal Technologist (LATG). The certification levels and minimum qualifications to sit for the certification exam are described in Figure 1.2. The duties of assistant laboratory animal technicians are primarily related to animal care and facility sanitation. Laboratory animal technicians are expected to have increased diagnostic and technical

Eligibility Requirements

Below are the minimum eligibility requirements for each exam. To be eligible for the exam you wish to take, you must meet one of the combinations of education and work experience.

		Current cert. level	Education level: HS/ GED or higher	Education level: AA/AS or higher	Education level: BA/BS or higher	Lab animal work experience (years)
ALAT Exam	❑					2
	❑		•			1
	❑			•	•	0.5
LAT Exam	❑		•			3
	❑			•		2
	❑				•	1
	❑	ALAT	•			0.5*
	❑	ALAT				2**
LATG Exam	❑		•			5
	❑			•		4
	❑				•	3
	❑	LAT	•			0.5*

* Work experience must be acquired after attaining the specified certification.
** Option for those without documentation of education level.

Fig 1.2. AALAS technician certification: Minimum eligibility requirements. (Source: AALAS.)

Fig 1.3. AALAS certification logos.

skills and research responsibilities. Laboratory animal technologists are frequently involved in supervisory capacities and carrying out portions of the research study. The achievement of certification at any level (Figure 1.3) denotes an individual who has dedicated himself or herself to the pursuit of a higher standard of technical skill and knowledge, and this often translates into a lifetime career in laboratory animal science. Many institutions now require or prefer AALAS certification as a prerequisite for obtaining jobs in their animal facility. Alternatively, many institutions encourage employees to pursue certification as a means of advancing their careers and offer classes as part of their training programs. AALAS offers training manuals for each of the three levels in both English and Spanish and suggests other materials appropriate for examination preparation. Employers often provide employees with

Table 1.2. Eligibility requirements for CMAR designation

Education Level	Total Work Experience	Total Management Experience
BA/BS	5 years	3 years
AA/AS	8 years	3 years
HS/GED	10 years	3 years

Source: AALAS

Note: Candidates meeting these requirements who pass the Animal Resources Exam and the Certified Manager (CM) exams will achieve the status of a Certified Manager of Animal Resources and will be able to use the CMAR acronym after their names.

financial support for the examinations and frequently reward the achievement of the various levels with a specific increase in salary.

A Certified Manager Animal Resources (CMAR) certification program is offered through the Institute for Certified Professional Managers (ICPM) and AALAS. The CMAR designation involves successfully completing a series of four examinations, three arranged through ICPM and one arranged through AALAS. The CMAR designation is a sign of professionalism in the field of animal resources management. The minimum eligibility requirements for CMAR designation are listed in Table 1.2. The Laboratory Animal Management Association, an association dedicated to advancing the quality of management of animals throughout the world, encourages members to become certified (Web site, www.lama-online.org).

American Society of Laboratory Animal Practitioners

The American Society of Laboratory Animal Practitioners (ASLAP) was founded in 1966, in response to the 1966 passing of the Laboratory Animal Welfare Act. It is a professional organization through which veterinarians engaged or interested in the practice of laboratory animal medicine can freely exchange ideas, experiences, and knowledge. In 1967, ASLAP was officially recognized as an ancillary organization of the American Veterinary Medical Association (AVMA). In 1986, ASLAP became an affiliate of AALAS. Both veterinarians and veterinary students make up the membership of ASLAP. According to its Web site, "the objectives of ASLAP are to (1) provide a mechanism for the exchange of scientific and technical information among veterinarians engaged in laboratory animal practice, (2) encourage the development and dissemination of knowledge in areas related to laboratory animal

practice, (3) act as a spokesperson for laboratory animal practitioners within the AVMA House of Delegates and to work with other organizations involved in the care and use of laboratory animals in representing their interests and concerns to the scientific community and the public at large, and (4) actively encourage its members to provide training for veterinarians in the field of laboratory animal practice at both the predoctoral and postdoctoral levels and lend their expertise to institutions conducting laboratory animal medicine training programs." For further information, contact ASLAP Coordinator, 9190 Crestwyn Hills Drive, Memphis, TN 38125-8538; phone, 901-333-0498; fax, 901-753-0046; email, aslap-info@aslap.org; Web site, www.aslap.org.

American College of Laboratory Animal Medicine

The College was originally established as the American Board of Laboratory Animal Medicine in 1957 to encourage education, training, and research in laboratory animal medicine; to establish standards of training and experience for veterinarians professionally concerned with the care and health of laboratory animals; and to recognize qualified persons in laboratory animal medicine by certification examination and other means. The name of the organization was changed to the American College of Laboratory Animal Medicine (ACLAM) in 1961. The ACLAM is a specialty board recognized by the AVMA. Veterinarians who have successfully completed the comprehensive certification examination and fulfilled other stated requirements earn the right to be board certified and to be called Diplomates of the American College of Laboratory Animal Medicine. ACLAM sponsors an annual education meeting, the ACLAM Forum, to highlight different topics of importance to the laboratory animal medicine community. In addition, ACLAM has developed a series of textbooks and programs to promote education about laboratory animal medicine. For further information, contact American College of Laboratory Animal Medicine, 96 Chester St., Chester, NH 03036; email, mail@aclam.org; phone, 603-887-2467; fax, 603-887-0096; Web site, www.aclam.org.

National Association for Biomedical Research

nabr National Association *for* Biomedical Research

The National Association for Biomedical Research (NABR) was founded in 1979. It is a nonprofit organization that advocates for sound public policy in support of ethical and essential animal use in biomedical research, higher education, and product safety testing. NABR serves as a unified voice in Washington, DC, for the scientific community on legislative and regulatory matters affecting laboratory animal research.

NABR supports the responsible and humane care and use of laboratory animals and believes that only as many animals as necessary should be used; that pain or distress animals may experience should be minimized; and that alternatives to the use of live animals should be developed and employed whenever feasible. NABR, however, recognizes that now and in the foreseeable future it is not possible to completely replace the use of animals. The study of whole, living organisms is an indispensable element of biomedical research and testing that benefits all animals. For more information, contact National Association for Biomedical Research, 818 Connecticut Ave. NW, Suite 900, Washington, DC 20006-2702; phone, 202-857-0540; fax, 202-659-1902; email, info@nabr.org; Web site, www.nabr.org.

Foundation for Biomedical Research

FOUNDATION FOR BIOMEDICAL RESEARCH

The Foundation for Biomedical Research (FBR), sister organization to NABR, was established in 1981. It is a nonprofit organization dedicated to improving the quality of human and animal health by promoting public understanding and support for the humane and responsible use of animals in scientific and medical research. The FBR provides information to teachers, students, the media, and the general public on the essential need for animals in medical research and scientific advancement. A wide variety of educational materials, including brochures, posters, reference papers, discussion papers, and videos to support their effort are available from the FBR. For further information, contact the Foundation for Biomedical Research, 818 Connecticut Ave. NW, Suite 200, Washington, DC 20006-2702; phone, 202-457-0654; fax, 202-457-0659; Web site, www.FBResearch.org.

Institute for Laboratory Animal Research

The Institute for Laboratory Animal Research (ILAR) was founded in 1952 under the guidance of the National Research Council (NRC) of the National Academy of Sciences. ILAR is made up of a staff that manages the daily activities of the organization and an ILAR Council composed of experts in laboratory animal medicine, medicine, bioethics, and other biomedical sciences, to provide advice on all aspects of ILAR's activities. ILAR develops guidelines and disseminates information on the scientific, technological, and ethical use of animals and related biological resources for research, testing, and education. ILAR promotes high-quality, humane care of animals and the appropriate use of animals and alternatives. ILAR functions as an advisor to the federal government, the biomedical research community, science educators and students, and the public. ILAR prepares

authoritative reports on subjects of importance to the animal care and use community, including the *Guide for the Care and Use of Laboratory Animals*. ILAR also publishes the *ILAR Journal*, a quarterly peer-reviewed publication on a variety of topics pertinent to the biomedical research community. For further information, contact ILAR, 500 Fifth Street NW, Washington, DC, 20001; phone, 202-334-2590; fax, 202-334-1687; Web site, www.dels.nas.edu/ilar.

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CHAPTER 1 REVIEW

Match Up

Match the following with their respective descriptions:

A. IACUC
B. ACLAM
C. AALAS
D. FDCA
E. ILAR
F. NIH
G. ASLAP
H. NABR
I. CMAR
J. FBR

1. ____ Professional organization for veterinarians engaged or interested in the practice of laboratory animal medicine
2. ____ Certification program for managers of animal facilities
3. ____ Passage of this law led to product safety testing.
4. ____ Develops guidelines and disseminates information on scientific, technological, and ethical use of animals
5. ____ Specialty board recognized by the American Veterinary Medical Association (AVMA)
6. ____ Nonprofit organization dedicated to advocating sound public policy that recognizes the vital role of animal use in biomedical research
7. ____ Prior to use of animals, a protocol must be approved by this committee.
8. ____ Main public granting agency for biomedical research
9. ____ Nonprofit organization that serves as the principal means of communication between individuals and organizations within the field of laboratory animal science
10. ____ Nonprofit organization dedicated to promoting public understanding and support for use of animals in research through educational materials

(*Continued*)

Fill in the Blank

Fill in the blank with one of the following:

REF = for refinement method
RED = for reduction method
REP = for replacement method

11. ____ Use cell culture in oncology study.
12. ____ Give nonsteroidal anti-inflammatory drug in food for pain relief.
13. ____ Use mice rather than dogs in study.
14. ____ Use Corrsitex *in vitro* method to determine the dermal corrosive potential of chemicals.
15. ____ Employ better statistical methods.
16. ____ Use tumor size as endpoint for study.
17. ____ Use excised porcine corneas that are normally discarded as waste in food production for ocular irritation assay.
18. ____ Use staircase design for study.
19. ____ Use computer model rather than rats.
20. ____ House primates in pairs or groups.
21. ____ Use multiple areas on back of pig for skin study.
22. ____ Fast rat for 8 hours rather than 16 hours before procedure.
23. ____ Use hollow fiber bioreactors for monoclonal antibody production instead of rabbit.
24. ____ Use Limulus Amebocyte Lysate Assay for pyrogen testing; blood is taken from horseshoe crabs, which are then returned to the ocean.

Suggested Activities

Use the Web site of the National Association for Biomedical Research http://www.nabr.org/uploadedFiles/nabrorg/Content/Biomedical_Research/FactMyth.pdf to initiate a discussion on common myths involving utilization of animals in research. For example:

MYTH—Animals are not needed for medical research. Most medical breakthroughs have resulted from epidemiological studies, computer models, and cell cultures.
FACT—Biomedical research involving lab animals has played a vital role in virtually every major medical advance of the last century.

Use the Web site North Carolina Responsible Owners Alliance www.ncraoa.com/AR or similar site and discuss the difference between the terms "animal rights" and "animal welfare." Do you place human beings or animals first? Would you go so far as to harm another human being to help an animal? Do you believe animals were put here for our use? Should our role be making sure animals are treated humanely?

Use the interactive Web site Animal Ethics Dilemma http://ae.imcode.com/en/servlet/StartDoc to explore various ethical dilemmas about our treatment of animals. There is no cost to use the Web site. You do need to register to use the site; however, the registration does not require you to provide any personal information.

Read *The Immortal Life of Henrietta Lacks*, by Rebecca Skloot.

Discuss the following:
1. Benefits of using HeLa cells in oncology research
2. Ethical implications of using cells without permission

2

Regulations, Policies, and Principles Governing the Care and Use of Laboratory Animals

Numerous regulations, policies, and guidelines impact the care and use of animals in research, testing, and education. Federal regulations such as the Animal Welfare Act, the Public Health Service Policy, and the Good Laboratory Practice Act are mandatory, where applicable. Several organizations exist that provide for voluntary membership, accreditation, or both. This chapter outlines the primary federal regulatory requirements as well as other important guidelines. Several of the more prominent documents used in the regulation of laboratory animals are depicted in Figure 2.1.

ANIMAL WELFARE ACT AND REGULATIONS

The Animal Welfare Act [AWA; see, e.g., APHIS (2010) and CRS (2010)], passed in 1966, was the first law that protected nonfarm animals in the United States. It was originally known as the Laboratory Animal Welfare Act, or PL 89-544, and was amended in 1970, 1976, 1985, 1990, 2002, 2007, and 2008. The requirements of the AWA are set forth under Regulations and Standards in the Code of Federal Regulations (CFR) and can be found in Title 9, CFR, Chapter 1, Subchapter A—Animal Welfare. These are commonly known as

Clinical Laboratory Animal Medicine: An Introduction, Fourth Edition.
Karen Hrapkiewicz, Lesley Colby, and Patricia Denison.
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Fig 2.1. Documents used in the regulation of laboratory animals.

the Animal Welfare Regulations, or AWRs. Part 1 defines the terms used, Part 2 contains the regulations, Part 3 specifies the standards, and Part 4 has the rules applicable to administrative and judicial decisions under the AWA. To further clarify the intent of the AWA, and to help maintain consistency in application of the regulations, the United States Department of Agriculture (USDA) periodically issues Animal Care Policies. These policies can be reviewed at aphis.usda.gov/animal_welfare/policy.php.

The responsibility for administration and enforcement of the AWA was delegated within the USDA to the Animal and Plant Health Inspection Service (APHIS), Animal Care program. A USDA veterinary medical officer performs unannounced inspections at least once yearly. The regulations apply to animal research facilities, animal dealers and exhibitors, operators of animal auction sales, and carriers and transporters of animals. Retail pet stores are exempt unless they sell animals to a research facility or wholesale dealer. The regulations describe humane handling, care, identification, record keeping, treatment, and transportation of animals. Mandatory minimal animal care standards were developed for dogs, cats, guinea pigs, hamsters, rabbits, nonhuman primates, and marine mammals. These standards include feeding, watering, sanitation, lighting, ventilation, shelter from extremes of weather and temperatures, separation by species, and adequate veterinary care. In private practice settings, dogs and cats are frequently housed in proximity. In research settings, species are housed separately unless justified. Maintenance of health records is an important component of adequate veterinary care. Health records must be current, legible, and sufficiently comprehensive to demonstrate the delivery of adequate health care. An animal's health records must be held for at least one year after its disposition or death.

Species that are regulated include any live or dead dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or other warm-blooded animal that the Secretary of Agriculture may determine is being used or is intended to be used in research, testing, or experimentation,

for exhibition purposes, or as a pet. Species that are not covered by the AWRs include birds, rats of the genus *Rattus*, and mice of the genus *Mus* that are specifically bred for research, teaching, or testing; horses not used for research purposes; and other farm animals intended for use in improving animal nutrition, breeding, management, or production efficiency or for improving the quality of food or fiber.

The purpose of the original Act was to protect owners of dogs and cats from theft of their pets, prevent the sale or use of dogs and cats that had been stolen, and ensure that certain animals intended for use in research facilities were provided humane care and treatment. The law required licensure of individuals or corporations that bought or sold dogs or cats for laboratory activities if the animals were transported across state lines. Organizations that used dogs or cats in biomedical activities were required to register with the USDA if they received federal funding or purchased dogs or cats transported across state lines. The original Act covered nonhuman primates, guinea pigs, hamsters, rabbits, dogs, and cats. It applied only to animals being held before or after actual research and testing, and not during the time the animals were being used.

The Act was amended in 1970, PL 91-579, and given the official title of the Animal Welfare Act. The amendments broadened the coverage of the law to include any warm-blooded animal designated by the Secretary of Agriculture. The amendment covered all dogs and cats regardless of intrastate or interstate transportation. The standards for animal care were extended to apply to animals throughout their stay in the research facility. The Act did not allow the Secretary of Agriculture to issue rules, regulations, or orders with regard to the actual research. It did require, however, that every research facility demonstrate at least annually that professionally acceptable standards governing care, treatment, and use of animals were being followed. Research facilities were also required to file an annual report listing the number of animals used or held for research and whether the animals required or received anesthetics, analgesics, or tranquilizers. The USDA Annual Report of Research Facility form is shown in Figure 2.2. The annual report covers the period October 1 through September 30 of the preceding year and is due December 1.

In 1976, the AWA was further amended, PL 94-279, to redefine the regulation of animals during transportation and to combat the use of animals for fighting. All carriers and intermediate handlers who were not required to be licensed under the AWA were required to register with the USDA. The Secretary of Agriculture also promulgated regulations that specifically excluded rats and mice bred for use in research, birds, horses, and farm animals intended for use as food or fiber or used in studies to improve production of food or fiber.

In 1985, the AWA was changed with the passage of the Food Security Act, PL 99-198. The Food Security Act contained an amendment entitled the Improved Standards for Laboratory Animals Act. This amendment required the chief executive officer of each research facility to appoint an Institutional Animal Care and Use Committee (IACUC). The expanded regulations detailed the membership and functions of the IACUC. A good source of information is IACUC's Web site (www.iacuc.org).

The AWA specifies that an IACUC must consist of at least three members, including a doctor of veterinary medicine with experience or training in laboratory animal medicine and one member who is not affiliated with the institution in any way. The IACUC is charged to act as an agent of the research facility to assure compliance with the AWA. Once every 6 months, the IACUC is required to inspect all animal facilities and study areas and to review

This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provide for in Section 21!

See attached form for additional information.

Interagency Report Control No.:

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER:

CUSTOMER NUMBER:

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS (Sites) - See Attached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS Form 7023A)

A. Animals Covered By The Animal Welfare Regulations	**B.** Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	**C.** Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	**D.** Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	**E.** Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for we the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, yes or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the research such drugs were not used must be attached to this report.	**F.** TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs					
5. Cats					
6. Guinea Pigs					
7. Hamsters					
8. Rabbits					
9. Non-human Primates					
10. Sheep					
11. Pigs					
12. Other Farm Animals					
13. Other Animals					

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary in brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional Office)

SIGNATURE OF C.E.O OR INSTITUTIONAL OFFICIAL	NAME & TITLE OF C.E.O OR INSTITUTIONAL OFFICIAL *(Type or print)*	DATE SIGNED

APHIS FORM 7023 (AUG 91) (Replaces VS FORM 18-23 (OCT 88), which is obsolete.)

Fig 2.2. USDA Annual Report form.

the research facility's program to assure that the care and use of the animals comply with the regulations and standards. The USDA Animal Care (Program of Veterinary Care for Research Facilities) form is shown in Figure 2.3. The IACUC must file a report of its inspection with the institutional official of the research facility. The report must distinguish significant deficiencies, those that threaten animal health or safety, from minor deficiencies. A reasonable and specific plan with dates for correction of the deficiencies must be included in the final report. If significant deficiencies or deviations are found during the inspection and review and are not corrected in accordance with the IACUC's specifications, the USDA and other applicable federal funding agencies must be notified in writing. Recommendations to the institutional official regarding any aspects of the animal program, facilities, or personnel training are included in the report. The report must be signed by a quorum or majority of the committee members and must include any minority views expressed. The IACUC is also required to review and, if warranted, investigate concerns involving the care and use of research animals raised by members of the public or by animal care or research personnel. Personnel must be provided a means to report concerns with anonymity and without fear of reprisal by the institution.

The IACUC must review and approve all proposed activities involving the care and use of animals in research, testing, or education not less than annually. The protocol is a detailed description of the procedures or proposed activities involving the use of the animals. The protocol must provide the following information: (1) the species and approximate number of animals to be used; (2) a rationale for involving animals and for the appropriateness of the species and number of animals requested; (3) a complete and detailed description of the proposed use of the animals; (4) a description of procedures and pharmacologic agents designed to assure that discomfort and pain to animals will be limited to that which is unavoidable for the conduct of scientifically valuable research; and (5) a description of the euthanasia method to be used. The protocol must provide assurance that animal discomfort, distress, or pain will be avoided or minimized. The 1985 amendment specifies that consultation with a doctor of veterinary medicine is necessary in planning any procedure that could cause pain to animals. For procedures that might cause more than momentary or slight pain or distress, a written narrative description of the methods and sources used to determine that alternatives are not available is required. The principal investigator of the protocol must also assure the committee that the proposed work does not unnecessarily duplicate previous experiments.

Survival surgical procedures must be performed using aseptic techniques, including at a minimum sterile instruments, masks, and surgical gloves. Major survival surgery on nonrodents may be conducted only in facilities intended for that purpose. Major surgery involves penetration and exposure of a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, and joint replacement). Minor surgery does not expose a body cavity and causes little or no physical impairment (such as peripheral vessel cannulation and suturing a wound). No animal may be used in more than one major operative procedure from which it recovers unless it is justified, required as a routine veterinary procedure, or required to protect the health or well-being of the animal.

For the first time, the 1985 amendment mandated training of all personnel using animals in research facilities. The institution was required to provide instruction on the care and

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the form. Send comments regarding this burden estimate or any other aspects of this collection of information, including suggestions for reducing the burden, to USDA, ORIM, Clearance Officer, Room 404-W, Washington, DC 20250. When replying refer to the OMB Number and Form Number in your letter.

The Animal Welfare Regulations, Title 9, Subchapter A, Part II, Subpart C. Section 2.33 and Subpart D, Section 2.40 requires a Program of Veterinary Care.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANIMAL CARE

(Program of Veterinary Care for Research Facilities of Exhibitors/Dealers)

FORM APPROVED OMB NO. 0579-0036

OFFICE USE ONLY

DATE RECEIVED

SECTION I. A PROGRAM OF VETERINARY CARE (PVC) HAS BEEN ESTABLISHED BETWEEN:

A. LICENSEE/REGISTRANT		B. VETERINARIAN
1. NAME		1. NAME
2. BUSINESS NAME		2. CLINIC
3. USDA LICENSE/REGISTRATION NUMBER		3. STATE LICENSE NUMBER
4. MAILING ADDRESS		4. BUSINESS ADDRESS
5. CITY, STATE AND ZIP CODE		5. CITY, STATE AND ZIP CODE
6. TELEPHONE NO. *(Home)*	TELEPHONE NO. *(Business)*	6. TELEPHONE NO. *(Business)*

This is a form that may be used for the Program of Veterinary Care. Also, this form may be used as a guideline for the written Program of Veterinary Care as required.

The attending veterinarian shall establish, maintain and supervise programs of disease control and prevention, pest and parasite control, pre-procedural and post-procedural care, nitrition, euthanasia and adequate veterinary care for all animals on the premises of the licensee/registrant. A written program of adequate veterinary care between the licensee/registrant and the doctor of veterinary medicine shall be established and reviewed on an annual basis. By law, such programs must include regularly scheduled visits to the premises by the veterinarian. Scheduled visits are required to monitor animal health and husbandry.

Pages or blocks which do not apply to the facility should be marked N/A. If space provided is not adequate for a specific topic, additional sheets may be added. Please indicate Section and Item Number.

I have read and completed this Program of Veterinary Care, and understand my responsibilities

Regularly scheduled visits by the veterinarian will occur at the following frequency: _____(minimum annual).

C. SIGNATURE OF LICENSEE/REGISTRANT	DATE
D. SIGNATURE OF VETERINARIAN	DATE

APHIS FORM 7002
(JUN 92)

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Fig 2.3. USDA Animal Care form.

handling of animals, humane methods of experimentation, aseptic surgery techniques, methods to minimize or eliminate the use of animals, and how to report deficiencies in animal care and treatment.

The amendment set standards for exercise of dogs and an environment adequate to promote the psychological well-being of nonhuman primates. The standards for exercise of dogs requires a plan be developed, documented, and followed to provide dogs over 12 weeks of age with the opportunity for exercise. The attending veterinarian must approve the exercise plan. The opportunity for exercise may be provided in a number of ways, such as providing access to a run or open area for a prescribed time and frequency or walking animals on a leash. Dogs that are individually housed with at least twice the minimum floor space required and dogs that are held in compatible groups maintained in floor space that provides the minimum space standards for each dog do not require additional opportunity for exercise. The attending veterinarian may approve exemptions to this exercise plan based on the dog's health, condition, or well-being. Such exemptions must be documented and reviewed at least every 30 days by the attending veterinarian unless the condition for exemption is a permanent one (e.g., chronic heart failure).

The standards for environmental enhancement to promote psychological well-being for nonhuman primates requires a plan be developed in accordance with the currently accepted professional standards and directed by the attending veterinarian, documented, and followed. The plan minimally must address social needs, environmental enrichment of primary enclosures, special needs of individual species, use of restraint devices, and exemption of certain primates due to health concerns or research needs. Group housing compatible individuals is an example of addressing the social needs of gregarious nonhuman primates. Environmental enrichment devices may include providing foraging boards, food treats, perches, swings, task-oriented food puzzles, videos, and manipulative-type toys.

In addition, the 1985 amendment established the National Agriculture Library's Animal Welfare Information Center (AWIC). The AWIC provides information pertinent to employee training, information that could prevent unintended duplication of animal experimentation, and information on improved methods of animal experimentation, including methods that could reduce, refine, or replace animal use and minimize pain and distress to animals.

The next amendment, called Protection of Pets, occurred in 1990. It was attached to the farm bill Food, Agriculture, Conservation, and Trade Act, PL101-624. This amendment mandated pounds and shelters, both private and public, to hold any live dog or cat for a minimum period of 5 days, not including the day of acquisition, before euthanizing or releasing the animal to a Class B USDA-licensed dealer. See Chapter 1 for further information regarding Class B dealers and random source dogs.

In 2002, the Farm Security and Rural Investment Act (PL 107-171) changed the definition of "animal" in the AWA to specifically exclude birds, rats of the genus *Rattus*, and mice of the genus *Mus* bred for use in research. The amendment also included verbiage to address animal fighting. It made it a misdemeanor to ship a bird in interstate commerce for fighting purposes or to sponsor or exhibit in a fight a bird shipped for that purpose.

The Animal Fighting Prohibition Enforcement Act of 2007 (PL 110-22) made violation of the animal fighting provisions of the AWA a felony, punishable by up to three years in prison. The law also makes it a felony to trade, sell, or ship select equipment designed for use in animal fighting, or to promote an animal fighting venture.

The Farm Bill (PL 110-246) was passed in 2008. It contains a number of AWA amendments to strengthen definitions of and penalties for activities related to animal fighting. It also requires regulations to limit the transport and resale of dogs unless they are at least six months of age, are in good health, and have all necessary vaccinations. Exemptions exist for research, veterinary treatment, or imports into Hawaii from certain countries. The monetary maximum penalty for a general violation of the act for each violation was also increased.

The Chimpanzee Health Improvement, Maintenance, and Protection Act

The Chimpanzee Health Improvement, Maintenance, and Protection (CHIMP) Act (PL 106-551) was signed into law in December 2000. It created a "sanctuary" retirement system for chimpanzees previously used, bred, or purchased for use in medical research. In 2002, Chimp Haven, located within the Eddie D. Jones Nature Park near Shreveport, Louisiana, was selected to operate the National Chimpanzee Sanctuary System. The CHIMP Act applies only to chimpanzees that were used in studies supported by the agencies of the federal government. The CHIMP Act also mandated that standards of care for chimpanzees in the sanctuary be developed to ensure the well-being and the health and safety of the chimpanzees. The law originally contained a clause that allowed use of the chimpanzees in further research under special circumstances. In December 2007, the Chimp Haven is Home Act was passed, which prohibits chimpanzees retired from medical research to be returned to laboratories.

Office of Laboratory Animal Welfare and Public Health Service Policy

The Health Research Extension Act 1985, PL 99-158, revised the Public Health Service (PHS) policy originally initiated in 1971. The PHS policy relates to the use of animals in research and other biomedical activities that are supported by grants, contracts, and awards from the U.S. Public Health Service. The PHS comprises multiple organizations, including the National Institutes of Health (NIH), the Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), Health Resources and Services Administration, and Alcohol, Drug Abuse and Mental Health Administration. The PHS policy extends to all vertebrates rather than just warm-blooded animals. Institutions must submit a written Animal Welfare Assurance to the Office of Laboratory Animal Welfare (OLAW) fully describing the institution's program for the care and use of animals and assuring that they are committed to following the "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training" (Table 2.1) and the *Guide for the Care and Use of Laboratory Animals* (the *Guide*; ILAR, 2011). In addition, the Assurance must include the names, position titles, and credentials of the IACUC chairperson and the members. Under the PHS policy, the IACUC must maintain oversight of its animal facilities and procedures and must consist of at least five members, including at least (1) one doctor of veterinary medicine, with training or experience in laboratory animal science and medicine, who has direct or delegated program authority and responsibility for activities involving animals at the institution; (2) one practicing scientist experienced in research involving animals; (3) one member whose primary concerns are in a nonscientific area (e.g., ethicist, lawyer, member of the clergy); and (4) one member who is not affiliated with the institution in any way other than as a member of the IACUC and is not a member of the immediate family of a person who is affiliated with the institution. An individual who

Table 2.1. U.S. government principles for the utilization and care of vertebrate animals used in testing, research, and training

I.	The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et seq.) and other applicable Federal laws, guidelines, and policies.
II.	Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.
III.	The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered.
IV.	Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
V.	Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.
VI.	Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.
VII.	The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.
VIII.	Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.
IX.	Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

Source: www.nal.usda.gov/awic/pubs/IACUC/vert.htm.

meets the requirements of more than one of the categories may fulfill more than one requirement; however, no committee may consist of fewer than five members. Under the PHS policy, protocols must be reviewed *de novo* (anew) at least once every 3 years.

Institutions are required to establish a mechanism to review their animal facilities and procedures for conformance with the *Guide*. Voluntary accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) is deemed to be the best method of demonstrating conformance to the *Guide*. An update to the Animal Welfare Assurance document must be submitted to OLAW annually, describing any changes or improvements in the facility's animal care and use program. For further information, visit http://grants.nih.gov/grants/olaw/contactus.htm.

In 2000, an addition to the PHS policy required that staff who are engaged in research or research training with PHS funds, or who work on PHS-supported research projects,

must receive instruction in the responsible conduct of research. Areas of instruction must include data acquisition, management, sharing, and ownership; mentor–trainee responsibilities; publication practices and responsible authorship; peer review; collaborative science; research involving human subjects; research involving animals; research misconduct; conflict of interest and commitment; and compliance with existing PHS and institutional policies.

Good Laboratory Practice Regulations

Good Laboratory Practice (GLP) regulations provide the framework for performing well-conducted, well-documented, and well-reported safety studies. The GLP regulations were adopted in 1978 for nonclinical safety studies funded by the FDA and in 1980 for studies funded by the Environmental Protection Agency (EPA). These regulations tighten the standards for research facilities that are engaged in product safety testing. The general concept is that inadequate animal facilities, treatment, or records are sufficient reasons to question the value or the validity of the data gathered. Quality assurance and strict adherence to standard operating procedures are very important components of the regulations. Each study must have an approved written protocol that defines the study title and purpose, the test article being studied, the testing facility, details about animal use, and study sponsorship. Laboratories must maintain extensive records of all steps of research and make them available to the FDA or EPA, as needed. The FDA and EPA have the legal authority to inspect the facilities where the studies are conducted.

Guide for the Care and Use of Laboratory Animals

The *Guide for the Care and Use of Laboratory Animals*, prepared by the Institute of Laboratory Animal Research (ILAR) of the National Research Council for the NIH, was first published in 1963 and most recently revised in 2011 (ILAR, 2011). According to the ILAR, "The purpose of the *Guide* is to assist institutions caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate. The *Guide* is also intended to assist investigators in fulfilling their obligation to plan and conduct animal experiments in accord with the highest scientific, humane, and ethical principles." The *Guide* makes recommendations for humane animal care and use based on published data, scientific principles, expert opinion, and experience with methods and practices proven consistent with high-quality, humane animal care and use. The *Guide*'s recommendations carry the force of law based on the Health Research Extension Act passed by Congress in 1985. The *Guide* defines laboratory animals as "any vertebrate (e.g., traditional laboratory animals, farm animals, wildlife, and aquatic animals) used in research, testing, or education."

The *Guide* is used throughout the world as a resource for laboratory animal research facilities (Box 2.1). Currently, the *Guide* is available in numerous translations, including Chinese, English, French, Japanese, Korean, Portuguese, Russian, Spanish, and Taiwanese. The *Guide* outlines and references the major components of an animal care and use program, including institutional policies and responsibilities; animal environment, housing, and management; veterinary medical care; and physical plant. Personnel qualifications and training, occupational health and safety of personnel, preventive medicine, surgery including post-surgical care, and euthanasia are addressed in detail. The *Guide* states that, unless a deviation is justified for scientific or medical reasons, the method of euthanasia should be consistent with the most recent *AVMA Guidelines for Euthanasia* (AVMA, 2013).

Box 2.1

The Guide for the Care and Use of Laboratory Animals *is used throughout the world as a resource for laboratory animal research facilities.*

The *Guide* encourages programs to adhere to the U.S. Government Principles (see Table 2.1). The *Guide* also clarifies that programs should function in accord with the USDA regulations, the PHS Policy, and other applicable federal, state, and local laws, regulations, and policies. The *Guide* also states that, in areas where the *Guide* differs from the AWRs or the PHS Policy, users regulated by the AWRs or the PHS Policy must comply with them. The *Guide* employs performance-oriented standards in addition to engineering standards. Recommendations that are performance-oriented direct the user to achieve a specific goal but do not specify the methods used to achieve that outcome. This approach allows flexibility and professional judgment and tends to result in greater enhancement of animal well-being. Engineering standards are specific and generally science-based, giving an exact requirement that must be met. When performance and engineering standards are balanced, programs achieve higher levels of care and use because professional judgment can be used to apply standards to meet a variety of situations. The latest edition of the *Guide* places increased emphasis on housing and enrichment, has updated recommendations regarding physical plant issues, introduces the concept of biosecurity, and contains a new section on aquatic species. To download a copy of the *Guide*, visit www.nap.edu/catalog.php?record_id=12910.

Guide for the Care and Use of Agricultural Animals in Research and Teaching

The agricultural community published the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (the *Ag Guide*) in 1988. The Federation of Animal Science Societies (FASS) revised the document in 1999 and most recently in 2010 (FASS, 2010). It is intended to supplement applicable federal and state laws, regulations, and policies and the *Guide for the Care and Use of Laboratory Animals* (ILAR, 2011). The *Ag Guide* provides guidelines for husbandry, veterinary care, facility construction and maintenance, and institutional policies for agricultural animals. Agricultural animals, for purposes of the *Ag Guide*, include any warm-blooded vertebrate animal used in agricultural research or teaching for which the scientific objectives are to improve understanding of the animal's use in production agriculture and that may require a simulated or actual production agricultural setting consistent with consideration of the animal's well-being. The *Ag Guide* is not intended to pertain to animals produced on farms and ranches for commercial purposes. AAALAC International uses the *Ag Guide* for relevant program assessment and accreditation purposes. To download a copy of this publication, visit www.aaalac.org/about/Ag_Guide_3rd_ed.pdf.

Occupational Health and Safety

Employees, students, and visitors in the course of their work with research animals may be exposed to hazards that could adversely affect their health. Physical hazards (e.g., animal bites, needle sticks, lifting), biohazards (infectious agents and toxins), chemical hazards (e.g., carcinogens, cleaning chemicals), and radiation (x-rays, lasers, radionucleotides) are all potential problems. Some hazards are inherent in animal use facilities such as allergens, zoonotic agents, use of cage-wash equipment, wet floors, and lifting. The *Guide* states, "Each institution must establish and maintain an occupational health and safety program (OHSP) as a part of the overall program of animal care and use. The OHSP must be consistent with federal, state, and local regulations and should focus on maintaining a safe and healthy workplace. The nature of the OHSP will depend on the facility, research activities, hazards, and animal species involved." Control and prevention are key to a successful program. Anyone who may enter the animal facility or have contact with animals and equipment used should be risk assessed. Common methods include the use of health questionnaires, physical exams, and self-reporting changes in health status. The workplace should be periodically assessed for potential hazards and risks. Developing standard operating procedures, use of appropriate safety equipment, and provision of personal protective equipment are ways to manage hazards and risks. ILAR offers an implementation handbook entitled *Occupational Health and Safety in the Care and Use of Research Animals* (ILAR, 2003). This book identifies principles for building an effective safety program and discusses the accountability of institutional leaders, managers, and employees for a program's success.

Association for Assessment and Accreditation of Laboratory Animal Care International

The AAALAC was organized by leading veterinarians and researchers in 1965. AAALAC is a private, nonprofit organization that promotes the humane treatment of animals in science by encouraging high standards of animal care, use, and well-being through assessment and accreditation programs (Figure 2.4). AAALAC comprises a management staff, a Council on Accreditation that performs the majority of site visits and program evaluations, and ad hoc consultants who accompany council members on site visits and make recommendations. AAALAC is supported by member organizations, which "are prestigious scientific, professional, and educational groups with an interest in advancing biomedical research and animal well-being in science." Each member organization appoints a representative to serve on the AAALAC International Board of Trustees.

The Council on Accreditation consists of leading animal care and use professionals and researchers from around the world. The Council currently has North American, European, Pacific Rim, and Southeast Asian regions. Council members and ad hoc consultants conduct peer review evaluations of laboratory animal care facilities and programs. These site visits occur once every 3 years. The number of site visitors and number of days for assessment are based upon the size and complexity of a program. One of the most valuable aspects of the accreditation process is the writing of the program description, which forces individuals to carefully describe the details of their animal care and use program and in the process to perform a self-assessment. The program description outline follows the chapters of the *Guide*, including animal care and use policies and responsibilities; animal environment, housing, and management; veterinary medical care; and physical plant. Accreditation by

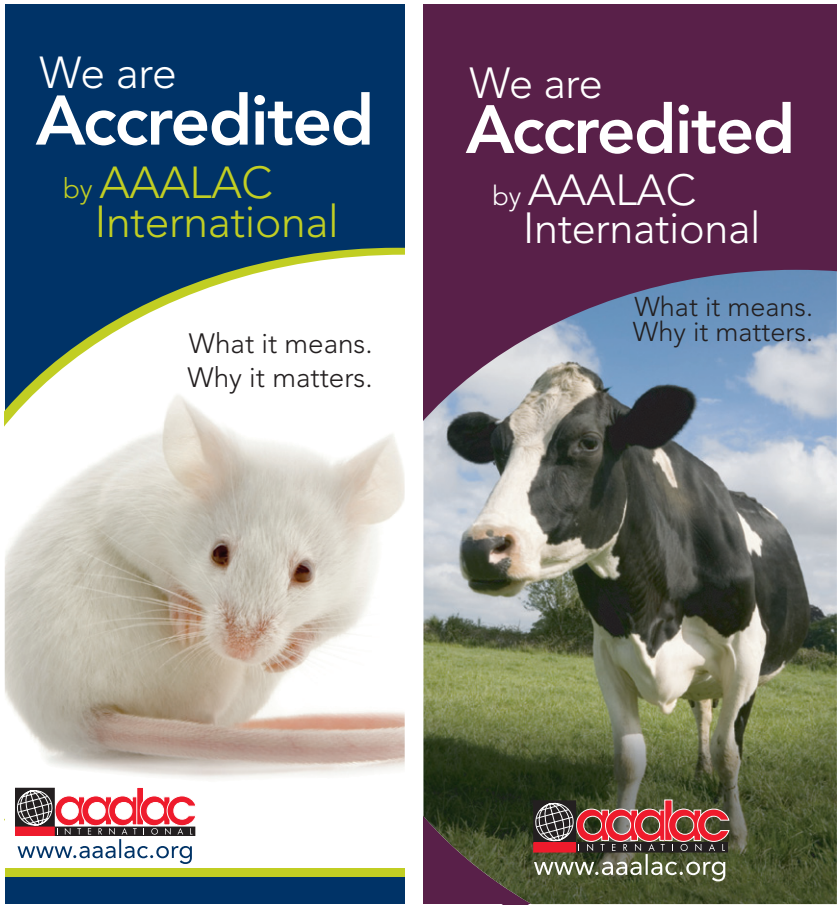

Fig 2.4. AAALAC brochures.

AAALAC is voluntary and is considered the "gold standard" of the industry because it demonstrates that an organization has achieved standards beyond the minimum required by law. There are presently over 850 accredited units in 35 countries, and the number continues to rise steadily as more institutions recognize the value of accreditation.

AAALAC uses three primary standards in conducting evaluations of laboratory animal care and use programs: the *Guide for the Care and Use of Laboratory Animals* (ILAR, 2011); the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2010); and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Council of Europe (ETS 123). AAALAC also refers to other specialty publications and reference resources for supplemental information about procedures or techniques related to the care and use of laboratory animals. A list of these resources and AAALAC International policy statements can be found at www.aaalac.org or by contacting AAALAC International, 5283 Corporate Drive, Suite 203, Frederick, MD 21703-2879; phone, 301-696-9626; fax, 301-696-9627; email, accredit@aaalac.org.

Animal Welfare Information Center

The AWIC is part of the National Agricultural Library located in Beltsville, Maryland. It was established in 1986 as mandated by amendments to the Animal Welfare Act. The AWIC combines personnel with subject expertise, state-of-the-art technology, and networking to assist those interested in learning more about methods for the humane care, use, and handling of animals in research, testing, and education. The AWIC provides the research community with specific information on employee training and identification of improved research methods that could reduce or replace animal use and minimize pain and distress to animals. It will assist researchers in conducting appropriate literature searches designed to identify animal alternatives and prevent unintended duplication of animal experimentation. The AWIC also provides educational opportunities through workshops, publications, and public exhibits. For further information, contact Animal Welfare Information Center, National Agriculture Library, Beltsville, MD 20705-2351; phone, 301-504-6212; Web site, http://awic.nal.usda.gov/.

State Regulations

In the United States, all 50 states and the District of Columbia have laws that protect animals. Most of these laws protect animals from cruel treatment and require that animals have access to food and water and be provided with shelter from extreme weather. Some states have public health and agriculture regulations that specifically cover animals used in research. Additionally, a number of states, cities, and towns regulate the release of impounded animals for research.

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CHAPTER 2 REVIEW

Match Up

Match the following with their respective descriptions:

A. 2008
B. 1966
C. 1990
D. 1950
E. 1985
F. 2002
G. 1976
H. 2007
I. 1970
J. IACUC
K. AVMA
L. AWA
M. AWIC
N. GLP
O. PHS
P. APHIS Animal Care
Q. *Guide for the Care and Use of Laboratory Animals*
R. 7
S. 3
T. 5

1. ____ Protection of Pets amendment passed in this year.
2. ____ NIH, FDA, and CDC are part of this.
3. ____ This organization publishes *Guidelines for Euthanasia*.
4. ____ The Animal Welfare Act (AWA) was originally passed in this year.
5. ____ Regulations used for preclinical safety studies funded by the FDA
6. ____ Amendment of this year extended standard of animal care to apply to an animal throughout its stay in the research facility.
7. ____ Amendment of this year created the IACUC.
8. ____ Farm Bill amendment passed in this year.
9. ____ Primary publication used by AAALAC for evaluation.

10. ____ Amendment of this year changed the definition of "animal" to specifically exclude birds, mice, and rats bred for research.
11. ____ According to the AWA, this is the minimum number of IACUC members.
12. ____ This is part of the National Agriculture Library.
13. ____ Animal Fighting and Prohibition Enforcement Act amendment passed in this year.
14. ____ Amendment of this year redefined the regulation of animals during transport.
15. ____ Must inspect animal facilities every 6 months
16. ____ Responsible for administration and enforcement of the AWA

Fill in the Blank

Fill in the blank with one of the following regarding the AWA:

C = AWA covered species
NC = AWA noncovered species

17. ____ BALB/c mouse
18. ____ Pig used in a dermatology study
19. ____ Sheep used in a wool study
20. ____ Guinea pig used in pregnancy toxemia study
21. ____ Rabbit used in gene therapy study
22. ____ Wild rat used in leptospirosis study
23. ____ Nude mouse
24. ____ Rhesus monkey used in vaccine study
25. ____ Sheep used in fetal human surgery study
26. ____ Dog used in cardiovascular study
27. ____ Cow used in milk production study
28. ____ Pigeon bred for research, used in behavioral study

chapter 3

3 Facility Design, Equipment, Housing, and Management

LABORATORY ANIMAL FACILITY DESIGN

The design of an animal facility is primarily determined by the nature of institutional research activities and the type of animals to be housed. Good animal care is dependent upon a well-planned, well-designed, well-constructed, and properly maintained facility. Animal facilities are usually divided into several functional areas: animal housing; receiving and storage areas for food and bedding; cage wash and sterilization; waste storage; specialized laboratories for activities such as surgery, intensive care, necropsy, radiography, and clinical pathology; containment facilities to support use of hazardous biological, physical, or chemical agents; locker rooms and change areas for personnel; break/lunch rooms; and administrative support. Personnel areas such as offices, conference rooms, and break/lunch rooms should be separated from animal facilities for health protection and human comfort. The facility should have a security system such as a card-key entry to limit personnel access. Systems to monitor heating, ventilation, and air conditioning can be linked with the security system.

There are several important considerations to address when the facility is being designed. Interior surfaces such as walls and ceilings need to be durable, moisture-proof, fire-resistant, and as seamless as possible. Surfaces must be able to withstand cleaning agents, high-pressure sprays, and impacts by carts and cage racks. Corridors must be wide enough to allow movement of equipment and animals. In most animal facilities, 6- to 8-foot-wide corridors are adequate. Doors should open into the animal room and be large enough, minimally 42 by

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Karen Hrapkiewicz, Lesley Colby, and Patricia Denison.
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84 inches, to easily accommodate passage of large pieces of equipment. Recessed handles and kickplates add longevity to the doors. Door windows can be used for safety or as a form of enrichment for some species such as nonhuman primates or dogs, but corridor lighting must be considered and excluded during the night phase of the animal's photoperiod. Hatch-type ports or red-tinted glass that does not transmit specific wavelengths of visible light may be used. Floors should be durable, nonabsorbent, impact-resistant, and as seamless as possible. They should be relatively smooth to aid in sanitation, yet slip proof. The floor should be sloped toward the drain and the drain should be of adequate size, at least 4 inches in diameter, for rapid removal of water and excrement. The heating, ventilation, and air conditioning (HVAC) system must be reliable to closely regulate temperature and humidity for a variety of housing situations. Temperatures should be capable of adjustments of plus or minus 2°F and the humidity kept within a range of 30%–70%. The general guideline of 10 to 15 air changes per hour is the acceptable standard for most animal housing rooms; however, it does not take into consideration different heat loads, animal numbers, or housing systems such as individually ventilated caging. Refer to the *Guide for the Care and Use of Laboratory Animals* (the *Guide*; ILAR, 2011) for more information on appropriate ventilation for animal housing rooms. Air pressure differentials or high-efficiency particulate air (HEPA) filters are recommended to control cross-contamination. For example, areas for quarantine and for the housing of nonhuman primates should be kept under negative pressure relative to the corridor or anteroom, whereas areas for surgery and for housing of pathogen-free animals should be kept under positive pressure. A time-controlled lighting system that provides sufficient illumination for the well-being of animals and safe working conditions is necessary. Adequate space should be provided for storage. Construction materials should be selected to minimize sound travel. Sound-absorbing media such as paint and baffles can be used. Ideally, cage wash and other noise-producing areas should be physically distanced from animal housing for sound control. Similarly, noisy species such as dogs and pigs should be separated from rodents to prevent breeding disruption or introduction of a nonexperimental variable.

BIOSECURITY

The degree of protection required from adventitious infectious agents and/or experimentally induced contagions is a major design consideration. If a high degree of protection is needed, the design used may include entry barriers and designated traffic flow patterns. Specialized equipment such as ventilated housing racks, biological safety cabinets, and extensive personal protective equipment (PPE) may be used in the facility as additional containment controls.

Conventional Facilities

Conventional facilities do not necessarily designate specific traffic circulation patterns; however, consideration must be given to the location of animal rooms and the cage-wash facility operations. Facility design may use a single-corridor system, a dual-corridor system, or a combination of single and dual corridors. Figure 3.1 depicts four examples of

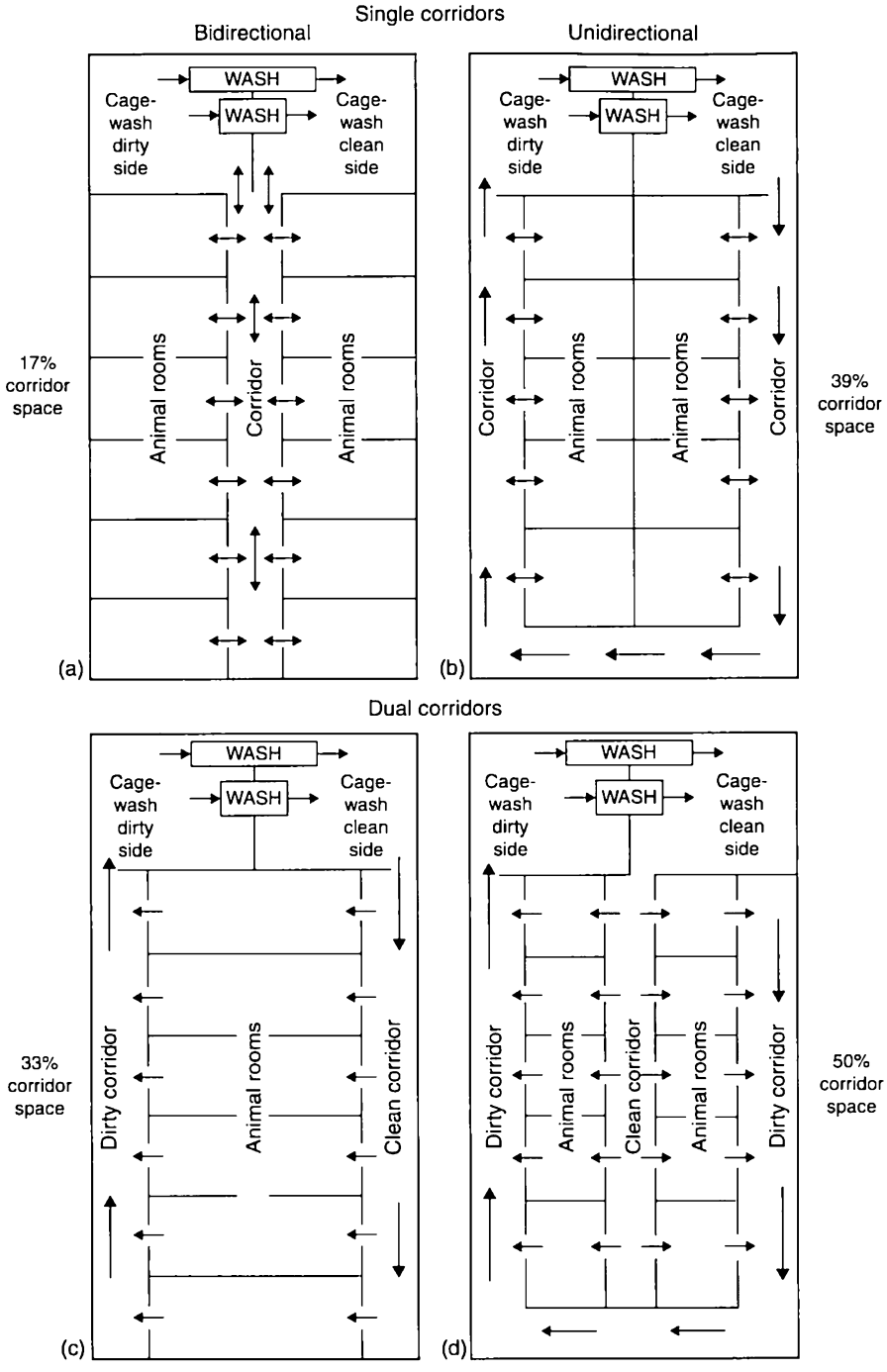

Fig 3.1. Four examples of circulation patterns within identical footprints are shown with arrows pointing in the direction(s) of traffic flow around the cage-washing area: (a) a single-corridor bidirectional flow pattern; (b) a single-corridor unidirectional flow pattern; (c) a dual-corridor flow pattern with large animal rooms; and (d) a dual-corridor flow pattern with smaller animal rooms. The percentage of the footprint occupied by corridors is shown for each pattern. These percentages only serve to illustrate the impact of circulation pattern choices, and do not apply to any specific floor plan. (Hessler and Lehner, 2009, *Planning and Designing Research Animal Facilities*, p. 102, Figure 9-2.)

circulation patterns. Single-corridor traffic flow may be unidirectional or bidirectional. In a unidirectional pattern, clean and soiled items enter and exit the animal room through the same door. In a bidirectional pattern, clean items enter the room through a clean corridor and soiled items exit the animal room into the soiled corridor. Dual-corridor traffic flow incorporates the use of a designated clean corridor and a designated soiled corridor. This pattern minimizes cross-contamination within the facility. Clean items are brought to the animal room from the clean corridor through one door, and soiled items must exit the animal room from another door to the soiled corridor. The soiled items are then taken to the cage-wash area via the soiled corridor. Airflow pressure differences are used in these facilities to promote directional airflow from clean to soiled areas and to prevent circulation of contaminated ventilation to other areas.

Barrier Facilities

Barrier facilities are designed to exclude unintentional contamination of an area, adding a higher degree of protection to animals housed in them. Barrier suites such as the one depicted in Figure 3.2 are used to maintain breeding and experimental animal colonies in a disease-free condition. Examples of colonies afforded such protection include transgenic, immunocompromised, and founding breeder lines. Dual-corridor traffic flow patterns and ventilated caging systems are routinely used in barrier facilities. Contamination-controlled flooring, adhesive mats, and shoe covers are frequently used to reduce organic contamination of barrier areas; shoe covers have been found most effective. Large capacity, pass-through autoclaves are used to provide sterile food, water, and microisolation housing for barrier-maintained animals. Personnel typically pass into the barrier by way of two or three compartment locks, allowing for a multistage transition from original garb through a wet, chemical, or air shower and into a uniform and required PPE.

Containment or Biohazard Facilities

Containment or biohazard facilities protect the general animal and human population by minimizing the opportunities for escape of experimentally induced contagions. Facilities employ HVAC systems designed to filter and contain hazardous agents and provide laminar airflow with HEPA filtration, using air locks, autoclaves, biological safety cabinets, and specific PPE protocols to minimize risk. Laminar or mass air displacement systems provide a uniform, unidirectional, continuous flow of large volumes of HEPA-filtered air over an area to reduce the incidence of airborne infections. A room with laminar airflow is shown in Figure 3.3. The number of air changes in this type of system can be 200 or more per hour. HEPA filters are 99.7% efficient in removing particulate matter as small as 0.3 microns and render air clean of bacterial and viral particles that meet or exceed this size. Access is restricted and, before entering the barrier, personnel are often required to shower, don clean uniforms, and employ additional PPE as dictated by the animal biosafety level (ABSL) designation of the room or area. This may include universal precautions such as a surgical cap, N95 respirator, and disposable gown; higher ABSLs mandate escalating increments of PPE, some with a requirement for full body, positive pressure ventilated suits constructed of PVC-coated polyester with attached boots. All supplies and equipment that enter an animal room must first be autoclaved and brought into the barrier by way of a portal located

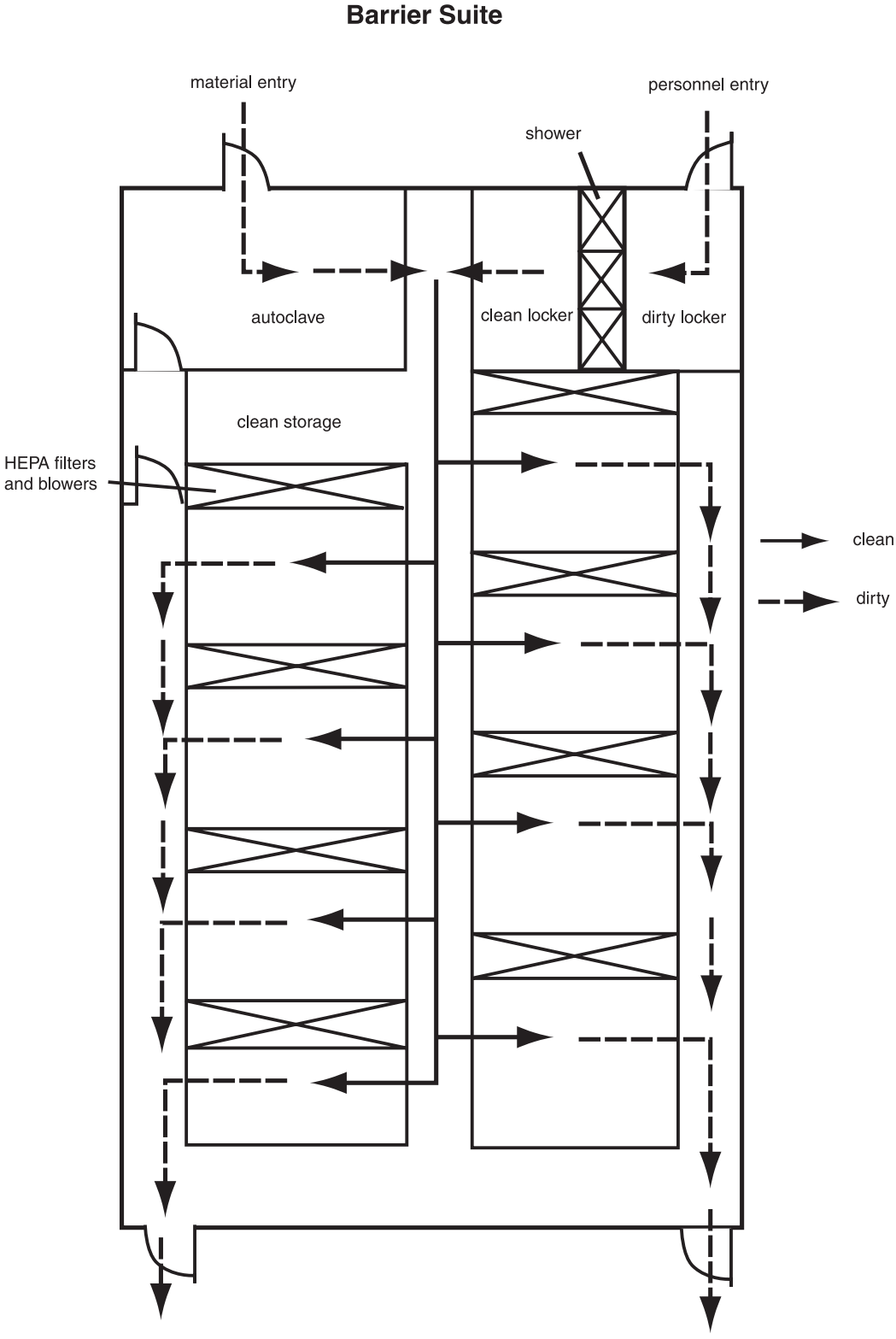

Fig 3.2. Barrier suite depicting air and traffic flow. (From AALAS, 1998, *Assistant Laboratory Animal Technician Training Manual*, p. 51, Figure 8.1.)

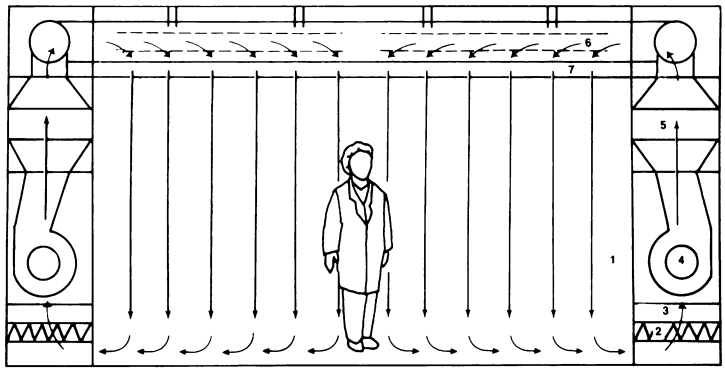

Fig 3.3. Laminar air flow room with HEPA filtration. The system operates as follows: (1) particulate matter produced in the room is caught by the downward flow of clean air, preventing its lateral displacement, and is carried out of the room by the action of the filters inside the return air grates; (2) contaminated air first passes through a prefilter, which removes gross particulate matter; (3) in some systems the air then passes through activated carbon rods or granules, which remove many types of molecular contaminants; (4) an internal centrifugal blower unit keeps air moving through the system at a rate sufficient to meet most operational requirements; (5) the prefiltered air passes through a HEPA filter, which removes particles as small as bacterial bacteria; (6) cleansed air is kept under constant pressure and passes through a plenum chamber in which temperature and humidity are controlled as needed; (7) the air is delivered back into the room through perforations in a suspended ceiling, which promotes turbulence-free distribution. (From AALAS, 2003, *Laboratory Animal Technician Training Manual*, p. 71, Figure 7.2.)

in the clean corridor. Figure 3.4 depicts a biohazard containment suite floor plan. All animal work is conducted within the confines of a biological safety cabinet (Figure 3.5) for areas designated as "Cabinet Laboratories" or with the use of personal, pressurized and ventilated suits for "Suit Laboratories." Waste material and products are usually removed from the facility via the soiled corridor.

Work conducted within these facilities employs a combination of established laboratory safety practices, containment equipment, and facility design. Experimental protocols and their subjects are designated as ABSL 1–4, with ABSL 1 representing the lowest risk level and ABSL 4 the highest risk level. This designation and the combination of escalating controls function to contain hazardous biological agents and protect involved staff members. All equipment, ventilation, and waste are treated via use of autoclaves, incinerators, and air filters before they leave the containment facility to ensure decontamination of potentially infectious material. Personnel are required to properly dispose of or treat PPE and to shower and don clean uniforms before returning to common areas. A containment facility is shown in Figure 3.6. HVAC and waste treatment areas are often designed with redundancy in the event of a system failure. Further guidelines and principles of biosafety can be found in the most recent edition of *Biosafety in Microbiological and Biomedical Laboratories* (Chosewood and Wilson, 2009).

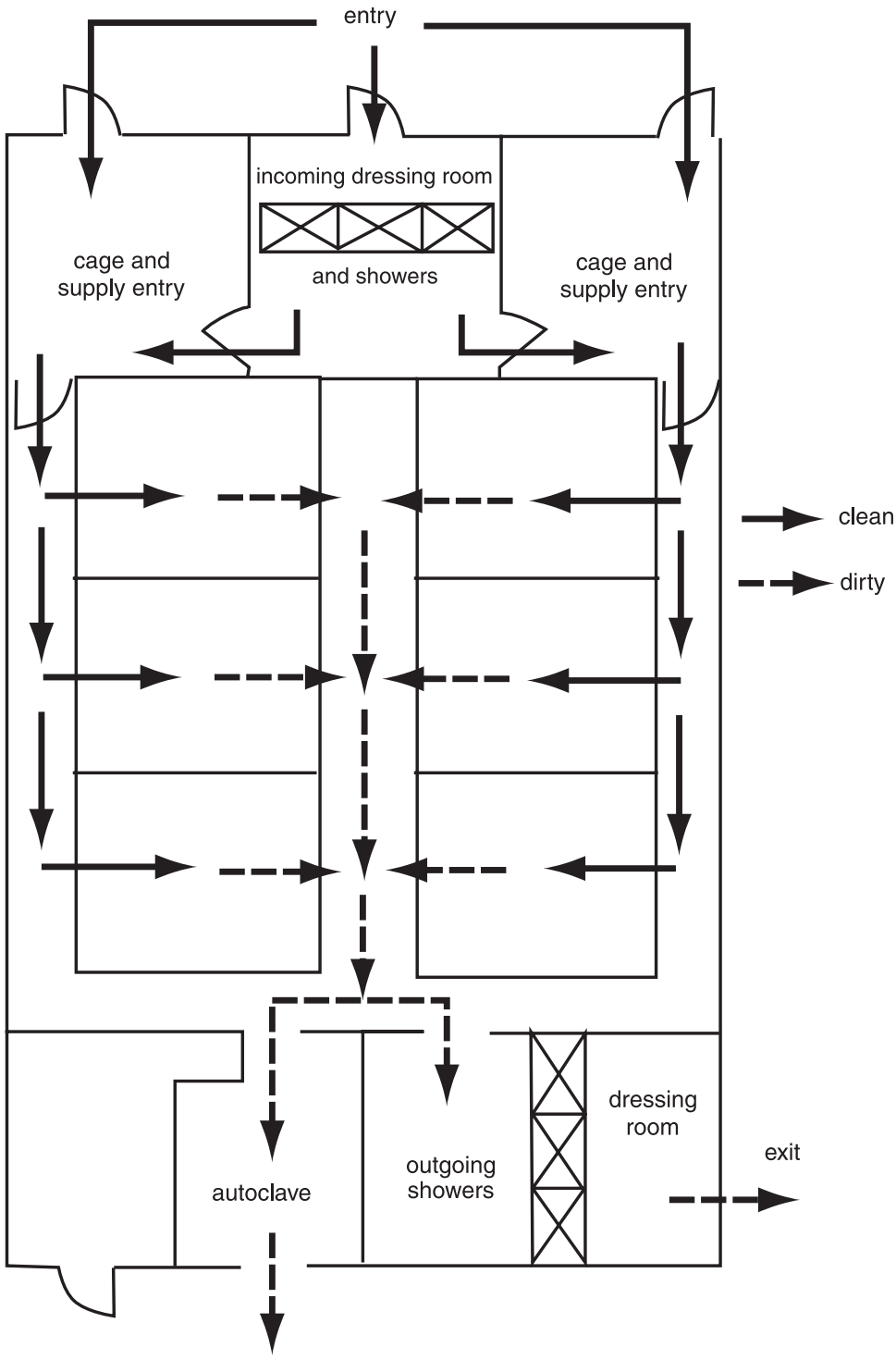

Fig 3.4. Biohazard containment suite floor plan. (From AALAS, 1998, *Assistant Laboratory Animal Technician Training Manual*, p. 55, Figure 8.3.)

Fig 3.5. Class II–Type A2 Biological Safety Cabinet. Biological safety cabinets provide HEPA filtration to air exiting the cabinets and entering the room. The difference between classes I, II, and III safety cabinets is the level of safety devices on them. Class III cabinets are used to contain the most severe biohazards. (Images courtesy of The Baker Company, www.bakerco.com.)

FACILITY EQUIPMENT

An animal facility requires the use of specialized equipment to provide high-quality care and sanitation in an efficient manner. Common pieces of equipment can be viewed on the Web site associated with this book.

Cage Washers

Three types of automatic cage washers are commonly found in animal facilities: rack, cabinet, and tunnel. Personnel place caging and equipment in the washer chamber; the machine goes through a series of washing and rinsing cycles, then a drying cycle. The rack washer allows personnel to sanitize large pieces of equipment such as caging racks, shelving units, or portable kennels. The cabinet washers are smaller versions of the rack washer and are constructed in the configuration of a large cabinet. Tunnel washers carry equipment through various stations on a conveyor belt; the sanitized items exit the opposite side of the tunnel, clean and dry. Cage washers are designed to control temperature, timing of cycles, and detergent and/or acid application, and they must possess emergency shutoff features. Bottle

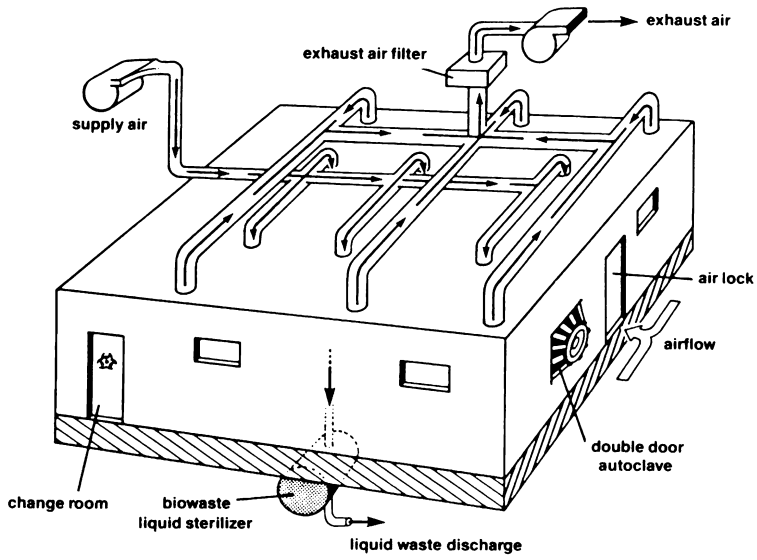

Fig 3.6. Containment facility provides treatment for all exiting air, fluids, equipment, and personnel. Nonexperimental-related functions such as lighting, air exchange, and air conditioning are best designed to permit servicing from outside the containment area. (From AALAS, 2003, *Laboratory Animal Technician Training Manual*, p. 82, Figure 7.10.)

washers that sanitize bottles and sipper tubes are also found in the cage-wash area. Traditionally, 180°F is the suggested temperature for achieving sanitation and refers to the rinse water. The effectiveness of sanitation should be monitored at regular, established intervals through use of temperature test strips and biologic tests. Two commonly used methods include RODAC plate testing and ATP bioluminescent technology. RODAC is an acronym for "replicate organism detection and counting"—a system that measures bacterial colonies remaining on sanitized equipment. ATP bioluminescent technology measures the presence of adenosine triphosphate, a molecule present in all living organisms, to assess effectiveness of sanitization procedures. Equipment logs should be kept to document sanitation effectiveness and equipment failure and repairs. Clean cages and equipment should be stored in an organized manner. Personnel working in cage-wash areas should be advised of safety issues that are inherent in these types of facility operations such as noise, chemical exposure, wet floors, and moving heavy equipment. Another concern is the occupational exposure to laboratory animal allergens. Procedures must be used to minimize exposure to aerosolized bedding material contaminated with rodent urinary proteins, animal dander and hair, and dust. Engineering controls such as negative pressure HEPA-filtered cage dumping systems and PPE should be employed.

Watering Equipment

Automatic watering systems are replacing water bottles in many facilities because they are less labor intensive and reduce ergonomic issues inherent in bottle handling. These automatic systems have pressure regulators and filters that need to be replaced at established intervals. Recoil hoses connect the mobile cage racks to the watering system pipes. Lixit valves are

attached to the cage rack to provide water to individual cages. Automatic water systems must be periodically sanitized by flushing with water or a bleach solution. Bottles with sipper tubes are used when water consumption must be monitored or measured, or when specific medications or compounds are given. Sipper Sacks or Hydrogel packs can be used in place of water bottles and are particularly useful when transporting or shipping rodents. To provide standardized water quality and eliminate extraneous contaminants from tap water, the incoming supply may be treated by a number of methods, including filtration, ultraviolet lighting, hyperchlorination, reverse osmosis, and autoclaving. Treatment plans frequently involve use of reverse osmosis combined with pretreatment ultrafiltration and posttreatment disinfection. Lixit valves and all other system components should be checked regularly to insure proper function and uninterrupted water delivery.

Food and Bedding Materials

Food and bedding materials must be obtained from vendors and suppliers who ensure the quality of their products. Areas in which laboratory animal diets and bedding materials are stored should be kept clean to minimize the introduction of disease, parasites, and potential disease vectors.

Animals should be fed a diet that is palatable, uncontaminated, and nutritionally adequate. Most dry diets that are properly stored can be used up to 6 months after manufacture. Refrigeration may be used to store larger quantities of food because it preserves the nutritional quality and lengthens shelf life of the product. Bags of dry pelleted food have a milling or manufacturing date that should be clearly visible when bags are stacked in the storage area. Stock should be rotated to ensure animals are fed fresh food. Unopened bags of food should be stored off the floor on pallets, racks, or carts and positioned away from the wall to facilitate cleaning of the area. Opened bags of food should be stored in sealed, vermin-proof containers that also protect against moisture or other environmental contaminants. Purified and chemically defined diets should be stored at 39°F or lower because they are less stable than natural-ingredient diets. The shelf life of these diets may be less than 6 months. Autoclavable and irradiated diets are adjusted in nutrient concentrations and ingredients to allow for degradation during sterilization but ensure that adequate nutritional value is maintained. Double wrapping of the irradiated or autoclaved food is suggested; the inner wrapper maybe disinfected and removed prior to entry into the barrier. The date of sterilization should be recorded on the autoclaved package. Perishable foods should be refrigerated and used quickly to ensure freshness of product.

Depending upon the species, housing method, and experimental conditions, the type of bedding material used varies. The bedding material should be selected based on its ability to provide animal comfort and its absorbency, availability, cost, low dust factor, and ease of disposal. In addition, bedding material should be nontoxic, nonnutritive, and nonpalatable. To minimize contamination and maintain quality, bedding materials should be stored off the floor on pallets, racks, or carts.

HOUSING

A variety of housing systems may be used in a laboratory animal facility. The system used depends upon the species being housed, the nature of the research, and the design of the facility.

Rodent Housing

Shoebox-style cages are used to house rodents. The shoebox cage is a solid-bottomed cage usually made of a plastic material that has a stainless steel lid with a V-shaped trough to hold food and a water bottle. Caging materials must be durable and able to withstand repeated sanitation. Plastic cages are made of polystyrene, polypropylene, polycarbonate, and polyphenylsulfone. Data suggests a potential concern for the leaching of bisphenols from plastics into the rodent's microenvironment that may pose an unwanted research variable. Polystyrene cages are disposable and intended for single use, most often with biohazard or chemical hazard protocols. Polypropylene cages are opaque and commonly used for rodents needing more seclusion, such as breeding animals. Polycarbonate cages are clear and have high-impact strength; an autoclavable version is used for protocols that require sterile caging. Both polypropylene cages and polycarbonate cages can withstand high cage-wash temperatures. Polyphenylsulfone caging comes in a variety of clear colors to allow for varying degrees of darkness and can withstand over 2000 autoclaving cycles.

Microisolation (MI) caging is a refinement of the shoebox cage system that uses a plastic top with inset filter over the shoebox cage (Figure 3.7). This allows greater control of the cage environment, further protecting the animal from adventitious agent contamination. MI cages can be used as static (without forced ventilation) or as ventilated cages. Individual cages are placed on a rack or suspended shelving unit. Ventilated caging systems, described in greater detail next, are becoming more popular as a means of providing rodents with additional protection from adventitious agents and decreasing human allergen exposure. Rodents that need superior protection are housed in semirigid or flexible film isolators. An isolator is a chamber made of clear, plastic material that is kept under positive pressure. Operators work inside the isolator through sleeved gloves sealed to ports of the isolator to provide a sterile environment. Figure 3.8 depicts an isolator.

Individually ventilated cages (IVCs) are a refinement to the static MI caging system. IVCs are constructed with a port and docking system in which an automatic watering lixit and an

Fig 3.7. Microisolation cage.

Fig 3.8. Isolator. (Photo courtesy of Harlan.)

air delivery port provide both *ad libitum* water and a direct, continuous flow of air to individual rodent cages. This system provides containment and protection for the rodent while constant air exchange mitigates ammonia and carbon dioxide levels often found in static MI caging. Cages may not need to be changed as frequently if the quality of the microenvironment is not compromised. Figure 3.9 depicts an IVC caging system.

Suspended cages are made of plastic, metal, or wire mesh and have perforated or solid bottoms. Each cage hangs from an aluminum or stainless steel runner that is built into a mobile rack. Perforated or wire mesh flooring allows the animal wastes to drop through to a collection pan located beneath the cage. Use of this type of flooring for housing rodents is discouraged because it may produce foot pathology in heavier animals housed for extended periods of time. In addition, rodents demonstrate a preference for solid-bottom caging with bedding substrate. Solid resting platforms may be offered to rodents or rabbits housed on wire flooring.

Space recommendations for individually housed animals are located in the Animal Welfare Act (see, e.g., APHIS, 2010) and in the *Guide* (ILAR, 2011). The space recommendations for housing mice and rats of various sizes are shown in Table 3.1. Figure 3.10 illustrates calculations used to determine acceptable occupant numbers per cage.

Fig 3.9. Individually ventilated caging (IVC) system.

Table 3.1. Recommended cage space for commonly used group-housed laboratory rodents

Species	Weight (grams)	Floor Area per Animal (in.^{2})	Height (inches)[a]
Mice	<10	6	5
	Up to 15	8	5
	Up to 25	12	5
	>25	≥15	5
	Female with a litter	51[b]	5
Rats	<100	17	7
	Up to 200	23	7
	Up to 300	29	7
	Up to 400	40	7
	Up to 500	60	7
	>500	≥70	7
	Female with a litter	124[b]	7
Hamsters	<60	10	6
	Up to 80	13	6
	Up to 100	16	6
	>100	≥19	6
Guinea pigs	Up to 350	60	7
	>350	≥101	7

Source: Guide for the Care and Use of Laboratory Animals (ILAR, 2011).

[a]From cage floor to cage top.

[b]Recommended space for housing group.

You measure your mouse cage and find it to be: 7.5 inches in width
11.5 inches in length
5.0 inches in height

You weigh the mice to be housed and find they weigh 35 g.

From Table 3.1 you see the cage needs to be at least 5 inches in height and that each adult mouse (>25 g) needs minimally 15 square inches of floor space.

Calculate the floor area by utilizing the formula: length x width.

$7.5 \times 11.5 = 86.25 \text{ in.}^2$

Then divide by 15 square inches per mouse to determine the number of mice you can house.

86.25 in.^2 divided by $15 \text{ in.}^2 = 5.75$

You can house 5 mice in this cage.

Fig 3.10. Calculations used to determine acceptable occupant numbers per cage.

Housing for Other Small Mammals

Front-opening cages are commonly used to house rabbits, chinchillas, and ferrets. They are available as individual cages or as multiple-cage racks and, as the name suggests, provide front rather than top access to the animal. Front-opening cages can be made of stainless steel or plastic and have slotted bar or perforated flooring with trays under the flooring to collect excreta. Drawer-type rabbit cages offer the advantage of being able to examine the animal without fully removing it from the enclosure. Figure 3.11 shows front-opening, drawer-type caging. A J-type feeder is often used in this type of caging system and must be routinely checked and sanitized to prevent accumulation of feed dust.

Specialized Housing Systems

Metabolism cages are used in studies where urine or feces collection is required. The metabolism cage has a funnel-like apparatus to separate urine and feces, and a drinking valve located on the outside of the cage to avoid contamination of the collected urine sample. *Exercise cages* are used as a form of enrichment or for housing animals undergoing research involving nutrition or exercise. *Squeeze cages* are used to house nonhuman primates and to safely restrain them. These cages have devices that move the false back of the cage forward to allow animal immobilization. *Gang cages* are used to house groups of the same animal species, such as primates. They can be mobile on rollers or stationary and commonly have resting boards or perches to make better use of the space. Food and water are provided in multiple locations to avoid food hoarding by dominant animals. *Transport cages* are used either to move animals from one part of the facility to another or for shipping. Shipping containers

Fig 3.11. Front-opening drawer-type rabbit caging.

have design features to ensure the comfort and well-being of animals in transit. Live animal signage and arrows delineate the correct cage positioning. The container has screened windows with filters to reduce airborne contamination, and overhanging box edges to help ensure adequate air circulation.

MANAGEMENT

A variety of administrative duties are required for the operation of an animal facility, including management of the budget and facility personnel, record keeping, preparation of standard operating procedures, executing the occupational health and safety program, and oversight of facility activities such as ordering of supplies and equipment and pest control. In most facilities, the researchers who use animals pay charges for animal housing that supports the majority of animal facility operating costs. The per diem, or per-day fee, is the cost to maintain one animal for one day, calculated by determining the cost incurred for husbandry and for other services provided by the animal facility. Figure 3.12 shows a sample calculation of rabbit per diem charges.

Accurate, up-to-date records with sufficient detail must be kept to assure good animal care. The management team oversees maintenance of records, including those required by regulations and sound veterinary medical practice. Sophisticated computerized programs such as radio-frequency identification systems now enable easy capture of most of the required information for an animal care and use program, including daily animal census, per diem charges, protocols, and animal health records.

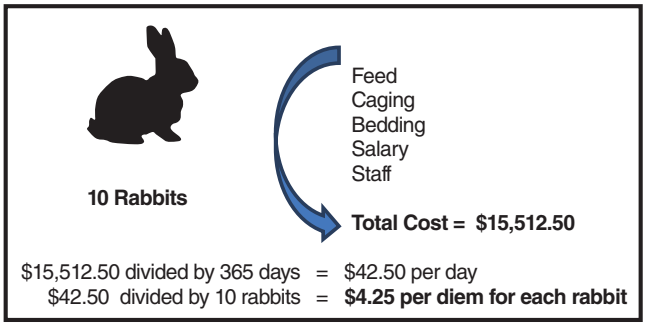

Fig 3.12. Calculation of per diems.

Resources, including personnel, must be used efficiently for the facility to operate effectively. In large operations, use of bedding dispensers, automatic watering, IVC systems, and robots for cage handling may increase efficiency. Personnel must be trained in standard operating procedures and have basic knowledge of the species employed, including humane handling, restraint, and euthanasia techniques, and they must understand the hazards and potential risks involved in carrying out their duties. Management must consider ergonomic issues associated with tasks that are done repetitively to reduce personnel injury. Although day-to-day responsibility for safety lies with the laboratory or facility supervisor, the institution's administration must be supportive of the occupational health and safety program as it relates to the use of animals in research.

Laboratory Animal Allergies

It must be noted that exposure to laboratory animal allergens is an occupational health concern for which each facility must establish a program of risk assessment and control. It is estimated that 6%–44% of laboratory animal handlers will develop laboratory animal allergies (LAAs) as a result of exposure to allergenic proteins found in the fur, dander, saliva, and urine of research animals, particularly mice and rats. Factors in the work environment, for example, the types of allergens and intensity and duration of exposure to the allergens, play a major role in developing LAAs. Preventive measures include engineering controls, administrative policies, and education and training of employees. Two of the most effective and yet practical measures to reduce allergen exposure involve use of engineering controls. Numerous studies have demonstrated the effectiveness of housing mice in negatively pressurized IVCs and changing cage bedding using pressurized ventilated changing tables. Personnel need to understand LAA progression and the importance of using PPE to reduce risk of developing disease. This program is usually operated jointly by animal facility administration and the institution's office of environmental/occupational health and safety in conjunction with an established program of medical surveillance for all potentially at-risk employees. Further guidelines and principles may be found in the latest editions of *Occupational Health and Safety in the Care and Use of Research Animals* (ILAR, 2003), *Biosafety in Microbiological and Biomedical Laboratories* (Chosewood and Wilson, 2009), and the *Guide for the Care and Use of Laboratory Animals* (ILAR, 2011).

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CHAPTER 3 REVIEW

Multiple Choice

1. Floor drains should be at least _____ inches in diameter for rapid water and waste removal.
   A. 6
   B. 5
   C. 4
   D. 8
2. Animal biosafety level designation that involves the highest level of risk is
   A. 3
   B. 1
   C. 2
   D. 4
3. Temperature suggested to achieve sanitation using an automatic cage washer is
   A. 180°F
   B. 160°F
   C. 250°F
   D. 280°F
4. A squeeze cage is routinely used for this species.
   A. ferret
   B. nonhuman primate
   C. rabbit
   D. chinchilla
5. Both the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* have information on
   A. specific nutritional needs of various species
   B. corridor width and door height recommendations
   C. housing systems to reduce allergen generation
   D. space recommendations for individually housed animals
6. The cage type used to separate and collect urine and feces is
   A. squeeze
   B. front opening
   C. metabolism
   D. microisolation
7. J-Feeders are used for this species.
   A. mice
   B. rabbits
   C. hamsters
   D. rats

(*Continued*)

8. Temperatures in the animal facility should be capable of adjustments of plus or minus
   A. 1°F
   B. 2°F
   C. 3°F
   D. 4°F
9. Humidity in the animal facility should be kept in the range
   A. 20%–50%
   B. 35%–60%
   C. 40%–70%
   D. 30%–70%
10. The guideline for ventilation in animal housing rooms is _____ air changes per hour.
    A. 8–10
    B. 4–8
    C. 10–15
    D. 15–20
11. A lixit valve is associated with
    A. water
    B. ventilation
    C. sanitation
    D. heating
12. Animal room doors should
    A. open toward the corridor
    B. have windows if housing rodents
    C. minimally be 42″×84″
    D. be made of wood
13. This room or area should be kept under negative air pressure relative to the corridor or anteroom.
    A. nonhuman primate room
    B. surgery facility
    C. rabbit room
    D. clean cage-wash area
14. HEPA filters are 99.7% efficient in removing particulate matter as small as ____ micron(s) and render the air clean of bacterial and viral particles that meet or exceed this size.
    A. 1
    B. 0.5
    C. 0.2
    D. 0.3

15. Type of cage-washing equipment designed to sanitize large pieces of equipment is
    A. cabinet
    B. rack
    C. bottle
    D. tunnel
16. Most dry animal diets that are properly stored can be used up to _____ after milling.
    A. 6 months
    B. 1 year
    C. 3 months
    D. 1½ years
17. The term "static" refers to microisolation cages that are
    A. autoclaved
    B. sanitized using bacteriostatic products
    C. used without forced ventilation
    D. locked into a rack system
18. Front-opening cage is used for this species.
    A. hamsters
    B. rabbits
    C. gerbils
    D. mice
19. The cost to maintain one animal for one day is called
    A. per capita
    B. perdu
    C. per annum
    D. per diem
20. ATP bioluminescent technology is used
    A. to determine facility air flow
    B. to calculate room humidity levels
    C. to balance room air pressure
    D. to check cage sanitation effectiveness

Critical Thinking

21. A shipment of rats has arrived at the animal facility. You check the paperwork and notice the rats weigh between 150 and 175 g. The cages you have available are 8" high, 8.5" wide, and 17" long. How many rats can you place in each cage?

4 Mice

The laboratory mouse, *Mus musculus*, commonly known as the house mouse, belongs to the order Rodentia and the family Muridae. There are currently thousands of outbred stocks and inbred strains of mice in the world, with the number increasing almost daily. Although highly influenced by the stock or strain, adult mice usually weigh between 25 and 40 g and come in a variety of colors, including albino, black, brown, agouti, gray, and piebald.

GENETICS

Multiple genetic categories of mice are used in biomedical research: outbred stocks, inbred strains, F_1 hybrids, and transgenics. *Outbred stocks* are produced and maintained in large populations where the genetic composition of the population as a whole remains stable and matings are carefully planned to minimize genetic changes such as inbreeding. Each outbred mouse should be unique, or heterogeneous, when compared with others in the population. To avoid genetic drift within a population, outbred stocks should not be subject to artificial selection for any characteristic (e.g., ease of handling, body conformation) other than possibly breeding efficiency. Common outbred stocks used in research include Swiss Webster, CD-1, and ICR.

In contrast to outbred stocks, all mice of an *inbred strain* are, by design, nearly genetically identical (Box 4.1). Strains are often produced to select for a specific trait that will be studied, such as diabetes mellitus or anemia. Following 20 generations of strategically planned brother × sister or parent × offspring matings, mice will exhibit at least 99% genetic homogeneity. With appropriate matings and genetic screening, established inbred strains can be maintained

Clinical Laboratory Animal Medicine: An Introduction, Fourth Edition.
Karen Hrapkiewicz, Lesley Colby, and Patricia Denison.
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Box 4.1

Outbred mice are bred to maintain genetic heterogeneity. Inbred mice are bred to maintain maximum genetic homogeneity within a population.

Fig 4.1. Strains of mice: BALB/c, C57BL/6J, nude. (Images courtesy of The Jackson Laboratory and Harlan.)

indefinitely. Three common inbred strains are C57BL, BALB/c, and C3H mice. Two common inbred mutant strains used are the nude (nu/nu) and severe combined immunodeficient (scid/scid) mice. Figure 4.1 depicts common strains. Nude mice are deficient in T-cell lymphocytes but they have B cells and natural killer (NK) cell lymphocytes. As suggested by their name, nude mice lack normal coats; however, they are not truly nude and may, at times, exhibit some hair growth. SCID mice lack both T cells and B cells but have NK cells. Both of these strains are used most frequently in oncology research because they readily accept many transplanted human and murine tumors. This characteristic allows researchers to study tumor growth, metastasis, and chemotherapeutic efficacy.

F_1 hybrids are the first progeny of a mating between two different inbred strains and are genetically identical at all loci. All F_1 hybrids produced from mating two inbred strains are identical if and only if the parental sex from each strain is consistent (e.g., female of inbred strain 1 mated to male of inbred strain 2). F_1 hybrids produced from the reverse mating (e.g., male of inbred strain 1 mated to female of inbred strain 2) are similar but not identical. F_1 hybrids have the advantage of hybrid vigor, which means they are heartier than either of the parental strains. Inbred strains and F_1 hybrids are often chosen for research purposes because their genetic homogeneity eliminates an important variable in experimental work. Common F_1 hybrids used in research include B6D2F1 (C57BL/6 female mated with a DBA/2 male) and CD2F1 (BALB/c female mated with a DBA/2 male).

Transgenic technology is founded on the ability to alter the genetic makeup of an organism. Precisely ordered nucleotides (i.e., adenine, cytosine, guanine, and thymine) form the backbone of mammalian DNA. DNA is, in turn, ordered to comprise the functional unit of the gene and determines inherited characteristics. Through interactions with other cell components, DNA is responsible for protein synthesis and cellular metabolism and therefore orchestrates the function of the cell, an organ, an organ system, and the organism as a whole (Figure 4.2).

Although transgenic technologies have existed for over 50 years, the number of transgenic mice used in the study of gene functions and as models of human and animal disease has

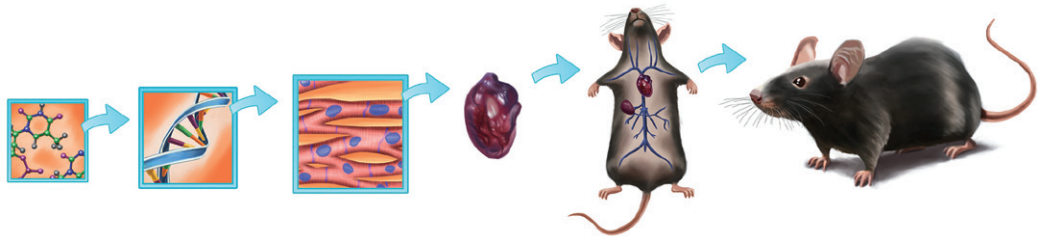

Fig 4.2. Nucleotides →DNA → cell → organ → organ system → organism. (Illustration by Stephen Ramsey.)

exploded since a draft of the mouse genome was published in 2002 (MGSC, 2002). Mice are referred to as *transgenic* if a foreign piece of DNA, a *transgene,* has been purposefully integrated in their genome. The DNA may originate from the mouse genome or from the genome of another organism. It may retain its original genetic sequence or be purposefully modified by the researcher.

Maintaining the genetic integrity of a strain or stock by controlling genetic drift and artificial selection of breeders is vitally important so that research results can be compared over the course of a study or between studies. The genetic composition of a strain or stock may vary between commercial animal vendors and between institutions. Therefore, to limit unintended genetic variability, all animals for a study should be obtained from only one source for the full duration of the study.

MICROBIOLOGIC CLASSIFICATIONS

In addition to being categorized by their genetic classification, mice are frequently classified by their microbiologic status: (1) germ-free or axenic, which are free of all detectable microflora; (2) gnotobiotic, which have associated known microflora; (3) specific pathogen–free, which are free of a defined list of pathogens; and (4) conventional, or animals with undefined microflora. While the number and prevalence of pathogens have declined considerably, many are still identified in laboratory animals and represent unwanted variables in research. Investigators using mice in biomedical experimentation should be aware of the profound effects that many of these agents can have on research. Animal care personnel and scientists must strive to ensure that research animals are free of disease to eliminate the unwanted variables that infections bring. Research institutions often institute a complex set of operational procedures designed to safeguard the microbiologic status of their existing mouse colonies. Of equal importance is the need for institutions to critically examine the microbiologic health status of animals obtained from other institutions or commercial vendors to ensure that the animals are suitable for their intended use and to minimize the health risk to the existing colonies.

Mice that either are infected with a known pathogenic agent or are of unknown health status may be bred and undergo cesarean rederivation to obtain pathogen-free mouse pups. To increase viability, cesarean rederivation should be performed as close as possible to the

expected time of parturition, day 19–21 of gestation, depending on strain. To prevent cross-contamination, the dam is euthanized and her abdomen opened to expose the uterus. A second set of sterile instruments is used to remove the intact uterus. The uterus is then dipped in a disinfectant, such as dilute iodine, and opened to remove the pups. Mouse pups must be gently stimulated to ensure they are breathing and then cross-fostered to a specific pathogen–free, lactating female that can provide appropriate care and nutrition. Another method to prevent transmission of select pathogens from dams to pups is through removal of the pups from the dam immediately postpartum. The pups are then cross-fostered to a lactating, specific pathogen–free female until weaning.

USES

More mice are used in research than any other mammal (Box 4.2). Mice have many attributes that make them valuable for research purposes, including a short life span, short gestation, large litter size, and great genetic diversity. The short gestation and large litter size make them valuable in studies of reproduction, teratogenicity, and genetics. A short life span permits the study of several generations over a period of a few years. Furthermore, the anatomy, physiology, and genetics of mice have been studied extensively and are well characterized, providing a wealth of information for researchers to build upon. Common uses of mice in biomedical research include studies of infectious disease, oncology, autoimmune conditions, immunology, drug discovery, and product safety. Mice can be easily manipulated if handled gently; however, they do have a tendency to bite when they are startled or mishandled. Much of the complex equipment and imaging modalities (e.g., MRI, PET, ultrasound) originally developed for use in humans or larger animal species have been miniaturized to permit their use in mice. Mutant and genetically modified strains of inbred mice provide investigators with a wide variety of animal models with which to study biologic processes and diseases.

Box 4.2

More mice are used in research than any other mammal.

The Food and Drug Administration requires the safety of a product to be proven prior to being marketed in the United States. Companies must use the most effective ways to test the safety of a product, which currently include animal testing. Relative to other species of research animals, mice are inexpensive to purchase and easy to maintain. Thus, they are frequently used for toxicity and carcinogenicity studies of various compounds for which large numbers of animals are required to provide statistically valid data.

BEHAVIOR

Mice are generally easy to handle and not aggressive toward humans. They are curious and social animals that normally sleep together in groups. They should be housed in same-sex

groups unless they are part of a breeding pair or harem or are behaviorally incompatible. Although rodents are generally considered to be nocturnal animals, when housed indoors mice tend to have both active and resting periods throughout the day and night. Mice build nests in which to sleep and keep their litters.

Rodents possess an innate preference for contact with the vertical perimeter of a bounded space (i.e., wall following, corner burrowing, and group aggregation), avoiding the perceived threat of open areas (this orientation response is called thigmotaxis). Mice tend to be territorial. Adult males of many strains will fight when housed together unless they were co-housed at or soon after birth. Certain strains of mice (e.g., SJL/J) are more prone to aggression. Mice can inflict severe bite wounds that are not readily apparent around the genitals and tails and along the backs of their foes. Severe fight wounds can lead to morbidity and death. Female mice of most strains and stocks rarely fight unless defending their litters. Aggression may be reduced if mice are provided with enrichment objects, huts, or areas for hiding. Enrichment items must be carefully evaluated to insure they will not themselves promote territorial or aggressive behaviors. If aggression persists, population densities must be reduced until fighting ceases or animals must be individually housed. Incompatible animals may *not* remain together.

When group housed, one or more mice may remove the hair and whiskers from the faces, heads, and bodies of the other mice, a behavior known as barbering. This is an abnormal behavior and many factors may be involved in this idiosyncratic hair chewing. A distinct line of demarcation usually exists between the hairless and the haired areas, and the skin has no wounds. The "barber" mouse typically has no hair loss. Contrary to popular belief, barbering is not a dominance behavior, and both dominant and subordinate mice barber. Females are 1.5 times more likely than males to engage in this behavior. Barbering has been noted to increase during pregnancy. It appears to be strain dependent, with C57BL mice showing a stronger likelihood to barber than do CD-1 mice. Barbering has been suggested as a model for the study of trichotillomania (compulsive hair pulling) in humans.

ANATOMIC AND PHYSIOLOGIC FEATURES

General biologic and reproductive data for the mouse are listed in Table 4.1. Mice have small bodies covered in soft, dense fur; short legs; and long, thin, hairless tails. Typical of other rodents, mice have a dental formula of 2(I 1/1, C 0/0, P 0/0, M 3/3). The incisors are open rooted, hypsodont (grow continuously throughout life), and are worn down by abrasion of the occlusal surface, whereas the molars have fixed roots.

Mice have a divided stomach consisting of a nonglandular forestomach and a glandular stomach. Their lungs consist of one large left lobe and four small right lobes. Brown fat tissue occurs in several places in the mouse, including between the scapulae. It is important in nonshivering thermogenesis during which the fat is metabolized to increase heat production in response to a cold environment.

Mice have five pairs of mammary glands: three thoracic and two abdominal. Mammary tissue is widely distributed in mice, with the glands extending well onto the sides and back.

Table 4.1. Biologic and reproductive data for mice

Adult body weight: Male	20–40 g
Adult body weight: Female	25–40 g
Life span	1.5–3 y
Body temperature	36.5°–38°C (97.7°–100.4°F)
Heart rate	325–780 beats per minute
Respiratory rate	60–220 breaths per minute
Tidal volume	0.09–0.23 mL
Food consumption	12–18 g/100 g per day
Water consumption	15 mL/100 g per day
Breeding onset: Male	50 d
Breeding onset: Female	50–60 d
Estrous cycle length	4–5 d
Gestation period	19–21 d
Postpartum estrus	Fertile
Litter size	6–12
Weaning age	21–28 d
Breeding duration	7–9 mo
Chromosome number (diploid)	40

Source: Adapted from Harkness et al. (2010) and Harkness and Wagner (1995).

Both male and female mice have mammary glands, but the nipples are more prominent in females. Mice have open inguinal canals their entire life; therefore, to avoid herniation of abdominal organs, care should be taken to close the canals when castrating males. Males also have an os penis.

The most reliable criteria for differentiating the sexes are that the genital papilla is more prominent in the male, and the distance between the anus and the genital papilla is about one-and-one-half to two times greater in the male (Figure 4.3). Sexing of neonatal mice requires practice but can be accomplished by comparing the anogenital distance; the size of the genital papillae; and in nonalbino mice, the presence of a pigmented spot on the perineum of the mouse pup between the genital papilla and the anus.

There are several distinctive characteristics of the hematologic and urinary profiles of mice. Lymphocytes are the predominant circulating leukocyte in mice. Basophils are rarely found in the circulating blood. Mature male mice have higher granulocyte counts than do female mice. Even such factors as site of collection and time of day can influence the number of leukocytes in peripheral blood. White blood cell counts, therefore, are of limited value in disease diagnosis. Mouse urine is excreted a drop at a time, is highly concentrated, and contains large amounts of protein. Taurine and creatinine are normal constituents of the urine while tryptophan is always absent. Urine pH is 7.3–8.5, with a mean specific gravity of 1.058. Hematologic and biochemical parameters for the mouse are listed in Appendix 1, "Normal Values."

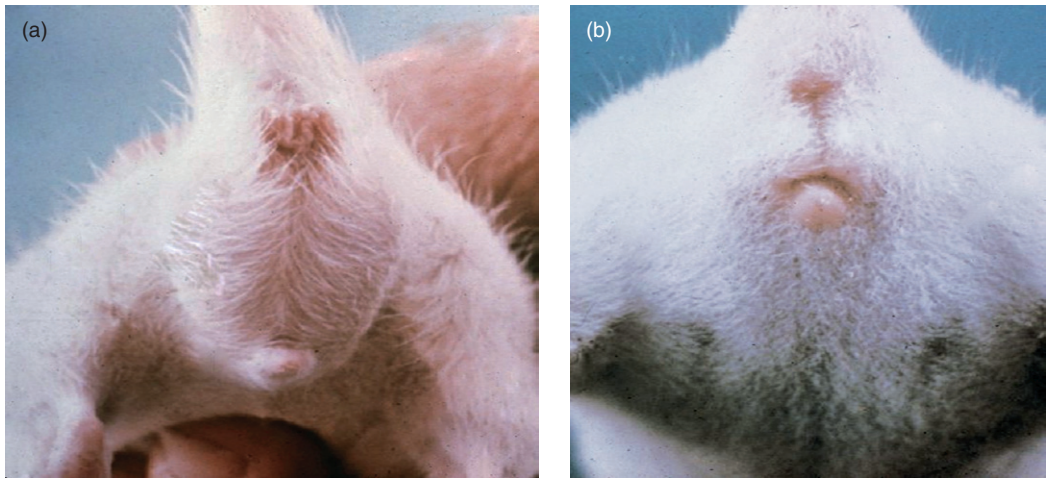

Fig 4.3. Sexing mice: (a) male; (b) female. (Photo courtesy of Wayne State University.)

BREEDING AND REPRODUCTION

Mice are continuously polyestrous without significant seasonal variations. The normal estrous cycle of mice is 4–5 days. Males and females can become reproductively active very soon after the recommended weaning age of 21 days. For this reason, sexes should be housed separately after weaning to prevent unintended pregnancies between littermates or parents and offspring. For maximum productivity, breeding should begin soon after animals reach sexual maturity (6–8 weeks, 20–30 g) and be continued throughout the breeding life of the female. It may be difficult to induce females to resume breeding if the breeding cycle is interrupted. Both monogamous (one male, one female) and polygamous (one male, multiple females) mating systems are routinely employed in mouse breeding. Monogamous systems may be preferentially selected when large numbers of offspring are not required and when defined mating pairs must be maintained. Polygamous mating systems may be selected to maximize breeding production and to maximize space utilization. The most commonly used polygamous breeding system is "trio breeding," in which two females are housed with one male. Depending on cage size, the adults and offspring may be co-housed until weaning or the male and/or a female and her offspring may be transferred to another cage when the offspring reach a designated body size or age. Reproductive performance decreases with age, and many commercial suppliers suggest replacement of breeders at 8–10 months of age. Some females may be territorial, so if they are not co-housed, it is best to bring the female to the male's cage for breeding. Once bred, females will sometimes not accept another male for 21 days.

Pheromones play an important role in the reproductive behavior of mice. Pheromones are chemical substances secreted from the body that elicit a specific behavioral reaction in the recipient by activating the olfactory system. Large groups of females housed together tend to go into anestrus and do not cycle. If these females are introduced to males or their odor, they begin to cycle, and 40%–50% of the females will be in estrus within 72 hours.

This synchronization of estrus is called the Whitten effect and can be attributed directly to pheromones. If a pregnant female mouse is exposed to the odor or presence of a strange male within 4 days of breeding, the existing pregnancy will often be aborted. This phenomenon is known as the Bruce effect and, like the Whitten effect, can be attributed to pheromones. Similarly, in what is known as the Lee–Boot effect, adult female mice housed in a group without exposure to a male will initially become synchronized in their estrus cycles. With prolonged absence of male pheromones, the females ultimately exhibit a suppressed estrous cycle.

Matings can usually be confirmed by the presence of sperm or a vaginal plug (firm whitish mass) in the female. Secretions from the vesicular and coagulating glands of the male form the plug. The plug may be deep in the vagina and difficult to observe. To look for a plug, lift the mouse by the base of the tail. If the plug is not obvious, a cotton-tipped swab may be used to gently spread the lips of the vulva. Vaginal plugs usually persist for 18–24 hours but may last up to 48 hours. Plugs do not guarantee pregnancy but do verify that mating occurred. Pregnant mice have an increased rate of weight gain by day 13 of gestation and marked mammary development and a noticeably increased abdominal size by day 14. The fetuses can be palpated in mid- to late gestation.

The usual gestation period in mice is 19–21 days. In lactating mice, gestation is prolonged by 3–10 days because of a delay in uterine implantation of the blastocysts. Nonfertile matings result in a pseudopregnancy, which lasts for 14 days, during which estrus and ovulation do not occur. A fertile postpartum estrus may occur 14–28 hours after parturition; otherwise, mice will resume cycling 2–5 days postweaning.

Litter size is strain and age dependent but usually ranges between 6 and 12 pups. Smaller litter numbers are often noted with transgenic animals. To minimize cannibalism, dams and their litters should be left undisturbed for at least 2 days postpartum. Mice are altricial in that they are blind, naked, and deaf at birth. Pups are often called pinkies because of their color. The "milk spot" in their stomach can easily be observed through their thin skin to determine whether they are nursing. By day 10, mouse pups have a full covering of fine hair and their ears are open, and by day 12, their eyes are open. Mouse pups can begin eating solid food and drinking water by 2 weeks of age. Figure 4.4 depicts mice from 0–14 days of age. The usual weaning age is 21 days but may be as long as 28 days in some smaller inbred strains. Since thermoregulatory ability is not fully developed at weaning, pups should be co-housed with other weaned pups or adults or provided nesting materials to support body temperature maintenance. Cage size requirements for mice, including dams with litters, are outlined in the *Guide for the Care and Use of Laboratory Animals* (the *Guide*; ILAR, 2011) and further described in the housing section of this chapter.

HUSBANDRY

Housing and Environment

Mice are most frequently housed in shoebox-style cages constructed of a durable plastic, such as polycarbonate, polypropylene, or polysulfone. These plastics differ in their ability to withstand high temperatures and exposure to chemicals. Shoebox cages are comprised of a plastic housing component with a solid bottom and a fitted metal (usually stainless steel)

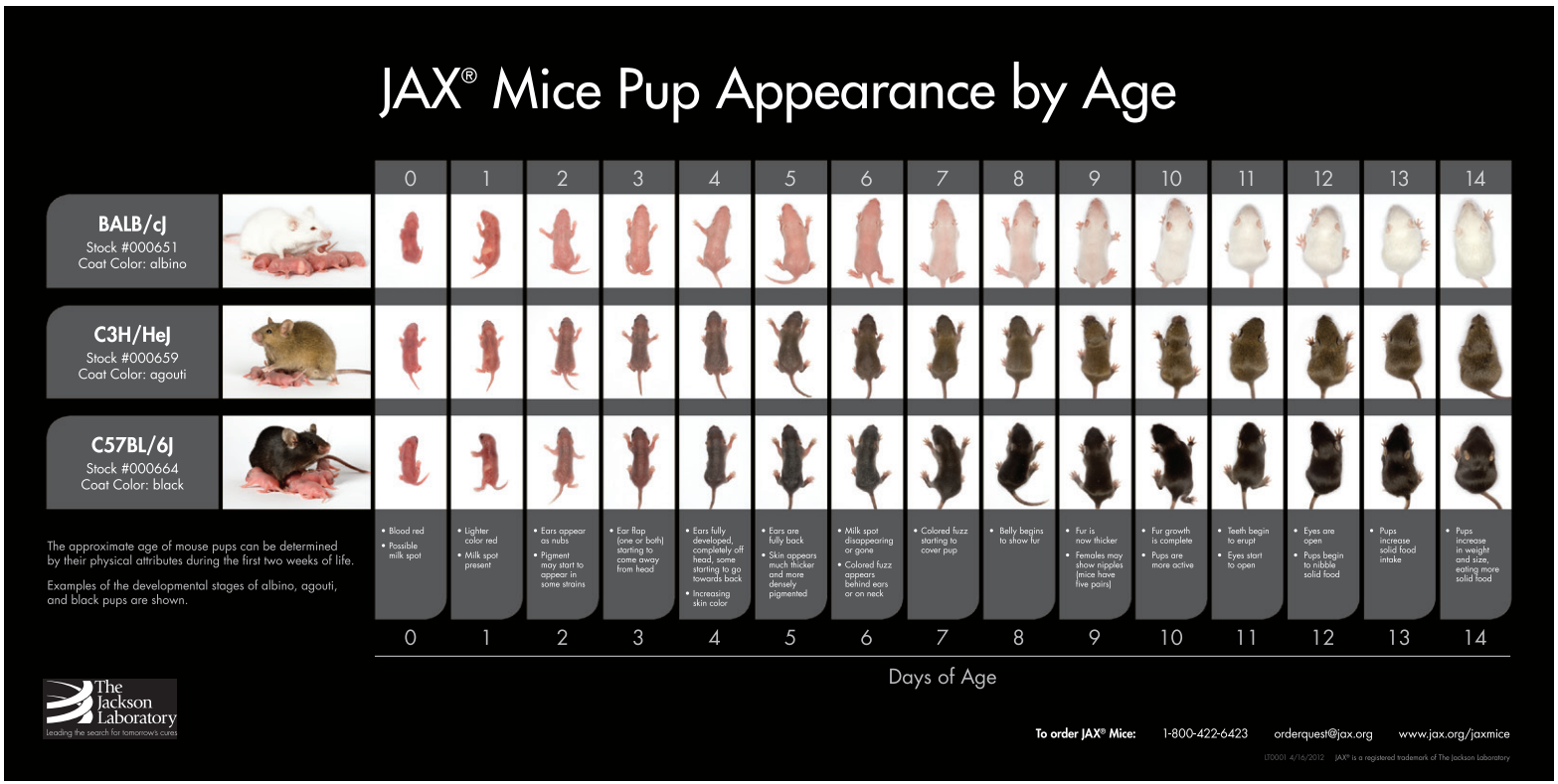

Fig 4.4. Mice pup appearance by age. (Image courtesy of The Jackson Laboratory.)

lid consisting of parallel bars. A depression in the lid accommodates the pelleted food supply; a metal divider separates feed from the area designated for the water bottle. Solid-bottom caging with bedding is recommended. Mice may, when scientifically necessary, be housed in suspended cages with grid floors. Use of wire-bottom cages should be strongly avoided, however, as they are less comfortable for mice and are associated with increased incidence of limb injuries and lesions. Body temperature regulation is also hindered.

Microisolation (MI) cages are a refined type of shoebox-style cage commonly used to house laboratory mice, especially immunosuppressed animals. These cages are made of a durable plastic and have plastic lids with filters to reduce airborne disease transmission between cages. One drawback to the use of MI cages is that airflow into and out of the cage occurs through passive diffusion across the filter, resulting in elevated humidity and ammonia levels that increase with the numbers of mice. Ventilated caging systems are frequently used as an alternative to MI caging. Ventilated caging systems consist of racks of shoebox-style MI cages designed with a ventilation port that attaches at the cage level to the ventilation system. The design allows individual cage ventilation and protection from airborne contaminants for the animals at the cage level. Additionally, the increased airflow inside the cage prevents buildup of humidity and ammonia to improve the microenvironment for the mice. Use of ventilated caging also benefits facility operations through a decreased frequency of necessary cage changes, the ability to house more animals in the same volume of room space (due to the more dense arrangement of individual cages), and containment of allergens that would otherwise be released into the room. These caging systems must be evaluated to ensure that intracage airflow rates are not excessive and associated motor systems do not produce significant cage vibrations, each of which could disturb cage occupants. Single-use, disposable caging is also available. Its use can be especially beneficial in small or remote facilities with limited access to large cage-wash systems and when animals are administered a substance hazardous to personnel (e.g., human pathogen, carcinogen, toxin).

As defined in the *Guide*, the minimal space requirements for group-housed adult mice larger than 25 g and without a litter are 96.7 cm^2 (15 $in.^2$) of floor space for each mouse and a cage height of 12.7 cm (5 in.). See Table 3.1 or the *Guide* for additional space requirements for smaller mice, females with litters, and larger mice. Mice tend to be proficient at escaping from their cages and do not return to them once they have escaped. To prevent escape, cages must have a secure cover such as a plastic lid or metal grid that is fine enough to contain the animals. A contact bedding material, such as hardwood chips, composite recycled paper pellets, or corncob particles, is placed in the bottom of solid shoebox cages. Softwood bedding, such as pine or cedar chips, should not be used for laboratory mice because they produce aromatic hydrocarbons that induce hepatic microsomal enzymes. It is highly recommended that nesting material be provided as a source of animal enrichment and to optimize animal comfort and thermoregulation.

In housing mice for research purposes, rigid control of room temperature, relative humidity, ventilation, and lighting is essential. A temperature range of 20°–26°C (68°–79°F) and a relative humidity range of 30%–70% are generally recommended. It is suggested that there should be 10 to 15 fresh air changes per hour in animal housing rooms to maintain adequate ventilation. This, however, is only a guideline, and determination of optimal ventilation should include careful consideration of the range of possible heat loads, including number of animals involved, type of bedding and frequency of cage-changing, room dimensions, and

efficiency of air distribution from the secondary to the primary enclosure. A constant cycle of darkness and light should be maintained in rooms housing mice. Typically, 12–14 hours of light per day should be provided and can be controlled through use of automatic timers. Fourteen hours is generally recommended for breeding mice although this may vary by strain or stock. Timer performance should be checked periodically to ensure proper lighting. During the dark portion of the light cycle, mice must experience complete darkness. Very short and/or low-intensity light exposure during the dark cycle can significantly alter their circadian rhythm and select physiological systems. Albino strains of mice should be housed under reduced lighting intensity as they are prone to retinal dysplasia.

Environmental Enrichment and Social Housing

Over its successive editions, the *Guide* has placed an increased emphasis on the appropriate provision of environmental enrichment and use of social housing to help meet the psychological and social needs of all species. The *Guide* states that social animals should be housed in physical contact with conspecifics when it is appropriate and compatible with the protocol. Mice are social animals and do best when housed in small, compatible groups. The provision of enrichment items may benefit mice. The types of enrichment items most frequently provided to mice are those that the animals can use for nesting materials (e.g., facial tissues, cotton nesting material, soft paper towels), items that the animal can manually manipulate or gnaw on, and items that provide increased cage complexity (e.g., partially enclosed structures or huts, running wheels). However, controversy exists regarding which enrichment items provide the greatest benefit and least risk to the animals. This is largely complicated by the significant difficulty of interpreting an animal's response to or desire for an object. It must be noted that mice may interpret an object in a very different way than a human may predict. Enrichment items may also induce unintended effects such as increased territoriality and aggression or physical trauma. For instance, a dominant mouse may attack its cagemates to protect a highly valued object. Cotton nesting material may traumatize the eyes of nude mice that do not possess eyelashes or may wrap around and strangulate their furless toes. Therefore, the use of all enrichment items must be critically evaluated before being routinely provided to a colony. In addition, scientific studies have shown that the physical and mental development of mice can be directly affected by social housing conditions (e.g., singly or group housed) and the absence or provision of enrichment items within their environment. Both consistency and caution must be exercised in the use of environmental enrichment with study animals where the changes may impact research results. For these reasons, formal approval should be secured from the principal investigator, the IACUC, and the facility veterinarian prior to the introduction of any enrichment items.

Feeding and Watering

Mice generally consume 4–5 g of solid food per day, although larger strains may consume more. Mice should be fed a nutritionally complete commercial rodent diet from a reputable company servicing the biomedical research community. There are a variety of diets available with a wide range in protein and fat composition depending on the needs of the animals. The diet should contain at least 16% protein and 4%–5% fat; higher fat levels may be indicated for breeding or nursing animals. Feed should be fresh—fed prior to the manufacturer's declared expiration date and stored in a cool (<70°F), dry location inaccessible to insects and

vermin. Open bags of food should be stored in a closed container. Feed is usually provided in the form of firm, dry pellets and fed *ad libitum* (free-choice). The food pellets should be placed in elevated hoppers (e.g., formed in the cage lid, attached to the cage wall) to protect it from contamination from the cage floor. Ill or very young mice may benefit from a container of moistened or gelatinous chow placed on the cage floor as they may not be able to access food or water near the cage top. If mice are fed a powdered or soft diet for research purposes, they likely will have an increased incidence of overgrown incisors since their teeth do not undergo normal wear from chewing. Provision of an object for chewing or occasional trimming may be needed to maintain the incisors at a normal length. Diet supplementation (e.g., fruits, seeds) is highly discouraged because mice may preferentially eat the supplements and not consume a well-balanced diet. Mice are coprophagic; consumption of their own feces serves as a significant source of B vitamins and other nutrients.

Adult mice drink 6–7 mL of water per day. Fresh, potable water should be available *ad libitum* through either water bottles with sipper tubes, sipper sacks (disposable, flexible plastic water containers), or automatic watering systems. Mice must be carefully observed for dehydration after shipping. Additional forms of hydration, including gelatinous diets or moistened food pellets, on the cage floor will help to prevent dehydration. Weanling mice must be carefully observed for signs of dehydration for multiple days after separation from the nursing female as the weanlings may have initial difficulty learning to access the water source. Ill or impaired mice may have difficulty obtaining water from a cage's primary water source. In these situations, additional easily accessible water sources (e.g., lixit valves that extend closer to the cage floor) should be provided.

Sanitation

The required frequency of cage cleaning depends, to some extent, on the number and size of mice caged together and the amount of air movement in the room or cage. One to two times weekly is usually adequate for conventional cages (caging which does not include a filtered cage top) or static MI cages housing up to five mice. More frequent cage changing may be required if the mice produce a large volume of urine (e.g., diabetic mice). Ventilated cages can be changed less frequently because of the improved environmental conditions.

Cages, water bottles, and feed hoppers should be disinfected with chemicals, hot water 61.6°–82.2°C (143°–180°F), or a combination of both. Wardrip et al. (1994) showed that, when hot water is used alone, it is the combination of water temperature and time applied to the surface, known as the cumulative heat factor, that disinfects. Thus, a range of temperatures is acceptable as long as the water is applied to the surface for a long enough period of time to ensure destruction of common vegetative pathogenic organisms. Higher temperatures require less contact time than lower temperatures. Detergents and chemical disinfectants enhance the effectiveness of hot water, but they must be thoroughly rinsed from all caging surfaces with fresh water. Cage racks used with automatic watering system manifolds (piping attached to cage racks) require special consideration. Before introducing the rack with attached manifolds into the cage washer, all residual water must be removed. Residual water within the pipes will adversely affect the rack washer's ability to achieve sanitization temperatures. It is recommended that both stainless steel and PVC types of manifolds be routinely treated with pressurized flushing and chemical agents or heat that are compatible with their chemical makeup. The pretreatment of the rack manifolds with physical or

chemical agents prior to introduction into the rack washer should also be considered. This process reduces the bioburden (biofilm) within the rack manifold prior to sanitization.

TECHNIQUES

Handling and Restraint

Exam gloves should always be worn when handling mice or the contents of their cages. To transfer a mouse from cage to cage, pick it up by the base of the tail with the fingers or with a pair of rubber-tipped forceps. Never grasp a mouse by the tip of its tail as this can result in a loss of skin and exposure of the caudal vertebrae.

To restrain a mouse, grasp the base of the tail with the thumb and forefinger of the dominant hand and place the mouse on a surface to which it will cling, such as a wire cage lid. As the mouse attempts to move forward, quickly scruff the loose skin at the back of the neck between the thumb and forefinger of the nondominant hand. When the skin is held properly, the mouse's head is immobilized so it cannot turn and bite. The mouse can be lifted and the tail secured between the pinky finger and palm of the same hand. The dominant hand is then free to give injections (Figure 4.5) or dose the mouse by oral gavage. Mechanical restrainers made of rigid plastic are commercially available and allow access to blood collection and injection sites. Use of a soft towel to enfold the mouse can provide enough restraint for brief access to the tail or rear limbs.

Identification

Cage cards may be used as a general means of identifying caged mice. Ear punching, ear tagging, ear or tail tattooing, or subcutaneous (SC) placement of a microchip (e.g., radiofrequency identification transponder) that allows electronic identification via a handheld scanner may be used for individual identification of mice. Temporary identification can be achieved by temporary ink tail markings and fur dyes. Toe amputation (toe clipping) is not allowed at most institutions, as less invasive methods of animal identification are available.

Blood Collection

Assuming the animal is mature, healthy, and on an adequate plane of nutrition, the blood volume of most species averages 7% of the body weight in grams. A typical rule of thumb for blood withdrawal suggests that up to 10% of the circulating blood volume (or 1% of

Fig 4.5. Restraint of the mouse. (Photo courtesy of ULAM, University of Michigan.)

the body weight) can be withdrawn every 2–4 weeks from normal, healthy animals with minimal adverse effect. A larger volume or more frequent blood collection may be possible if fluid replacement is provided. Collecting blood from mice can be challenging because of their small size.

One of the best sites for blood collection is the retroorbital sinus. Individuals must be familiar with the anatomy of the retroorbital sinus and receive training prior to attempting this blood collection technique. When performed correctly, multiple blood samples can be safely collected over time with no negative effect on vision. Mice must be anesthetized for this procedure and an ophthalmic anesthetic (e.g., proparacaine) can be applied. Sterile ophthalmic ointment should be used if the selected anesthetic will decrease the blink reflex for more than a brief period. Once the mouse is anesthetized, the forefinger and thumb of one hand are used to place pressure on the top and bottom lids of one eye to keep the eye open and slightly proptosed. Alternatively, the anesthetized mouse may be held by the scruff as described for general restraint. With the other hand, place the tip of a glass microcapillary tube in the medial or lateral canthus at approximately a 30°–45° angle toward the back of the eye (Figure 4.6). Using firm, steady forward pressure, rotate the tube between the thumb and forefinger of the hand. This rotation will allow the tube to act as a cutting instrument through the conjunctiva at the back of the eye and enter the retroorbital sinus. Once the conjunctiva has been bypassed, the tube will come up against bone; slightly withdraw the tube, and blood should flow into the tube by capillary action. A steady hand is required because additional movement of the tube tip can cause severe damage to the eye and the optic nerve, a clearly painful outcome. After collecting the blood sample, withdraw the tube, close the eyelids, and place slight pressure with a gauze square to stop the flow of blood. The eye will not be affected when proper technique is used because the capillary tube merely passes along the side and back of the globe. If mice show signs of ocular damage or pain, they should be promptly euthanized or provided analgesia with treatment to the eye injury.

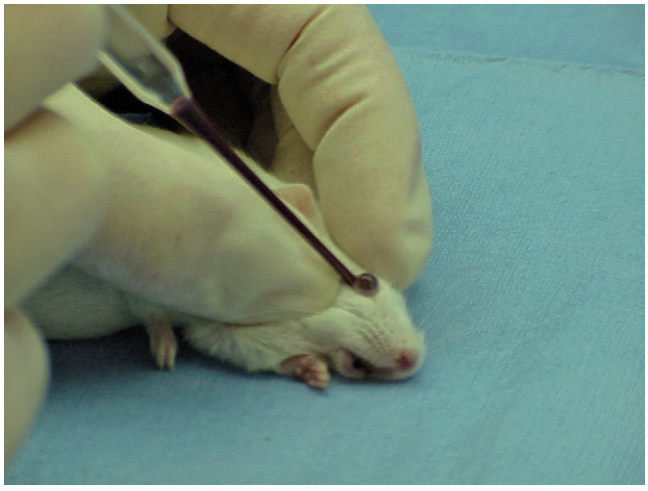

Fig 4.6. Retroorbital bleeding using the lateral canthus approach. (Photo courtesy of ULAM, University of Michigan.)

If mice will be bled over several days to weeks by this method, consideration should be given to alternating eyes and using sterile capillary tubes to minimize the chances for retro-orbital abscesses.

Another collection method has recently been described using the facial vein that courses approximately from the lateral canthus of the eye to the point of the jaw before joining with the jugular vein. For the procedure, an unanesthetized mouse is restrained by grasping the skin over the neck and back, and the puncture site is identified at the point of the jaw. The puncture is made by fully inserting the point of an appropriately sized sterile lancet through the skin and vessel and up to 200 microliters (µL) of blood is allowed to drip freely into a collection tube. A 22- or 23-gauge hypodermic needle can sometimes be used in place of a lancet although this may be more difficult. Hemostasis is achieved by direct pressure on the site. Animals may die from blood loss if adequate hemostasis is not achieved. A video tutorial of the technique is available at www.medipoint.com.

The lateral saphenous vein is an excellent site for blood collection from a conscious mouse. Restraint is accomplished by placing the mouse head first into a 50 mL tissue culture conical tube with perforations to allow air movement and then grasping the skin between the caudal aspect of the thigh and the tail to help extend the leg. The hair over the lateral aspect of the distal thigh is shaved with an electric clipper or scalpel blade. A sterile, oil-based ointment is applied topically to prevent blood from dispersing over the surface of the skin. Using a 23- or 25-gauge needle, a puncture is made into the vessel, and the drops of blood are collected into a microcapillary or other appropriate tube. Up to 100 µL can be collected. Multiple samples can be taken at a later date by removing the scab that forms over the collection site.

Small amounts of blood can also be collected by capillary tube collection from the hub of a needle placed in the dorsal tail artery or lateral tail veins or from a clipped toenail. Larger blood samples can be collected from a cardiac puncture. It must be performed when the mouse is fully anesthetized and usually only when the animal will be euthanized prior to anesthetic recovery. The cardiac puncture technique is described in more detail in the rat chapter. To collect a terminal blood sample in euthanized mice, the axillary muscles and vessels of the front leg can be transected and a Pasteur pipette used to collect the blood as it pools. Approximate blood volumes for mice are listed in Table 4.2. It should be noted that hematological and blood chemistry values may be significantly influenced by the site of blood collection.

Table 4.2. Adult mouse blood volumes and suggested sample size

	Volume (mL)
Total blood	1.6–3.2
Single sample	0.2–0.3
Exsanguination	1–1.5

Source: Adapted from Harkness et al. (2010).

Note: Values are approximate.

Table 4.3. Maximum dosing volumes for a 30 g mouse

Route	Amount (mL)
IP	2.0
SC	2.5
IM	0.05 per injection site [not recommended due to small muscle mass]
IV	0.125
PO	1.25

Source: Adapted from Harkness and Wagner (1995), Lukas (1999), and Diehl et al. (2001).

Urine Collection

Mice may be stimulated to urinate upon handling or upon placement on a cold surface or in a cooled plastic bag. A urine sample is best collected with a specially designed metabolic cage, which separates fecal pellets from urine. The estimated daily volume production of a healthy, adult mouse is 0.5–1.0 mL.

Drug Administration

Most drug administration techniques used in small animal practice can be used with rodents when modified to fit their small size. The tendency to overdose mice and other small rodents is attributable to overestimation of their body weight. Small digital laboratory scales are useful for obtaining accurate body weights. To increase dosing accuracy of small drug volumes, substances may be diluted to larger, more easily measured volumes and syringes designed for small volume administration (e.g., tuberculin syringe) used. Dosing volumes are most accurate when calculated on the basis of milliliters per kilogram (mL/kg) and not simply "per mouse." Table 4.3 lists the maximum recommended dosing volumes for an average, adult 30-g mouse.

Medication is frequently administered orally and is most easily accomplished by mixing the medication into the water or feed. Many factors may affect a mouse's willingness to drink or eat medicated water or food. Therefore, administration of all substances, especially analgesics, in feed or drinking water is discouraged unless it can be shown that all animals will consume required amounts. Specialized gavage needles available in metal as well as flexible plastic are used to deliver a substance directly into the stomach for greater accuracy or for administering unpalatable liquids. Flexible gavage needles may be considered a refinement to the rigid type. The ball at the end of the gavage needle helps ensure that the needle does not pass into the trachea. The gavage technique can be rapidly accomplished in mice through proper restraint and extension of the head although the technique requires training and practice. The maximum recommended volume to administer is 1.25 mL.

Smaller hypodermic needles, such as 25 or 23 gauge, should be used when giving injections. Medication can be administered through subcutaneous (SC) injections with a maximum volume of 2.5 mL. The injection is made under the loose skin over the shoulders. Intramuscular (IM) injections can be given in the quadriceps muscles of the hind legs, but this is not a preferred route because of the small muscle mass. Volume should not exceed 0.05 mL per

Box 4.3

As mice have a small muscle mass, the intraperitoneal route is best for administration of ketamine and other irritating drugs.

site with a maximum of two muscle sites. Certain drugs, such as ketamine, can cause muscle damage and nerve irritation that can lead to self-mutilation of the affected limb. As mice have such a small muscle mass, the intraperitoneal (IP) route is best for administration of ketamine and other irritating drugs (Box 4.3). The maximum injection volume for intraperitoneal injection is 2 mL. To administer an intraperitoneal injection, the mouse should be securely restrained to minimize movement with the head tilted slightly downward, and the injection administered in the lower abdominal quadrant just off of midline. Care should be used in male mice to avoid testicular injections. Before injection, the syringe should be aspirated to ensure that the needle has not entered the bladder or intestines of the mouse. Intravenous (IV) injections may be given into the lateral tail vein of mice using a 25-gauge or smaller needle at a maximum dose of 0.125 mL. Video tutorials for mouse injection techniques are available at www.procedureswithcare.org.uk/.

Anesthesia, Surgery, and Postoperative Care

Preanesthetic fasting is not recommended in mice unless an empty stomach is inherent to the success of the procedure. A variety of anesthetic and tranquilizing agents used in mice are listed in Table 4.4. Because of the small volumes of anesthetics required for mice, dilution of all injectable anesthetics is recommended. A dilution ratio of at least 1 part anesthetic to 10 parts either sterile distilled water or physiologic saline, based on manufacturer recommendations works well. The age, sex, strain, health, and body condition of the animal influences the dose of anesthetic needed. Thus, a conservative dose is best given initially. The combination of ketamine and xylazine is frequently chosen as an injectable regimen for surgical procedures. They can be mixed and given in one injection to minimize handling stress. Solutions should be mixed to the quantity required for short-term use. Mixed solutions have been reported to lose stability and effectiveness over time. This combination provides about 30–45 minutes of general anesthesia.

Tribromoethanol was once frequently used to induce a surgical plane of anesthesia in mice such as during embryo transfer procedures for transgenic production. However, its use is decreasing as more information is discovered regarding associated negative health effects (e.g., induced peritonitis with repeated administration) and unpredictable drug stability. If used, the drug must be freshly mixed, protected from light, and stored in a refrigerator.

Pentobarbital was commonly used in the past, but its use has significantly decreased due to its respiratory and cardiovascular depressant effects, variable anesthetic duration, and prolonged recovery partially due to induced hypothermia.

With the creation of anesthetic machines with multiple flow meters and nonrebreathing systems, use of inhalation anesthesia in rodents is common and often ideal in the research environment. Isoflurane and sevoflurane have high inherent vapor pressures and are best

Table 4.4. Anesthetic agents and tranquilizers used in mice

Drug	Dosage	Route	Reference
Inhalants			
Carbon dioxide	50%–70% mixed with O_2	Inhalation	Urbanski and Kelley (1991)
Isoflurane	1%–4% to effect	Inhalation	Markovic et al. (1993)
Sevoflurane	~5%	Inhalation	Cesarovic et al. (2010)
Injectables			
Acepromazine	2–5 mg/kg	IP	Flecknell (2009)
Alphaxalone/alphadolone	10–15 mg/kg	IV	Flecknell (2009)
Chloral hydrate	370–400 mg/kg	IP	White and Field (1987)
Diazepam	5 mg/kg	IP	Flecknell (2009)
Fentanyl	0.06 mg/kg	SC	Harkness et al. (2010)
+ metomidate	60 mg/kg	SC	
Fentanyl/fluanisone	0.3–0.6 mL/kg	IP	Redrobe (2001)
Fentanyl/fluanisone	0.4 mL/kg	IP	Redrobe (2002)
+ diazepam	5 mg/kg	IP	
Ketamine	100 mg/kg	IP	Flecknell (2009)
+ acepromazine	5 mg/kg	IP	
Ketamine	100 mg/kg	IP	Harkness et al. (2010)
+ diazepam	5 mg/kg	IP	
Ketamine	75 mg/kg	IP	Flecknell (2009)
+ medetomidine	1 mg/kg	IP	
Ketamine	75 mg/kg	SC	Flecknell (2012)
+ medetomidine (mixed, diluted for easier volume)	1 mg/kg	SC	
Ketamine	100 mg/kg	IP	Flecknell (2009)
+ midazolam	5 mg/kg	IP	
Ketamine	80–100 mg/kg	IP	Harkness et al. (2010)
+ xylazine	10 mg/kg	IP	
Ketamine	80–100 mg/kg	IP	Flecknell (2009)
+ xylazine	10 mg/kg	IP	
+ acetylpromazine	3 mg/kg	IP	
Midazolam	5 mg/kg	IP	Flecknell (2009)
Pentobarbital (diluted 1:9 in saline)	40–80 mg/kg	IP	Hughes (1981)
Propofol	26 mg/kg	IV	Harkness et al. (2010)
Tribromoethanol (1.2% solution)	0.2 mL/10 g (240 mg/kg)	IP	Papaioannou and Fox (1993)

IM = intramuscular; IP = intraperitoneal; IV = intravenous.

used in a precision vaporizer with a mask or nose cone apparatus in conjunction with a nonrebreathing anesthetic system. Alternatively, very brief (~1 minute) inhalant anesthesia can be induced independent of a precision vaporizer, through use of an anesthetic chamber. A simple anesthetic chamber can be fashioned by placing a cotton ball soaked with 300 microliters of isoflurane in the bottom of a 500 mL glass beaker. A platform of hardware cloth or similar material should be placed over the cotton ball to separate the mouse from

the anesthetic. Placing the mouse in the beaker and covering the beaker with a lid then induces anesthesia. When limb movement ceases and respiration is relatively slow and steady, the mouse is removed from the chamber. Use of volatile anesthetics should always be performed with appropriate scavenging systems or under a fume hood to avoid exposing personnel to the anesthetic vapors.

Ether is flammable, forms explosive vapor mixtures with air or oxygen, and is an irritant to the respiratory tract. Ether use is strongly discouraged, disallowed at many institutions, and should only be permitted when absolutely necessary for the research study.

Contrary to some theories, rodents are no more resistant to infection than are other mammals, and surgeries should be performed using aseptic technique. Some research projects require multiple-rodent surgical procedures to be performed in 1 day. Sterilizing several small surgical packs is not always possible when surgical instruments are limited. In these situations, use of a hot bead sterilizer is ideal as it provides a rapid method to achieve sterilization of instruments, is easy to use, keeps the instruments dry and clean, and requires minimal bench space. Instruments must be cleaned of tissue and blood prior to placement in the bead sterilizer or the tissues will bake onto the instruments and potentially damage them. Instruments should be allowed to cool for several seconds prior to using on tissues. In situations where use of multiple surgical packs or a hot bead sterilizer is not possible, one sterilized pack can be used for a small number of animals (<5) with the instruments cleaned of gross debris with a cold sterilant and then rinsed between animals.

Likewise, adequate intraoperative and postoperative care is important for all animals. Rodents are particularly prone to hypothermia because of their small size. A small circulating water blanket, a medical-grade heat pack or heating system is essential in maintaining the normal body temperature of a mouse during anesthesia. To help prevent drying of a rodent's eyes, sterile ophthalmic ointment must be used in animals that lose their blinking reflex for more than a momentary period such as with injectable anesthetics or continually administered inhalant anesthetics.

Because of the high ratio of evaporative surface area to body mass, mice have a greater sensitivity to water loss than do most mammals. Administering warmed saline or lactated Ringer's solution subcutaneously (1–2 mL) to mice that have undergone a prolonged anesthetic period or surgical procedure helps prevent hypothermia and dehydration and assists with recovery.

It is an ethical imperative that analgesics be administered to all animals, including mice, for control of surgical pain (Box 4.4). Procedures that are known to cause pain in humans must be assumed to cause pain in other animals unless proven otherwise. Rodents are difficult to assess for signs of pain. Studies of postsurgical mice have shown that decreases in both water and food intake as well as decreases in activity and piloerection (hair erect) are strong indicators of pain. More recently, a facial-expression-based pain coding system,

Box 4.4

It is an ethical imperative that analgesics be administered to all animals, including mice, for control of surgical pain.

similar to ones used with humans, has been developed and validated for use with mice. One reason clinical signs of pain may be overlooked is that the normal active phase of rodents is during the dark cycle, when employees are usually not present. Analgesics should be provided sufficiently *prior to* the initiation of a painful procedure to allow the analgesic to reach effective levels at the time of the painful stimulus.

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as flunixin meglumine, inhibit the production of the chemical mediators that activate peripheral pain receptors. Care must be taken when using NSAIDs because they may cause gastric ulceration, renal toxicity, and platelet inhibition. Meloxicam, ketoprofen, and carprofen are newer NSAIDs that produce fewer side effects and have longer durations of efficacy in most species. NSAIDs may be sufficiently potent to treat musculoskeletal, incisional, inflammatory, and acute, mild-to-moderate visceral pain.

The opioids are effective analgesics for moderate to severe pain. Opioids are controlled drugs, and careful documentation of use is required as directed by Drug Enforcement Administration (DEA) regulations. Butorphanol is recommended for moderate postoperative discomfort, whereas buprenorphine works well to control severe acute or chronic visceral pain but causes more sedation. A sustained release formulation of buprenorphine (Buprenex SR LAB) is available. Table 4.5 lists several analgesics that can be used in mice.

Table 4.5. Analgesic agents used in mice

Drug	Dosage	Route	Reference
Acetaminophen	200 mg/kg	PO	Flecknell (2009)
Acetylsalicylic acid	100–120 mg/kg q4h	PO	Harkness et al. (2010)
Buprenorphine	0.05–0.1 mg/kg q12h	SC	Flecknell (2009)
Buprenorphine sustained release	0.15–1.0 mg/kg(one 72-hour injection)	SC	Carbone et al. (2012)
Butorphanol	1–2 mg/kg q4h	SC	Flecknell (2009)
Carprofen	5 mg/kg q24h	SC	Flecknell (2009)
Codeine	10–20 mg/kg q6h	SC	Smith and Burgmann (1997)
Diclofenac	8 mg/kg	PO	Flecknell (2009)
Flunixin meglumine	2.5 mg/kg q12–24h	SC	Heard (1993)
Ibuprofen	30 mg/kg	PO	Flecknell (2009)
Ketoprofen	5 mg/kg	SC	Flecknell (2009)
Meloxicam	5 mg/kg	PO, SC	Flecknell (2009)
Meperidine	20 mg/kg q2–3h	SC	Heard (1993)
Syrup	0.2 mg/mL drinking water		Huerkamp (1995)
Morphine	2.5 mg/kg q2–4h	SC	Flecknell (2009)
Nalbuphine	4–8 mg/kg q3h	IM	Heard (1993)
Oxymorphone	0.2–0.5 mg/kg q4–6h	SC	Flecknell (2009)
Pentazocine	10 mg/kg q2–4h	SC	Heard (1993)
Piroxicam	3 mg/kg q24h	PO	Harkness et al. (2010)
Tramadol	5 mg/kg	SC, IP	Flecknell (2009)

IM = intramuscular; IP = intraperitoneal; PO = per os; SC = subcutaneous.

Anesthetized mice should be placed on a dry paper or cloth towel in a safe and enclosed area for recovery. An anesthetized mouse should not be placed back into a cage with animals that are not anesthetized until it is conscious as rodents may cannibalize animals that appear nonresponsive. Anesthetized mice should never be placed on small-particle bedding because it can be aspirated. Use of an incubator, circulating water blanket under half of the cage, or heat lamp directed at one corner of the cage is critical to preventing hypothermia in recovering mice. Nonambulatory mice should be turned approximately every 30 minutes to prevent hypostatic lung congestion. Provision of moistened chow or a critical care supplement can assist in the recovery phase. Select, commercially available supportive and critical care products for laboratory animals are provided in Appendix 3.

Imaging Techniques

Great advances have been made in rodent and small animal imaging over the last decade. Many imaging modalities once used only in humans [e.g., digital x-ray imaging, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), dual-energy x-ray absorptiometry (DEXA) scan, and ultrasound] have been adapted for use with these species, producing images of exquisitely fine detail. In addition, novel imaging techniques have been developed for use in animal research, including *in vivo* optical imaging. Some x-ray units (e.g., Faxitron animal imaging and irradiation systems) have been designed for use specifically with small rodents. These units produce a highly magnified and clear resolution image of the field of interest.

For conventional x-ray imaging, an x-ray machine with high milliamperage, such as a 200- or 300-mA unit with the capability for low settings of peak voltage delivered (kVp) and small incremental changes, is ideal. High-detail film–screen combinations work well. Short exposure times of 1/40 second or less should be used to decrease the chance of motion artifact. Suggested exposure factors are 42–46 kVp, 300 mA, 1/40 second, 40 SID, 7.5 mA·s. Alternatively, nonscreen film can be used with standard x-ray or dental units. Nonscreen film produces high-detail radiographs, but longer exposure times are needed. Digital imaging techniques offer substantial flexibility in image enhancement and delivery application. The best method of restraint is to sedate the animal and use adhesive tape to secure it to the cassette in the appropriate position. A radiolucent tube or stockinette with the patient positioned inside can be used for restraint of conscious animals.

Euthanasia

The term euthanasia literally translates as "good" (*eu*) and "death" (*thanatos*). Euthanasia should provide a rapid death with little to no pain or distress. Individuals must be appropriately trained to use the various methods of euthanasia to minimize the chances of pain or distress if performed inappropriately. Methods must be consistent with the most current American Veterinary Medical Association (AVMA) *Guidelines for the Euthanasia of Animals* (AVMA, 2013), performed by trained personnel, detailed in the research protocol, and approved by the IACUC. Per AVMA Guidelines, "Carbon dioxide is acceptable with conditions for euthanasia in those species where aversion or distress can be minimized. Carbon dioxide exposure using a gradual fill method is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness; a displacement rate from 10% to 30% of the chamber volume/min is recommended. Whenever gradual displacement

methods are used, CO_2 flow should be maintained for at least 1 minute after respiratory arrest. If animals need to be combined, they should be of the same species and, if needed, restrained so that they will not hurt themselves or others. Oxygen administered together with CO_2 appears to provide little advantage and is not recommended for euthanasia. The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO_2, is unacceptable. Carbon dioxide and CO_2 gas mixtures must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate [author note: e.g., dry ice]. As gas displacement rate is critical to the humane application of CO_2, an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size of the container being utilized is absolutely necessary."

The euthanasia of mouse feti and neonates requires special consideration. Neonates are resistant to hypoxia and require greater duration of exposure; for this reason, other methods may be preferred for this age group. Fetal mice (unborn animals who have not breathed) under 15 days of gestation may be euthanized by allowing them to remain *in utero* following routine euthanasia of the dam. Alternatively, euthanasia can be induced by removal of the fetus from the uterus. However, feti removed from the uterus and allowed to breathe air must be promptly euthanized by a physical method of euthanasia (e.g., cervical dislocation or decapitation). Neonatal mice under 10 days of age may show marked resistance to CO_2. CO_2 euthanasia may still be used with these animals although prolonged exposure time may be required. Alternate methods of euthanasia of neonatal mice include injection of anesthetics, decapitation, or cervical dislocation. Death must always be verified prior to carcass disposal.

Other inhalant anesthetics may also be used for euthanasia, including isoflurane and sevoflurane. Anesthetic waste gas must be captured and diverted away from personnel. Another method that may be used to euthanize small numbers of mice is intraperitoneal administration of an overdose of an injectable barbiturate at three to four times the anesthetic dose or commercial euthanasia solution.

Occasionally, it is necessary to euthanize animals without the use of anesthetics or tranquilizers that have been documented to complicate research results, such as by cervical dislocation or decapitation. Investigators must fully justify the need for using such a euthanasia method, personnel training must be insured, and the method must be approved by the IACUC. A preventive maintenance program must be in place for guillotines to ensure proper functioning and adequate sharpness of the blades.

Euthanasia of rodents inside an animal room should be carefully considered because under some conditions doing so may cause stress in the remaining animals. Per the AVMA Guidelines, "Distress vocalizations, fearful behavior, and release of certain odors or pheromones by a frightened animal may cause anxiety and apprehension in other animals. Therefore, for sensitive species, it is desirable that other animals not be present when individual animal euthanasia is performed."

There should always be a method to ensure death of animals, especially when noninvasive euthanasia procedures are used. Examples of assurance methods include performing a bilateral pneumothorax, cervical dislocation, removal of a vital organ, or exsanguination by

transection of a major vessel. This step will ensure that death has occurred rather than just a deep plane of anesthesia from which the animal might later recover.

Humane Endpoints

To support humane animal treatment, an animal may require premature removal from a study due to its impaired physical condition or imminent death. The *humane endpoint* constitutes the discrete criteria used to determine when euthanasia should occur and is designed to occur prior to the time that an animal's potential suffering outweighs the value of the experimental data that may be acquired. The appropriate humane endpoint must be determined for each type of experiment performed and often for each strain of mouse utilized. Common criteria considered when determining a humane endpoint include loss of a percentage of body weight; inability to eat, drink, or ambulate; loss of consciousness; severe or uncontrollable neurologic symptoms; large tumor mass; and hypothermia.

SPECIAL TECHNIQUES: TRANSGENIC PRODUCTION TECHNOLOGY

Two primary methods are used to create transgenic mice: pronuclear injection (the injection of DNA into fertilized eggs) and gene targeting (the injection of genetically modified embryonic stem cells into preimplantation embryos).

Pronuclear Injection

One method of producing transgenic mice requires injection of foreign DNA into the egg- or sperm-derived pronuclei prior to their fusion within the fertilized egg (Figure 4.7). The basic steps for producing a transgenic animal through pronuclear injection are as follows:

1. Foreign DNA containing a gene of interest (the transgene) is injected into the male or female pronuclei of a fertilized egg. The injected DNA integrates at a random location and in variable numbers (up to hundreds of copies) in the chromosomes of the developing egg. However, since some cell division may have occurred prior to the DNA injection, *not all* cells present may carry the transgene.
2. Multiple fertilized eggs, each injected with the foreign DNA, are surgically implanted in a recipient, pseudopregnant female (female previously mated to a sterile male) in whom the eggs develop to pups.
3. Following birth, a tissue sample from each pup is genetically tested to identify the pups whose genome has successfully incorporated the foreign DNA. Each of these pups is considered a genetic "founder." Each founder is genetically unique; founder animals produced even from the same litter are not genetically identical due to the random nature of DNA integration following pronuclear injection.
4. Each founder animal is then backcrossed to an inbred strain to produce a unique mouse strain that is homozygous for the transgene.

The FVB mouse strain is frequently used in pronuclear injection as their pronuclei tend to be larger and more well-defined than in other strains. Also, FVB females produce large numbers of embryos and are good mothers. The common complications of this technique

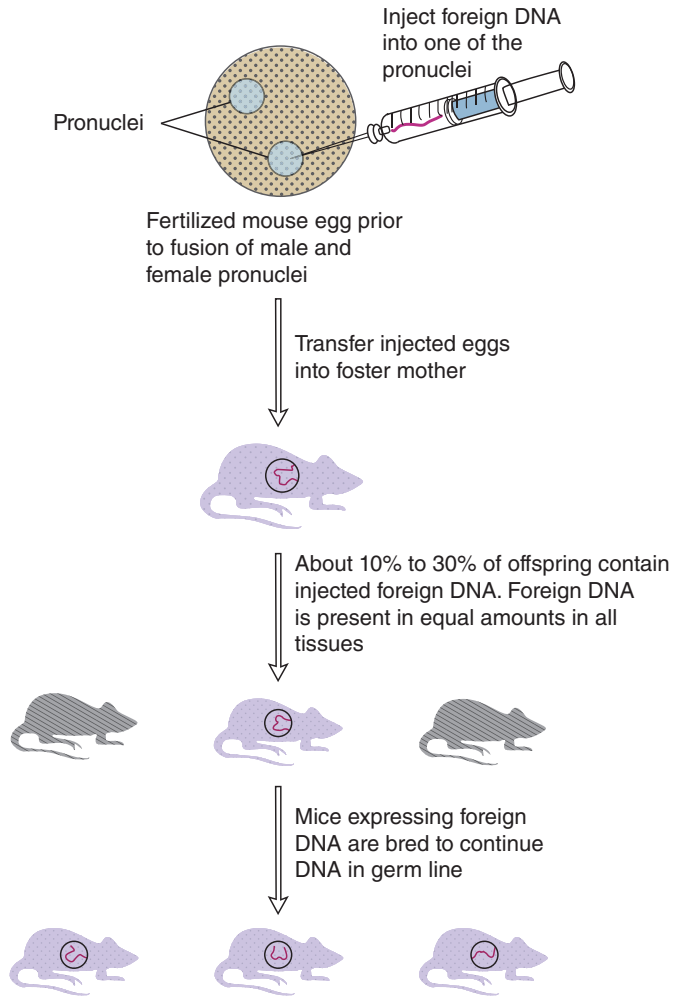

Fig 4.7. Pronuclear injection for the creation of transgenic mice. (From *Molecular Cell Biology*, 6th ed., by Lodish et al., © 2008 by W. H. Freeman and Company. Used with permission.)

are attributed to the random (i.e., nontargeted) insertion of an uncontrolled number of transgenes into an animal's genome. For instance, the random insertion of a transgene may disrupt a gene responsible for development, viability, or reproduction. Alternatively, transgene insertion may disrupt a necessary biochemical pathway or prevent production of a specific protein.

Gene Targeting

Gene targeting of mouse embryonic stem (ES) cells is most frequently used to alter the mouse genome at a specific location (Figure 4.8). The basic steps of gene targeting are as follows:

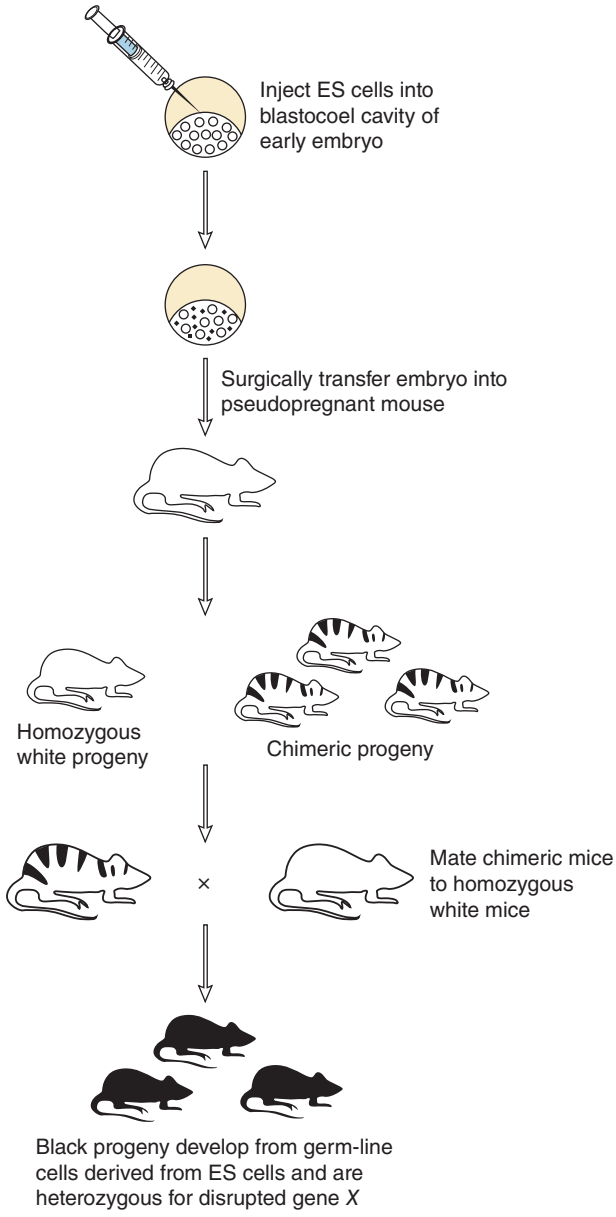

Fig 4.8. Gene targeting for the creation of transgenic mice. (From *Molecular Cell Biology*, 6th ed., by Lodish et al., © 2008 by W. H. Freeman and Company. Used with permission. Adapted from M. R. Capecchi, 1989, *Trends Genet* 5: 70 with permission from Elsevier.)

1. A preimplantation embryo is harvested from a donor female. At this stage, the embryo is composed of ES cells that possess the ability to differentiate into any cell type (e.g., skin, muscle, nervous tissue) and can be maintained and propagated in culture.
2. The ES cells are mixed with DNA segments containing the transgene flanked on either side with a genetic sequence that is identical, or homologous, to the targeted gene in the

ES cells. Through manipulation of the culture environment (e.g., passing an electric current through the cell thereby increasing the cell's membrane permeability), the DNA segments enter the ES cells and insert in the chromosome. A high proportion of insertions occur randomly, at unintended locations throughout the genome. A smaller number of targeted insertions occur through homologous recombination where the genetic sequences flanking the transgene correspond with the genetic sequence of the ES cell.

3. The now genetically modified ES cells are screened in culture to select those in which the transgene was successfully inserted in the targeted location in the cell's genome. Genetically modified ES cells with nontargeted insertions are discarded.
4. The ES cells with the desired genetic mutation are injected into a host preimplantation embryo, where the modified ES cells and the host cells mix prior to their differentiation and proliferation during embryo development.
5. The embryo is surgically implanted in a surrogate, pseudopregnant dam (a female mouse previously mated to a sterile male), where it develops normally to a pup. The tissues of the pup will have derived either exclusively from the normal embryo (if ES cell injection was unsuccessful) or from a mixture of normal and modified ES cells. Animals with tissues derived from both ES cell lines are termed *chimeras*. If the coat color of the normal and modified ES cells' strains were different (e.g., normal ES cells from white strain and modified ES cells from black strain), then chimeras can be visually identified by altered coat color and pattern (e.g., white and black fur patches).
6. Chimeras are bred to select those with incorporation of the desired mutation in their germline (cells that give rise to eggs or sperm). These individuals are then bred to yield a population of animals each possessing the identical, targeted genetic mutation.

The two strains of mice frequently used in gene targeting are strains 129 and C57BL/6. Selection of these strains is useful as their coat colors allow ready differentiation of each strain and the resulting chimeras. The white strain 129 mice are used as a source of ES cells. The black strain C57BL/6 are used as a source of host embryos.

There are many practical applications to gene targeting. For instance, *knockout* (KO) mice can be engineered in which a specific gene is deleted or disrupted. By examining the abnormalities induced by the absence of the gene, scientists can infer the gene's normal function.

While this is simple in concept, many possible complications need to be considered. For instance, select strains of KO mice exhibit no observable change from their wild-type (natural genetic composition of the standard strain) counterparts due to compensatory replacement of the knocked-out gene's function by other unaltered genes or body systems. In these instances, a different strategy is required to determine gene function. Similarly, the function of a gene may change over time so that the primary function in young animals may differ from that in older animals. In other KO mice, high mortality rates or negative health complications may arise if gene expression is necessary for proper physiologic function during all or part of the animal's life. If the health complications manifest at an earlier age than the researcher wishes, the study may be compromised. Conditional gene modification systems (e.g., the loxP-Cre recombination system) can be used in these situations to effectively knock out the gene of interest in only select tissues or during a specific developmental stage or age.

Another application of gene targeting is the creation of *knock-in* (KI) mice, in which a transgene is inserted in a specific location in the genome without deletion or inactivation of the normal genetic sequence.

Many health complications may arise in transgenic mice, including compromised immune function, decreased reproductive ability, altered growth patterns, and development of painful musculoskeletal conditions. Many of these can initially go unnoticed by researchers who depend on husbandry and veterinary staff to monitor animal health and development. Regardless of the method used to create a transgenic animal strain, the original mouse strains utilized can significantly influence phenotypic expression as well as the disease conditions and pathologic lesions that can develop in the transgenic strain over time.

Genotyping

To fully characterize the genetic makeup of breeding or genetically modified rodents, a sample of DNA is usually collected from the tail tip. This involves removal of a few millimeters of the distal tip of the tail with sharp scissors or a scalpel blade. Minimal bleeding usually occurs, but if needed, hemostasis is achieved by applying pressure to the site. Anesthesia is used if mice over 21 days are biopsied; additional analgesia may be warranted and should be provided sufficiently *prior to* the initiation of a painful procedure to allow the analgesic to reach effective levels at the time of the painful stimulus. Alternative tissues that may also be collected include an ear punch, a swab of oral or rectal mucosa, feces, saliva, hair follicles, or an amputated toe. The use of ear punches as a genetic sample continues to increase in popularity. Toe amputation (toe clipping) is strongly discouraged but may be allowed on neonates less than 10 days of age with specific criteria that may include sampling not more than one toe per foot and amputation of only the distal-most phalange. The tissue collected from animal identification procedures (e.g., tail or ear punch) can and should be the tissue sample used for genetic testing.

Prior to performing any tissue collection method, investigators should contact the genetic testing laboratory to make sure they collect an appropriate amount of tissue and ship it in optimal conditions for maximum testing accuracy.

THERAPEUTIC AGENTS

Suggested mouse antimicrobial and antifungal drug dosages are listed in Table 4.6. Antiparasitic agents are listed in Table 4.7, and miscellaneous drugs are listed in Table 4.8.

INTRODUCTION TO DISEASES OF MICE

In the past 50 years, research and improved environmental controls and husbandry have led to the identification and eradication of a large number of infectious agents in laboratory rodents. Most laboratory rodent colonies today are relatively free of the viruses, bacteria, parasites, and fungi that cause clinical disease. However, these agents still cause sporadic outbreaks of disease in the laboratory setting. The evaluation of clinical signs in mice is difficult because of their small size. In many cases, serology, histopathology, microbiology, and

Table 4.6. Antimicrobial and antifungal agents used in mice

Drug	Dosage	Route	Reference
Amikacin	10 mg/kg q12h	SC, IM	Morrisey and Carpenter (2011)
Ampicillin	20–100 mg/kg q12h	PO, SC, IM	Anderson (1994)
	500 mg/L drinking water		Matsushita and Suzuki (1995)
Cephalexin	60 mg/kg q12h	PO	Flecknell (2009)
Cephaloridine	10–25 mg/kg q24h	SC	Harkness et al. (2010)
Cephalosporin	30 mg/kg q12h	SC	Harkness et al. (2010)
Chloramphenicol	0.5 mg/mL drinking water		Burgmann and Percy (1993)
	30–50 mg/kg q8–12h	SC	Burgmann and Percy (1993)
Chlortetracycline	25 mg/kg q12h	PO, SC	Morrisey and Carpenter (2011)
Ciprofloxacin	7–20 mg/kg q12h	PO	Harkness et al. (2010)
Doxycycline	2.5-5 mg/kg q12h	PO	Harkness et al. (2010)
Enrofloxacin	0.05–0.2 mg/mL drinking water for 14 d		Harkness et al. (2010)
	5–10 mg/kg q12h	PO, SC	Harkness et al. (2010)
Erythromycin	20 mg/kg q12h	PO	Morrisey and Carpenter (2011)
Gentamicin	2–4 mg/kg q8–12h	SC	Harkness et al. (2010)
Griseofulvin	25–50 mg/kg q12h for 14–60 d	PO	Harkness et al. (2010)
	1.5% in DMSO for 5–7 d	Topical	Harkness et al. (2010)
Ketoconazole	10–40 mg/kg q24h for 14 d	PO	Harkness et al. (2010)
Metronidazole	2.5 mg/mL drinking water for 5 d		Burgmann and Percy (1993)
	20–60 mg/kg q8–12h	PO	Harkness et al. (2010)
Neomycin	2.6 mg/mL drinking water		Anderson (1994)
Oxytetracycline	0.4 mg/mL drinking water		Burgmann and Percy (1993)
	10–20 mg/kg q8h	PO	Burgmann and Percy (1993)
Sulfadimethoxine	10–15 mg/kg q12h	PO	Harkness et al. (2010)
Sulfamerazine	1 mg/mL drinking water		Anderson (1994)
	500 mg/L drinking water		Matsushita and Suzuki (1995)
Sulfamethazine	1 mg/mL drinking water		Anderson (1994)
Tetracycline	2–5 mg/mL drinking water		Burgmann and Percy (1993)
	10–20 mg/kg q8–12h	PO	Burgmann and Percy (1993)
Trimethoprim/sulfa	30 mg/kg q12h	PO, SC	Harkness et al. (2010)
Tylosin	0.5 mg/mL drinking water		Collins (1995)
	10 mg/kg q24h	PO, SC	Harkness et al. (2010)

DMSO = dimethyl sulfoxide; IM = intramuscular; PO = per os; SC = subcutaneous.

polymerase chain reaction (PCR) are required for a definitive diagnosis. The introduction of a pathogen into a facility may stem from many sources. For instance, infectious organisms may arrive in association with personnel, contaminated equipment, infected animals, or biologic materials. To improve biosecurity, some laboratory animal programs have prohibited employees from owning pet rodents, although the value of such a policy is in question. Facilities routinely prohibit sharing of equipment between institutions, facilities, and areas

Table 4.7. Antiparasitic agents used in mice

Drug	Dosage	Route	Reference
Carbamate (5%)	Twice weekly	Topical	Harkness et al. (2010)
Fenbendazole	20 mg/kg q24h for 5 d	PO	Allen et al. (1993)
	150 mg/kg of feed; provide for 7 d on, 7 d off for 3 treatments		Boivin et al. (1996)
Fipronil	7.5 mg/kg q30–60d	Topical	Richardson (1997)
Ivermectin	Use 1% ivermectin diluted 1:10 with tap water, delivered as a mist spray; 1–2 mL per cage once weekly for 3 wk	Topical	Le Blanc et al. (1993)
	Use 1% ivermectin at 2 mg/kg delivered as micro-dot on skin between scapulae at 10 d intervals for 2 treatments	Topical	West et al. (1992)
	8 mg/L drinking water for 4 d on, 3 d off, for 5 wk		Klement et al. (1996)
	0.2–0.5 mg/kg q7d for 3 wk	PO, SC	Harkness et al. (2010)
Mebendazole	40 mg/kg q7d for 21 d	PO	Adamcak and Otten (2000)
Metronidazole	30–40 mg/kg q8–12h	PO	Harkness et al. (2010)
Moxidectin	0.5 mg/kg of 0.5% pour-on solution applied to the dorsum	Topical	Pullium et al. (2005)
Moxidectin	2 mg/kg repeated once in 15 d	PO	Harkness et al. (2010)
Permethrin	4 g of 0.25% dust once weekly in bedding for 4 wk		Bean-Knudsen et al. (1986)
	Cotton balls with 5%–7.4%		Mather and Lausen (1990)
	In bedding (w/w) active permethrin, used as nesting material for 4–5 wk		Hill et al. (2005)
Piperazine citrate	2–5 mg/mL drinking water for 7 d, off 7 d, repeat		Anderson (1994)
Piperazine sulfate and ivermectin	2.1 mg/mL in drinking water for 2 wk, then 0.007 mg/mL ivermectin for 2 wk; prepare medicated water weekly		Lipman et al. (1994)
Praziquantel	6–10 mg/kg	PO	Harkness et al. (2010)
	140 ppm in feed for 5 d		Harkness et al. (2010)
Pyrethrin powder	3 times a week for 3 wk	Topical	Harkness et al. (2010)
Thiabendazole	100–200 mg/kg	PO	Harkness et al. (2010)
	0.3% in feed for 7–10 d, use repeated treatments		Sebesteny (1979)

PO = per os; SC = subcutaneous.

within a facility. Biologic material (e.g., cell cultures, serum) are screened for contamination. There is also a recognized threat of spreading infectious agents via the increasing transportation and sharing of rodents and their tissues both nationally and internationally. Some infectious agents, notably those causing a gradual cumulative or subtle disease, may be present in an enzootic (constantly present) state in many research colonies. Finally, the use of immunocompromised and genetically altered strains of mice is on the rise. These animals are more susceptible to disease, and this population increases the likelihood of outbreaks from a

Table 4.8. Miscellaneous agents used in mice

Drug	Dosage	Route	Reference
Atipamezole	1 mg/kg	SC, IP, IV	Flecknell (1997)
	1–2.5 mg/kg	IP	Cruz et al. (1998)
Atropine	0.05–0.1 mg/kg	SC	Harkness and Wagner (1995)
	10 mg/kg q20min (organophosphate overdose)	SC	Harkness and Wagner (1995)
Cimetidine	5–10 mg/kg q6–12h	PO, SC	Allen et al. (1993)
Dexamethasone	0.5–2 mg/kg then decreasing dose q12h for 3–14d	PO, SC	Harkness and Wagner (1995)
Diphenhydramine	1–2 mg/kg q12h	PO, SC	Morrisey and Carpenter (2011)
Doxapram	5–10 mg/kg	IP, IV	Flecknell (2009)
Furosemide	1–4 mg/kg q4–6h	IM	Harrenstien (1994)
Glycopyrrolate	0.01–0.02 mg/kg	SC	Huerkamp (1995)
Naloxone	0.01–0.1 mg/kg	IV, IP	Harkness et al. (2010)
Oxytocin	0.2–3 IU/kg	SC, IV	Anderson (1994)
Prednisone	0.5–2.2 mg/kg	SC, PO	Anderson (1994)
Sucralfate	25–50 mg/kg	PO	Morrisey and Carpenter (2011)
Vitamin B complex	0.02–0.2 mL/kg	SC	Anderson (1994)
Vitamin K	1–10 mg/kg q24h for 4–6d	IM	Harkness and Wagner (1995)
Yohimbine	0.5–1 mg/kg	IV	Harkness and Wagner (1995)

IM = intramuscular; IP = intraperitoneal; IV = intravenous; PO = per os; SC = subcutaneous.

Box 4.5

Immunocompromised and genetically altered strains of mice are generally more susceptible to disease, increasing the likelihood of outbreaks from a variety of adventitious agents.

variety of adventitious agents (Box 4.5). Depending on the competency of their immune systems, identifying infected but subclinically infected immunocompromised mice can be especially challenging. Basic knowledge of the variety of infectious agents that may infect a rodent population is imperative to being able to quickly and appropriately respond to a disease outbreak. Presented here is a general overview of common bacterial, viral, fungal, and parasitic agents of mice and some standard diagnostic and treatment modalities.

Bacterial Diseases

Pneumonia and Respiratory Diseases

Primary bacterial pneumonia is uncommon in mice unless the animals are immunologically deficient or stressed. Infection with *Mycoplasma pulmonis*, a bacterium that lacks a cell wall, is one causal organism, but it is a much more serious disease issue in rats. Refer to Chapter

5 for a more detailed description of mycoplasmosis. Concurrent infection with Sendai virus or the cilia-associated respiratory (CAR) bacillus may result in a fatal pneumonia. Bacteria are often cultured from pneumonic lungs of mice with primary viral or mycoplasmal infections.

Cilia-associated respiratory bacillus (CAR bacillus) is a Gram-negative bacillus that has been found between the cilia lining the respiratory tract of mice and other laboratory and domestic species. In mice, it appears to be opportunistic, for it has only been found in association with other respiratory pathogens. A histologic change noted in mice is peribronchitis. Sulfamerazine or ampicillin, administered at a dose of 500 mg per liter of drinking water, has been reported to eliminate colonization of the organism as well as reduce the severity of the peribronchitis. Other bacteria occasionally associated with respiratory disease in mice include *Klebsiella pneumoniae*, *Corynebacterium kutscheri*, *Bordetella bronchiseptica*, and *Pasteurella pneumotropica*.

Clinical signs associated with pneumonia in mice include teeth chattering, labored respiration, weight loss, and conjunctivitis. Treatment with antibiotics is frequently not practical in a research colony; however, antimicrobials such as ampicillin, oxytetracycline, or trimethoprim sulfa may be effective when circumstances justify their use.

Helicobacter spp. Infection

Helicobacter spp. are small spiral- to rod-shaped, Gram-negative, motile bacteria that inhabit the gastrointestinal tract from the stomach to the colon in a variety of species, including mice, rats, hamsters, ferrets, cats, dogs, and humans. They are generally but not always host-specific. To date, at least 12 different *Helicobacter* species have been identified in mice, but this list will likely expand as the ability to recognize these agents improves. Transmission occurs by the fecal–oral route, and infections are persistent. Mouse strains vary greatly in their susceptibility to infection, with A/JCr mice being highly susceptible and C57BL mice being resistant to *H. hepaticus* infection. In addition to the differences in strain susceptibility, the different rodent helicobacters vary in pathogenicity. Infection with *Helicobacter* spp. cannot be equated with disease.

Helicobacter bilis and *H. hepaticus* have been documented as pathogens in a wide range of mouse strains and should therefore be regarded as pathogens of susceptible hosts. They have been associated with disease syndromes, including chronic active hepatitis, hepatic neoplasia, inflammatory bowel disease, proliferative colitis, and rectal prolapse. Mice infected with these pathogens should not be used in long-term studies or housed near immunodeficient animals. Although the organism can be readily transmitted between animals in physical or close contact, the organism is sensitive to desiccation, thus it is not readily transmitted between rooms or facilities employing routine biosecurity practices. Clinical signs are usually not seen until the liver disease is nearing end stage. Immunodeficient mice and aged mice have demonstrated a higher frequency of intestinal and hepatic lesions as well as an increased incidence of hepatocellular tumors, which may confound interpretation of experimental results. Gross lesions include multiple white foci in the liver, sites of hepatic necrosis. *H. hepaticus* serves as a model for *H. pylori*–induced chronic gastritis, gastric ulcers, and gastric adenocarcinoma in humans.

Helicobacter rappini, *H. muridarum*, and *H. rodentium* have rarely been associated with disease and should probably be regarded as undesirable contaminants of mice. Their presence

might be considered conditionally acceptable for some programs. Programs with substantial populations of susceptible immunodeficient, inbred, or transgenic mouse strains should consider excluding all *Helicobacter* spp. from the facility.

Sentinel mice exposed to soiled bedding can be used to screen for the presence of *Helicobacter* spp. Diagnosis of *Helicobacter* spp. is possible in several ways, including culture, histology, and PCR. No serologic assays are commercially available due to difficulties with assay specificity. PCR testing of fecal pellets or intestinal tissue samples is currently the most accurate and sensitive diagnostic tool available.

Eradication of *Helicobacter* spp. from a colony can be achievable by transferring neonates to *Helicobacter*-negative foster dams on the first day of life. Additionally, use of good microisolation technique can effectively prevent the horizontal transmission of *Helicobacter* spp. to other cages in the same room. Infection may be treated with a multi-antibiotic regimen although complete elimination of the organism is not always successful.

Tyzzer's Disease

Mice, rats, hamsters, gerbils, guinea pigs, rabbits, dogs, cats, several nonhuman primate species, and other animals are susceptible to Tyzzer's disease caused by *Clostridium piliforme*. *C. piliforme* is a long, thin, Gram-negative spore-forming bacterium. Tyzzer's disease occurs more frequently and is usually more severe in recently weaned animals, immunosuppressed animals, and those housed in unfavorable conditions. Different strains of mice have varied susceptibility to this organism. Clinical signs may include diarrhea, dehydration, and anorexia. Animals may be found dead in the absence of obvious signs of illness. Gross lesions seen at necropsy include miliary, pale foci throughout the liver, and at times a slightly reddened lower intestinal tract. Spores can remain stable up to 1 year at room temperature and are a likely means of transmission, especially through contaminated food or bedding. Diagnosis is made by histologic detection of intracellular organisms in the liver or intestine, by enzyme-linked immunosorbent assay (ELISA) or, ideally, by PCR. Treatment with antibiotics is frequently not effective because of spore formation; however, favorable results have been reported using tetracycline in the drinking water at high doses for 4–5 days. Barrier housing, good husbandry practices, and strict quarantine help to prevent outbreaks or control spread of infection.

Colonic Hyperplasia Disease

Citrobacter rodentium causes a disease syndrome in mice called transmissible murine colonic hyperplasia. Infection induces enterocolitis that leads to clinical signs of diarrhea, retarded growth, ruffled fur, soft feces, and occasionally rectal prolapse. The mortality rate is variable and depends on age and strain susceptibilities with recently weaned mice having a higher mortality. The characteristic gross finding at necropsy is thickening of the distal half of the colon. Microscopically, mucosal hyperplasia, infiltration of inflammatory cells, and mucosal erosions may be evident in the affected portion of the colon. Diagnosis can be made by culture, but PCR offers a more sensitive diagnostic assay. Recommended treatments include neomycin, tetracycline, and sulfamethazine.

Hyperkeratosis-Associated Coryneform (HAC)

Corynebacterium bovis has been associated with a severe hyperkeratotic dermatitis in nude mice of all ages and in neonatal SCID mice. The infection is often referred to as scaly skin

disease. Transmission is by direct contact, fomites, and cell lines. People can serve as the source of infection for mice. The disease may cause high mortality in suckling nude and SCID mice. Clinical signs include dry, white flaky skin, dehydration, and pruritis that typically last 1 week. Mice that recover from the clinical signs may be persistently infected. Definitive diagnosis requires culturing the organism on select media or by PCR identification. There is no known treatment, and affected animals should be euthanized to prevent spread of the organism within a clean colony. Strict adherence to microisolation technique is necessary to limit spread of disease in immunodeficient mice.

Miscellaneous Bacterial Diseases

Staphylococcus aureus is a Gram-positive coccus that commonly infects the skin and mucous membranes of animals. Staphylococci are prevalent and can be found on the skin and in the nasopharynx and gastrointestinal tract. *S. aureus* is a pathogen that is most frequently associated with abscesses, conjunctivitis, and superficial pyoderma, particularly around the head and face. Bite wounds are often secondarily infected with *S. aureus*. Suppurative dermatitis or furunculosis caused by *S. aureus* is a particularly troublesome condition in nude mice and treatment is rarely successful. Diagnosis is usually by culture.

Staphylococcus xylosus is a Gram-positive coccus that is ubiquitous in nature and a common commensal of the skin and mucous membranes of many species, including immunocompetent rodents and humans. The organism has been identified as the causative agent for morbidity and mortality in select strains of transgenic mice. Mice with clinical infections frequently exhibit abscesses or other skin lesions. Modified husbandry procedures may decrease the rate of clinical disease in susceptible strains.

Streptococcus spp. infections are associated with dermatitis, pharyngitis, cervical lymphadenitis, bacteremia, and a variety of other disease processes. Immunodeficient mice are more susceptible to infection. Systemic antibiotics and improved sanitation may be beneficial in treatment.

Pseudomonas aeruginosa is frequently found in association with water and is not part of the mouse's normal microflora. In animal facilities, contaminated, untreated drinking water is the most common source of *P. aeruginosa* and has been shown to be a source of infection in animals. As an opportunist, this organism, once established, may be found chronically in the oropharynx, nasopharynx, and gastrointestinal tract and is frequently isolated from preputial gland abscesses. Mice that are irradiated or otherwise immunologically compromised are susceptible to developing a *P. aeruginosa* septicemia. The septicemia is usually rapid, and clinical signs may include listlessness, equilibrium disturbances, conjunctivitis, serosanguinous nasal discharge, edema of the head, skin lesions, anorexia, and death. Diagnosis is usually by culture of the nasal mucosa or water sipper tubes. Decontamination of water prior to presentation to animals can be accomplished by a number of techniques, including reverse osmosis, deionization, microfiltration, reverse osmosis–deionization–ultrafiltration, autoclaving, hyperchlorination, and acidification. Infections can be avoided but not eliminated by chlorinating the drinking water with sodium hypochlorite to achieve 10–13 ppm of free chlorine or by acidifying to a pH of 2.5–3.0 with hydrochloric acid. Acidity of the water should be tested before use.

Corynebacterium kutscheri is a Gram-positive organism that is usually inapparent in mice, but it produces a disease in stressed or immunosuppressed mice termed pseudotuberculosis.

Transmission occurs by multiple routes from asymptomatic carriers. The disease is characterized by septicemia with high mortality and produces disseminated abscesses, especially in the kidneys and liver, in surviving mice. Treatment is usually not successful, but prevention can be achieved by barrier housing and good husbandry practices.

Salmonella spp. infections were extremely common in research colonies at one time but are now rare as a result of the institution of proper management practices. Mice can be intermittent shedders for several months without showing clinical signs of disease. Salmonella infection is significant due to its ability to infect and cause disease in humans.

Streptobacillus moniliformis infections are not common in mice. Epizootics are most likely to occur in mice that are housed near rats that may harbor the organism in the nasopharynx. The importance of this organism is that it has been identified as one of the causes of the zoonotic disease rat-bite fever, which is an influenza-like disease in humans.

Viral Diseases

Parvoviruses

Parvoviruses are small and nonenveloped DNA viruses; thus they are resistant to heat, lipid solvents, and acidic environments and can persist in the environment for months to years. Mouse parvovirus (MPV) type 1 and mouse minute virus (MMV) are the two parvoviruses known to naturally infect laboratory mice and are among the most prevalent infectious agents found in contemporary laboratory mouse colonies. Both viruses target rapidly dividing cells including those of the intestinal tract and lymphoid tissue. However, the intestinal tract is not usually significantly affected in infected animals. Neither of these viruses typically causes clinical disease in adult mice although runting and cerebral hypoplasia may be noted in neonates infected with MMV. MPV is a greater concern because of its known effects on the immune system of mice. There are differences in strain sensitivities to these infections. C57BL/6 mice are known to have increased resistance to infection. Transmission of MPV is relatively slow and is thought to be primarily fecal–oral although transmission can also occur through administration of contaminated biologic materials (e.g., cell lines, tumor cells, and serum). It appears to require direct contact with contagious mice or contaminated fomites. MPV is different from MMV in at least three ways: adult mice are as susceptible as neonates to infection, MPV causes immune dysfunction because of its tropism for lymphocytes, and MPV can infect and persist in immunocompetent mice but no one knows for how long. Diagnosis is usually made by serological screening of sentinel mice, but sentinels may not seroconvert if exposed to resistant strains like the C57BL/6. PCR testing can also be used to confirm diagnosis by screening infected tissues such as mesenteric lymph nodes. PCR assays have been developed to detect the virus' genetic material in the environment. Knowledge of MPV's influence on physiologic systems and biologic pathways is continually expanding. Due to the virus' known persistence in lymphoid tissue and effects on the immune system, institutions devote significant efforts to excluding the virus from their colonies. Control of infection generally includes depopulation of infected mice and/or cesarean rederivation or embryo transfer for valuable mice. Environmental decontamination is considered important by some, and the use of a chemical disinfectant such as chlorine dioxide or hydrogen peroxide that can be vaporized to reach all surfaces should be considered.

Sendai Virus

Sendai virus (SV) is a parainfluenza I virus. While once very common, the virus is rarely detected in modern laboratory colonies. Viral infection can cause clinical respiratory disease in adult immunocompetent mice. Rats, hamsters, and guinea pigs are also susceptible to infection. It usually exists as an inapparent infection. It may, however, produce acute disease that is sometimes fatal, particularly in suckling or weanling mice. There is a distinct strain variation in susceptibility to infection. Immunosuppressed mice are highly susceptible and develop a wasting disease. Transmission is by aerosol, and the virus is highly contagious. Recent outbreaks have been associated with the administration of biologic agents (e.g., blood, serum, tissue, cell cultures) contaminated with the virus. Sendai virus infection suppresses the normal antibacterial activity of the lungs, thereby predisposing infected mice to secondary bacterial pneumonia. Clinical signs include a hunched posture, ruffled or erect hair coat, rapid weight loss, dyspnea, crusty eyes, and chattering sounds. Sendai virus has also been associated with otitis media and interna as well as exacerbations of pneumonia due to *M. pulmonis*. Gross lesions include consolidated, plum-colored lungs. The severity of disease is intensified by secondary bacterial infection. Diagnosis is generally made by ELISA serology, but reverse transcriptase–PCR can also be used to detect virus in the lungs. Sendai virus infections are self-limiting in immunocompetent mice. Susceptible animals must not be introduced into a colony for 4–6 weeks to allow "burnout" of the organism. Barrier housing will easily prevent spread of infection. Antibiotics may be beneficial in preventing or eliminating secondary bacterial infection.

Mouse Hepatitis Virus

Mouse hepatitis virus (MHV) is a coronavirus that is ubiquitous and highly contagious. Mice shed the virus in their feces and respiratory secretions. Transmission occurs through direct contact, fomites, and airborne particles. Although it is much less common in research colonies today, it remains one of the more common viral contaminants of conventionally housed mice due to its ease of transmission. There are many related strains of MHV that generally fall into two disease patterns: the enteric pattern and the respiratory pattern. Enterotropic strains are much more common.

Mouse hepatitis virus infections are usually enzootic and subclinical, with epizootics occurring in naive suckling mice. Clinical signs in nursing mice may include severe diarrhea, runting, an empty stomach or disappearance of the milk spot (unlike nursing young with rotavirus infection), encephalitis with tremors, and high mortality. Infection of nude mice is associated with a severe wasting syndrome. Gross signs in adult mice include multifocal miliary white foci throughout the liver due to the necrotizing hepatitis that occurs with some strains of MHV. The most distinctive histologic finding is syncytial cells in the small intestinal mucosa and liver parenchyma. Diagnosis of MHV is most often achieved by use of ELISA with diagnosis confirmation through use of immunofluorescent assays (IFA) or PCR testing. MHV does not persist in immunocompetent mice and can be eliminated from a colony by cessation of breeding and no introduction of susceptible mice for at least 4 weeks to allow all infected mice to clear the infection. This eradication method may fail in transgenic mice if the genetic modification has altered immune function—an unintended and often unrecognized effect of genetic modification.

Epizootic Diarrhea of Infant Mice

Epizootic diarrhea of infant mice (EDIM) is a rotavirus that causes disease in suckling mice less than 2 weeks of age. It is characterized by the presence of soft, yellow feces or an accumulation of dried feces around the anus. Affected mice usually continue to nurse, as evidenced by the milk spot (milk outwardly visible within the stomach), but their growth is often stunted. Diagnosis is usually by ELISA serology. Spread of EDIM within a colony can be prevented by use of microisolation cages. No treatment exists other than supportive care.

Murine Retroviral Infection

All mice harbor both endogenous murine leukemia virus (MuLV) and endogenous murine mammary tumor virus (MuMTV) in their genome. Expression of disease is mouse strain-, age-, and cell-type-specific. The proviruses are transmitted genetically as inherited Mendelian characteristics and, as such, cannot be eradicated by traditional methods (e.g., test-and-cull, rederivation, cross-fostering of pups).

Lymphocytic Choriomeningitis Virus

Lymphocytic choriomeningitis virus (LCMV) is an arenavirus and is the only latent virus of mice that naturally infects humans, causing an influenza-like disease. The natural reservoir of LCMV is wild mice although many other rodent species can be infected. Mice may serve as long-term asymptomatic carriers of the virus, so care should be taken when contacting tissue, feces, or urine of suspect animals.

Ectromelia (Mousepox)

Ectromelia is caused by a poxvirus and is very uncommon in the United States yet still persists in other countries. It is significant when it occurs in research mice because it may be associated with high morbidity and mortality without additional clinical signs. Mice that survive the initial infection may have clinical signs, including conjunctivitis and a poxlike rash that can develop anywhere on the body. Severe infection of the feet and tail can lead to amputation, hence the name infectious ectromelia (*ectro*=loss of; *melia*=limbs). The disease is usually introduced through imported mice, tumor transplants, or other biologic materials. This underscores the need to screen all imported biologic materials by PCR or other methods prior to using them in animal studies.

Pneumonia Virus of Mice

Pneumonia virus of mice (PVM) is a pneumovirus that naturally infects mice, rats, guinea pigs, hamsters, and probably other rodents. Infections are asymptomatic, but they may cause dyspnea, listlessness, and a wasting disease in immunodeficient mice. PVM has been shown to have a worldwide distribution and may be prevalent in conventional colonies. It replicates only in the respiratory tract epithelium. Diagnosis is usually by ELISA screening of sentinel mice. Infections are acute and self-limiting in immunocompetent mice, thus control and elimination are similar to those described for SV.

Murine Norovirus

A recently identified murine calicivirus is the murine norovirus (MNV). Noroviruses are important human pathogens worldwide and cause more than 90% of all cases of nonbacterial epidemic gastroenteritis. Currently over 40 strains of MNV have been detected worldwide; none are known to be zoonotic. MNV is a common viral infection in laboratory mice although a growing number of research institutions have eliminated the virus from their animal colonies by rederivation and cross-fostering very young neonates with uninfected dams. Transmission is primarily via the fecal–oral route. Certain infected immunodeficient strains of mice have high mortality with encephalitis, pneumonia, and hepatitis and may remain persistently infected. Immunocompetent mice frequently exhibit no clinical signs and are only transiently infected. Further studies are required to fully determine the consequences of infection; the virus has been shown to frequently affect the immune system although the effects of MNV infection varies significantly across mouse strains and with each MNV strain. Tests used to detect animal infection include ELISA, IFA, and PCR. Growth of many strains in cell culture has been challenging. A 1:10 dilution of bleach is the only disinfectant currently known to kill the virus.

Miscellaneous Viral Infections

Mice are susceptible to a vast number of viral infections, including K virus, mouse polyoma virus, reovirus type 3, Theiler's encephalomyelitis virus, mouse adenovirus, and lactic dehydrogenase elevating virus. These rarely cause disease in immunocompetent mice and are primarily of concern in immunodeficient mice and as potential variables in biomedical research. They are most often introduced into colonies as contaminants of cell cultures or in subclinical mice being transported from a noncommercial source. Clinical signs are rarely seen in animals beyond the age of weaning. Quarantine of incoming mice with serologic and/or PCR screening should routinely be performed to ensure that only viral-free animals are used for research.

Parasitic Diseases

Mites

Mites are sporadically detected in research colonies. The more common species of pelage-inhabiting mites are *Myobia musculi*, *Radfordia affinis*, and *Myocoptes musculinus*. The degree of pathogenicity is highly variable, but *Myobia musculi* is considered the most pathogenic because it feeds on skin secretions and interstitial fluid. Light infestations are usually inapparent, whereas heavy infestations cause dermatitis, alopecia, greasy-appearing hair, and self-inflicted trauma. Some transgenic strains are especially sensitive to infection presumably due to often-unrecognized alterations of their immune systems. An allergic component may also be involved. Mite infestations can induce immunologic changes with marked systemic consequences that act as a variable in research. Mite lesions must be differentiated from fight wounds, ringworm, and other skin disorders.

Premortem diagnosis can be accomplished by several methods, including skin scrapings, fur plucks, and applying cellophane tape to the fur and examining the tape under the microscope for nits (eggs) or mites. The body site with the heaviest infestation, and therefore the highest chance of detection, varies with mite species and most frequently includes the dorsal

neck and shoulder regions, the dorsal caudal thoracic or cranial pelvic region, the ventral abdomen, and the axilla regions. In addition, PCR assays have been developed as a diagnostic method. Each diagnostic method has varying degrees of sensitivity. All three stages of the life cycle—the egg, nymph, and adult—attach to the hairs on the host. Euthanizing the mouse and either collecting the mites as they leave the cold carcass or removing the mouse skin, placing it in a petri dish, and examining the bottom of the dish under low magnification after several hours usually achieves more conclusive results. If mites are present, they should be transferred to a glass slide for identification.

Psorergates simplex is a follicle-inhabiting mite that produces dermal pouches that appear as small white nodules on the visceral surface of the reflected skin. These mites are rarely seen today.

Acaricides can reduce and even eliminate the mite population. Complete eradication of mites in a large mouse population, however, is difficult and requires an intensive treatment regimen. Ivermectin given by a number of routes, and to a lesser extent permethrin dusts, have been shown to be effective in eliminating mites. Cotton balls impregnated with permethrin have been successfully used to eliminate infestations after 4–8 weeks. Caution should be used when treating suckling mice because they may be more sensitive to topical applications of acaricides.

The most reliable means to eradicate mites from a colony is surgical rederivation. This method is more technically demanding and costly but highly effective. Cross-fostering within 36 hours in combination with topical ivermectin therapy has also been used to effectively eradicate murine fur mites. Extreme caution must be used with ivermectin administration as death or neurologic disease can be induced if the drug passes into the central nervous system through an incomplete or damaged blood–brain barrier. Ivermectin treatments should be administered to representative animals (e.g., varying age and sex) to evaluate safety prior to colony-wide administration.

Pinworms

Syphacia obvelata and *Aspiculuris tetraptera* inhabit the cecum and colon and are the most common nematode parasites that affect mice. A high proportion of conventional colonies are estimated to house mice infected with these parasites. *S. obvelata* is more prevalent and troublesome. In most cases, clinical signs are absent; however, heavy infestations may be associated with poor hair coat, mucoid enteritis, anal pruritis, rectal prolapse, and intestinal impaction or intussusceptions. Pinworm infection can alter the humoral immune response. Mice strains differ in their susceptibility to infection with pinworms, and this difference may be partially related to differences in immune function. Neither species of pinworms is transmissible to humans.

Premortem diagnosis of infection can be challenging, especially given the intermittent shedding of eggs in an infected mouse's feces, the need to have a mixed-sex infection to induce egg production, and the reduction in parasite load with age. The banana-shaped ova of *S. obvelata* can occasionally be detected by pressing a strip of cellophane tape to the perianal area, applying the tape to a glass slide, and examining the tape under a microscope. The more oval eggs of *A. tetraptera* may be detected by fecal examination. The most definitive diagnostic procedure involves examining the cecal contents for adults under a dissecting microscope. PCR assays are being developed to detect infection.

Anthelmintics effective in treating pinworms include fenbendazole, ivermectin, piperazine citrate, thiabendazole, and mebendazole, many of which can be formulated in the feed or drinking water. Caution must be used with the administration of each medication as many may induce severe adverse effects in some strains of mice or may alter experimental results. The eggs may remain infectious in the environment for prolonged periods. Complete elimination of *S. obvelata* without repopulation and strict sanitation is difficult to achieve.

Lice

Polyplax serrata, the house mouse louse, is a bloodsucking louse that causes skin irritation, anemia, and debilitation. It is highly uncommon in laboratory mice. Ivermectin has been effectively used to eliminate lice. Dusts, such as permethrin dust, applied to the animals and the bedding are also reported to be effective against lice.

Tapeworms

Mice may harbor *Rodentolepis nana*, the dwarf tapeworm, and *Hymenolepis diminuta*, the rat tapeworm. Both species are potentially transmissible to humans. Only *R. nana*, however, presents a practical public health concern because *H. diminuta* requires an intermediate host such as a grain beetle, whereas *R. nana* can directly infect its definitive host. Tapeworms are uncommon in modern mouse colonies.

Diarrhea and retarded growth may occur with heavy infestations, but most cases are clinically silent. Infestations can be detected by the presence of ova upon fecal flotation or by observing the adults in the small intestine at necropsy. Treatments may include thiabendazole or praziquantel, but treatments are generally not recommended because of the zoonotic hazard. Eradication by means other than repopulation is extremely difficult.

Flagellates

Spironucleus muris and *Giardia muris* are flagellates that occur in the small intestine and cecum. In young animals, the parasites may cause diarrhea and occasionally death, but older animals are generally asymptomatic. Giardia may be treated with metronidazole that controls outbreaks, but does not eliminate the parasite. There are no available treatments for spironucleosis.

Neoplasms

The incidence and types of tumors occurring in mice are highly strain- and age-related. Retroviral infections play a key role in the development of most neoplasms of the lymphoreticular and hematopoietic systems as well as of the mammary gland. Most mammary tumors are adenocarcinomas, which can produce large, firm masses on the abdomen, sides, or back of a mouse because of the wide distribution of mammary tissue. The C3H strain has a high incidence of mammary tumors; AKR mice have a high incidence of lymphoma.

Miscellaneous Conditions

Bite Wounds

Some strains of mice, including BALB/c, SCID, SJL, and FVB, are prone to aggression and can inflict severe bite wounds to their cagemates. Aggression is seen most often in males. In general, cohousing of multiple adult males not raised and maintained in the same litter is not

possible. Housing males together prior to weaning can sometimes decrease aggressive behavior, but this is challenging when research often requires randomization of mice. Wounds are seen primarily around the tail and perineum but also along the back, shoulders, and head. These wounds may become secondarily infected with *S. aureus*, which can lead to an ulcerative dermatitis with moist eczematous lesions. The stress from fighting likely impacts the physiology of the mice, and infection with *S. aureus* has been shown to alter immune responses. Bite wounds over the body must be differentiated from self-mutilation that results from mange mite infestation.

Treatments are usually ineffective, and euthanasia is indicated in more severe cases. For animal welfare and scientific reasons, aggressive mice should be identified and removed from cages to decrease wounding and stress (Box 4.6). Removing the aggressor may resolve the problem, but a new dominant male may emerge and fighting may continue. Addition of enrichment, such as nesting material, huts, and chewing objects, may result in decreased fighting by promoting other natural behaviors and providing an escape for subordinate mice.

Box 4.6

For animal welfare and scientific reasons, aggressive mice should be identified and removed from cages to decrease wounding and stress.

Ulcerative Dermatitis

Idiopathic ulcerative dermatitis (IUD) is a genetically linked skin syndrome found most frequently in C57BL/6 mice and strains of mice on the C57BL/6 background (Figure 4.9). Clinical signs include severe pruritis with excoriation, ulceration, and loss of skin segments. Lesions are most common over the dorsal cervical and scapular regions, although lesions

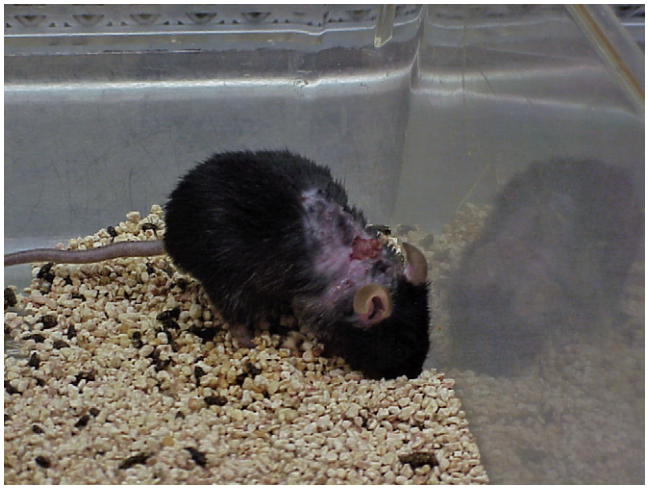

Fig 4.9. Ulcerative dermatitis. (Photo courtesy of ULAM, University of Michigan.)

may occur over any aspect of the body. Histologically, mice with IUD frequently have splenomegaly and peripheral lymphadenopathy secondary to ulceration and inflammation. A diagnosis of IUD should be made only after eliminating other etiologies (e.g., ectoparasite infestation, fighting wounds). Treatment options have included antibiotics, corticosteroids, antihistamines, antifungal and antibacterial ointments (e.g., zinc oxide, silver sulfadiazine), and dietary vitamin (e.g., vitamin E) supplementation all with limited therapeutic efficacy. Some success has been reported with trimming an affected animal's nails early in the disease process. Left untreated, IUD may lead to such severe lesions that euthanasia is required for animal welfare reasons.

Dehydration

Dehydration should not be overlooked as a possible cause of acute death in mice. Water may be unavailable because of an air lock in the sipper tube even when a water bottle is full. Malfunction of drinking valves in automatic watering systems can also result in a lack of water. In addition, mice may be slow in learning how to use a novel watering system when moved to a new environment. Mice undergoing surgical procedures should routinely be provided an IP or SC injection of warm saline or lactated Ringer's solution at 1–2 mL per adult mouse to offset the decrease in water consumption that often occurs postoperatively.

Hair Loss

Mice frequently develop bilaterally symmetrical areas of alopecia on the muzzle, which can be caused by friction from bars on the feeder. A more common cause of hair loss is barbering by a cagemate and most frequently involves hair chewing of the vibrissae or head region. Although long believed to be a show of dominance, studies have revealed that both dominant and submissive mice exhibit this behavior, and its causes are multifactorial. Additional information on barbering is provided in the behavior section of this chapter.

Malocclusion

Overgrowth of the incisors can occur when the jaw is malformed, there are missing opposing teeth, or the diet is too soft. If severe and undetected, overgrown incisors can lead to emaciation and death due to an inability to eat. A common clinical sign in later stages is excessive drooling of saliva with matting of the fur around the chin and neck. Incisors can be trimmed with suture removal scissors or toenail clippers, but this practice may lead to fractures of the teeth. A dental bur is a better tool to trim teeth without causing fractures. The use of a mouth speculum will protect the animal and the handler during examination and trimming. Some malocclusions have a genetic basis. Variable incidences are seen between strains, with some transgenic strains being severely affected. In these cases, it is recommended that affected animals be culled (removed) from the breeding population to decrease the incidence of malocclusion in future generations.

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CHAPTER 4 REVIEW

Multiple Choice

1. Gestation time of the mouse is
   A. 15 days
   B. 21 days
   C. 30 days
   D. 42 days
2. Albino inbred strain
   A. BALB/c
   B. Swiss Webster
   C. C3H
   D. ICR

3. Strain prone to aggression
   A. C3H
   B. nude
   C. C57BL
   D. SJL
4. Outbred stock
   A. C57BL
   B. BALB/c
   C. C3H
   D. ICR
5. Strain lacks T cells and B cells
   A. nude
   B. SJL
   C. SCID
   D. BALB/c
6. The term *altricial* means
   A. free choice
   B. young are born helpless
   C. to remove from litter
   D. independent from birth
7. Minimum number of generations of brother–sister crosses to create an inbred strain
   A. 20
   B. 35
   C. 15
   D. 50
8. Administration route *not* suggested in the mouse
   A. IV
   B. SC
   C. IP
   D. IM
9. False statement regarding the mouse
   A. It has a divided stomach.
   B. It has 4 pairs of mammary glands.
   C. It has brown fat between scapulae.
   D. Its incisors grow continuously.
10. Transgenic mice are frequently created from this background strain.
    A. BALB/c
    B. C3H
    C. C57BL/6
    D. SJL

(*Continued*)

Match Up

Match the following with their respective descriptions:

A. Ectromelia virus
B. Mouse hepatitis virus
C. Mites
D. Tapeworms
E. Pinworms
F. *Pseudomonas aeruginosa*
G. Ulcerative dermatitis
H. Murine norovirus
I. *Corynebacterium bovis*
J. Pneumonia virus of mice
K. Mouse minute virus
L. Sendai virus
M. Barbering
N. *Helicobacter hepaticus*

11. ____ Bacteria associated with chronic active hepatitis
12. ____ Parvovirus that targets intestinal and lymphoid tissue
13. ____ Hair chewing of vibrissae on head region
14. ____ *Syphacia* and *Aspiculuris*
15. ____ Poxvirus that can lead to amputation of feet and tail
16. ____ *Myobia* and *Mycoptes*
17. ____ Coronavirus that is highly contagious; enteric and respiratory patterns
18. ____ Genetically linked skin syndrome found frequently in C57BL/6 mice
19. ____ Cause of scaly skin disease in nudes
20. ____ Found in association with water, not part of normal flora

5 Rats

The laboratory rat strains most commonly used in research are believed to be domesticated albino strains of the Norway rat (*Rattus norvegicus*) that originated in Asia and migrated to Europe and then North America. Rats, like mice, belong to the order Rodentia and the family Muridae. Adult rats generally weigh between 250 and 500 g, with males being significantly larger than females by 9 weeks of age. Most laboratory rats are albino with white coats and pink eyes. There are several other color varieties, including brown, black, and hooded (black or brown head and shoulders with white bodies).

GENETICS

Like mice, rats are most frequently genetically classified as outbred stocks, inbred strains, or genetically modified. Refer to Chapter 4, "Mice," for a description of each type. Three of the most common outbred stocks of rats used in laboratories are the Sprague-Dawley, Wistar, and Long-Evans (Figure 5.1). Sprague-Dawley and Wistar rats are albinos, and the Long-Evans is hooded (color on the head and shoulders with a pigmented dorsal stripe). Inbred rats are frequently selected to limit experimental variability and to capitalize on a desired characteristic inherent to the strain. Just as a strain may have a desirable characteristic, strains may also have characteristics that may conflict with or complicate experimental interpretation. Several examples of inbred strains include the Fischer 344 (F344), the Spontaneously Hypertensive (SHR), Wistar-Kyoto (WKY), and the Buffalo (BUF). F344 rats have a high incidence of testicular tumors, large granular lymphocytic leukemia, and retinal degeneration. They are commonly used in gerontology and long-term toxicity studies. SHR

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Fig 5.1. Long-Evans, outbred hooded rat. (Photo courtesy of Taconic.)

rats develop hypertension and are used to study therapies for this disease; the WKY has normal blood pressure and functions as the control for the SHR. Buffalo rats have a high incidence of autoimmune thyroiditis, also known as Hashimoto's disease. Strains have also been developed that possess a spontaneous mutation, such as the nude rat and the Zucker Diabetic Fatty (ZDF) rat. The nude rat, similar to the nude mouse, lacks a thymus and thus is deficient in T-cell lymphocytes; they are used in oncology studies because they readily accept xenografts of human tumors. "Nude" is somewhat of a misnomer as they frequently possess a sparse, but noticeable, coat. The ZDF, with a body weight of 400–600 g by 15 weeks of age, develops hyperlipidemia and hyperglycemia at 8 weeks and diabetes at 12 weeks when fed a high-fat diet. The number of specifically designed, genetically modified strains continues to increase, although it is still significantly less than that of mice. Two common genetically modified strains are the p53 knockout used in oncology research and the RIP-HAT developed for use in diabetes research. Mutant strains of inbred rats provide investigators a large variety of animal models with which to study biologic processes and diseases.

MICROBIOLOGIC CLASSIFICATIONS

In addition to being categorized by their genetic classification, rats, like mice, are frequently classified by their microbiologic status. These are (1) germ-free or axenic, which are free of all detectable microflora; (2) gnotobiotic, which have associated known microflora; (3) specific pathogen free, which are free of a defined list of pathogens (including viruses, bacteria, and parasites); and (4) conventional, or animals with undefined microflora. The majority of rodents used for research are specific pathogen free. A description of each type is covered in Chapter 4.

USES

Rats rank second only to mice in the number of mammals used in biomedical research (Box 5.1). Rats and mice combined account for more than 95% of all mammalian species used. Rats share most of the attributes of mice that make them valuable for research purposes, including a short life span, a short gestation length, a large litter size, great genetic diversity, and ease of maintenance. Hypertension, neoplasia, teratology, toxicology, embryology, and aging are just a few of the research areas for which these animals are used.

Box 5.1

Rats rank second only to mice in the number of mammals used in biomedical research.

BEHAVIOR

Despite the fear that rats invoke in people, they are generally quiet, gentle animals that are intelligent and easily trained. They seldom bite unless provoked and do not have the distinct musty odor that is characteristic of mice. Rats appear to tolerate single housing but social housing in pairs or small groups is recommended whenever possible; the use of single housing should be scientifically justified. Unlike mice, rats rarely fight, and younger males can be housed together with few problems. Older males may be aggressive toward other males and therefore group housing of males after weaning age should be done with severe caution. Females with litters may not tolerate the company of other females. Most strains are docile and curious, and easily adapt to various environments. Rodents possess an innate preference for contact with the vertical perimeter of a bounded space (i.e., wall following, corner burrowing, and group aggregation), avoiding the perceived threats of open areas (this orientation response is called thigmotaxis). Knowledge of this behavioral preference can be employed to more quickly acclimate animals to handling by holding them securely with as much surface contact as possible. Some strains (e.g., Long-Evans and F344 strain) have a reputation for being more difficult to handle than other strains. Docile behavior can be markedly improved by frequent gentle handling, especially if initiated with young animals. Rats will bite out of fear if they are handled roughly.

Rats are nocturnal, sleeping much of the day. They like to burrow and, although there is some variability among strains, females may build nests if pups are present and they are provided with nesting material. Rats will escape from their cages if the lids are left off but will often return to them after a brief exploration period. Pups left alone by the dam emit high-frequency (22–80 kHz) vocalizations. Adults routinely vocalize under a variety of circumstances; nearly all of these are far beyond the range of human hearing.

ANATOMIC AND PHYSIOLOGIC FEATURES

Biologic and reproductive data for the rat are listed in Table 5.1. Rats are much larger than mice, having conical heads and longer, cylindrical bodies covered in thick, short fur. Their legs are short; their tails are sparsely haired and comprise approximately 85% of the length of the body.

The rat gastrointestinal system is anatomically similar to that of the mouse with a few notable exceptions. The dental formula 2(I 1/1, C 0/0, P 0/0, M 3/3), the continuously growing incisors, and a divided stomach (aglandular forestomach, glandular stomach) are all similar. The rat cannot vomit due to the presence of a fold in the limiting ridge that separates the two regions of the stomach and covers the entrance of the esophagus. They also lack the required neural connections between the brain stem and the viscera. Other unique features of the digestive system include the absence of a gallbladder, the presence of a diffuse pancreas, and numerous salivary glands in the head and neck. The liver has four lobes: median, left lateral, right lateral, and caudate. The cecum is highly developed and has a rumenlike function for microbial digestion of cellulose. In germ-free rats, the cecum may become greatly distended and occasionally twists on its long axis, resulting in fatal cecal torsion.

Behind each eye lies a large, pigmented, horseshoe-shaped lacrimal structure called the Harderian gland. The substance secreted by this gland contains high levels of porphyrin. During periods of illness or stress, chromodacryorrhea ("red tears") is frequently observed. These red crusts around the eyes and nose may be mistaken for blood. Unlike blood, por-

Table 5.1. Biologic and reproductive data for rats

Adult body weight: Male	450–520 g
Adult body weight: Female	250–300 g
Life span	2.5–3.5 y
Body temperature	35.9°–37.5°C (96.6°–99.5°F)
Heart rate	250–450 beats per minute
Respiratory rate	70–115 breaths per minute
Tidal volume	0.6–2 mL
Food consumption	5–6 g/100 g per day
Water consumption	10–12 mL/100 g day
Breeding onset: Male	65–110 d
Breeding onset: Female	65–110 d
Estrous cycle length	4–5 d
Gestation period	21–23 d
Postpartum estrus	Fertile
Litter size	6–12
Weaning age	21 d
Breeding duration	350–440 d
Chromosome number (diploid)	42

Source: Adapted from Harkness et al. (2010).

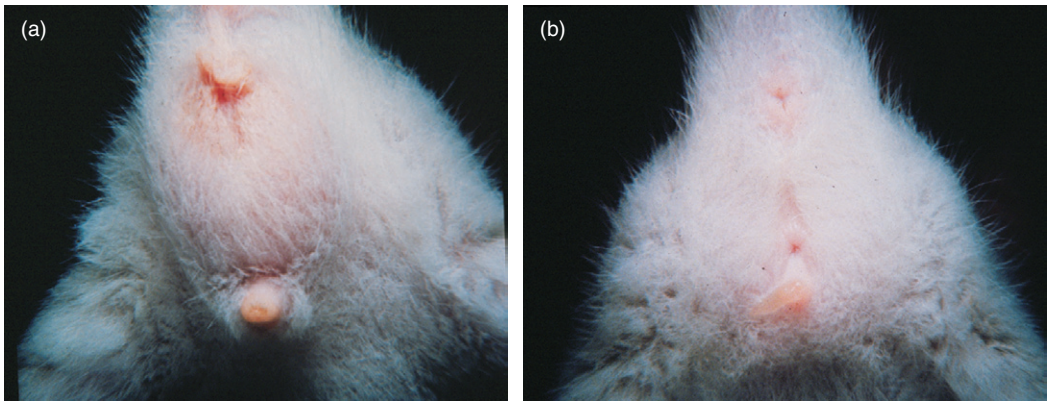

Fig 5.2. Sexing rats: (a) male; (b) female. (Photo courtesy of ACLAM.)

phyrin fluoresces under ultraviolet light. The lungs are similar to those in mice, consisting of one left lobe and four small right lobes.

The rat possesses paired auditory sebaceous glands (Zymbal's glands) that are located anterior and ventral to the base of the external ear canal. The ducts of the glands open into the external ear canal; their specific function remains unknown. Tumors may develop in the glands of older animals and most frequently manifest as masses near the ear base that often ulcerate. As with other rodents, brown fat is important in thermogenesis and is found between the scapulae and in the ventral cervical region. On necropsy, brown fat can be confused with salivary glands or lymph nodes.

Sexing of neonates is accomplished by comparing the anogenital distance and the size of the genital papillae. A larger genital papilla and greater anogenital distance are seen in males as compared with females (Figure 5.2). Adult rats are readily distinguishable, as the testes are obvious in the scrotum of males. Testes descend into the scrotum at about 15 days of age. The testes remain retractable throughout life due to open inguinal canals. Males have paired bulbourethral glands, seminal vesicles, and a large prostate gland, as well as an os penis.

Females have a bicornate (double-horned) uterus with two distinct uterine horns and associated cervices that communicate with the vagina. At birth, the vaginal opening is closed by the vaginal plate consisting of compact epithelium. This epithelium begins to degenerate and cornify at 3–5 weeks of age, and the vagina is completely open between 40 and 80 days. Rats, like mice, have extensive mammary tissue, ranging ventrally from the neck to the inguinal region and onto the sides and back. They have six pairs of mammary glands: three thoracic and three abdominal.

There are several distinctive characteristics of the hematologic and urinary profiles of rats. Lymphocytes comprise about 80% of the peripheral blood leukocyte population. Basophils are rarely seen in circulation. Males generally have higher granulocyte and lymphocyte counts than do females. Rats have the ability to concentrate urine twice that of humans. Urine pH is 7.3–8.5, and the specific gravity is 1.040–1.070. Hematologic and biochemical parameters for the rat are listed in Appendix 1, "Normal Values."

BREEDING AND REPRODUCTION

The following is a general overview of breeding practices. Either a monogamous or a polygamous breeding system may be followed. With a monogamous mating system, one male and one female are paired, and the offspring are removed when they reach weaning age. A polygamous mating system typically entails the housing of one male and two or more females together. Pregnant females are removed a few days prior to parturition to minimize cannibalism and abandonment of litters. They are returned to the breeding cage after their litters are weaned.

The timing of puberty varies depending on sex, stock, or strain. Rats commonly reach puberty between 2 and 3 months, but they are normally not bred until about 3 months of age. They are continuously polyestrous, spontaneous ovulators, and have an estrous cycle length of 4–5 days. Vaginal plugs, formed by coagulated seminal vesicle and coagulating gland secretions, may persist in the vagina for 12–24 hours after coitus and are useful to confirm matings. Conception rates of 85% and higher are commonly observed in outbred stocks, slightly less in inbred strains.

Pheromones appear to play a less important role in the reproductive behavior of rats compared with mice. The pheromone-mediated Bruce effect described in mice does not occur in rats. Synchronization of estrus, or the Whitten effect, does occur in rats but is less pronounced than it is in the mouse.

The usual gestation period of a rat is 21–23 days but may be prolonged for 3–7 days in lactating rats because of delayed implantation. Developing fetuses are palpable between 10 and 12 days of gestation. Rarely, nonfertile matings will result in a pseudopregnancy duration of approximately 13 days. There is a fertile postpartum estrus; however, it is normally not used in breeding rats as the males may disturb the young. Females resume cycling 2–4 days postweaning.

The average litter size is between 6 and 12 pups but varies with strain and age of rat. It may be preferable to isolate the female for a few days before and after parturition as disturbances may cause the female to cannibalize her pups. Rat pups are altricial (hairless, blind, deaf, and completely dependent on the dam) at birth. They are fully haired at 7–10 days, their ears open between 2.5 and 3.5 days, and eyes open between 14 and 17 days. Incisors erupt at 6–8 days, and pups start eating solid food at about 2 weeks of age. The usual weaning age of rats is 20–21 days.

HUSBANDRY

Housing and Environment

Rat cages should provide an average adult rat (300–400 g) with at least 258 cm^2 (40 $in.^2$) of floor space and a height of at least 18 cm (7 in.). Rats are most commonly housed in solid-bottom shoebox-type cages constructed of a durable plastic (usually polycarbonate) with bedding (Box 5.2). In special circumstances such as toxicology studies, stainless steel cages with wire-mesh bottoms may be used. Studies have shown that rats prefer solid-bottom cages with bedding to wire-mesh flooring. Housing on wire is not recommended, especially

if rats will be maintained beyond one year, as this flooring increases the likelihood of foot injuries. Shoebox cages are covered with a special plastic cagetop with filter to form a microisolation (MI) cage. The cagetops reduce infectious disease transmission and prevent allergenic particulates from becoming airborne. The value of MI housing is offset to some extent by the high ammonia concentrations and increased temperature and humidity that may build up in the interior of the cage because of decreased airflow. Ventilated caging systems are being used with increased frequency to provide the greatest level of protection and a better microenvironment compared with static MIs.

Box 5.2

Rats are most commonly housed in solid-bottom shoebox-type cages constructed of a durable plastic with bedding.

A contact bedding material such as corncob particles, composite recycled paper pellets, or select hardwood chips should be placed in the bottom of solid shoebox cages. Cedar wood chips or shavings should never be used due to the adverse physiologic effects (e.g., increased tumor incidence, liver disease) induced by volatile compounds emitted from this type of wood. Breeding animals and pups are best housed in solid-bottom cages containing suitable bedding material and should not be housed in wire-bottom cages. Nesting material, facial tissue, or soft paper towels may be provided to pregnant dams although any item placed in a cage should first be evaluated to ensure it will not introduce biologic or chemical contaminants into the cage.

The room temperature should be maintained within a range of 20°–26°C (68°–79°F) and the humidity should be between 30% and 70%. Ten to fifteen air changes per hour are suggested to maintain adequate ventilation. This, however, is only a guideline, and determination of optimal ventilation should include careful consideration of the amount of heat, humidity, and volatile compounds expected to be produced within the housing room that will be influenced by the number of animals present, the type of bedding and frequency of cage-changing, room size, and the ease with which air will circulate between the room and animal cages. Rats are nocturnal and must be provided with regular light–dark cycles for normal behavior and physiologic processes. In the laboratory, a timing device should be used to ensure provision of 12–14 hours of light per day. If regular light cycles are not maintained, the circadian rhythm is disrupted, resulting in inconsistent responses to experimental manipulation. Albino strains of rats should be housed under reduced lighting intensity as they are prone to retinal dysplasia.

Environmental Enrichment and Social Housing

Over its successive editions, the *Guide for the Care and Use of Laboratory Animals* (the *Guide*; ILAR, 2011) has placed an increased emphasis on the appropriate provision of environmental enrichment and use of social housing to help meet the psychological and social needs of all species. The *Guide* states that social animals should be housed in physical contact with conspecifics when it is appropriate and compatible with the protocol. Rats are social

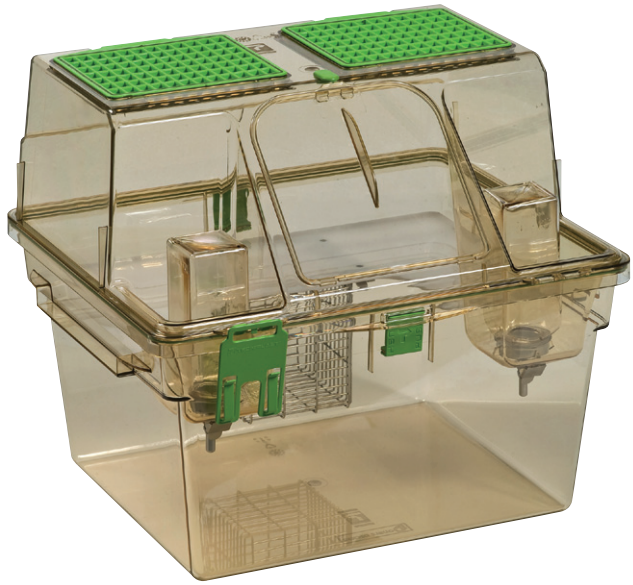

Fig 5.3. Double-level enrichment cage. (Photo courtesy of Tecniplast.)

animals and do best when housed in pairs or small, compatible groups. The provision of enrichment items may benefit rats; objects should be nontoxic, nondigestible, sanitizable, and safe. The type of enrichment items most frequently provided to rats are those that the animal can manually manipulate or gnaw, such as tissues, shredded papers, or Nylabones. Additional items that provide increased cage complexity include elevated shelves to increase usable floor space, resting platforms, tunnels, and huts, and are commercially available for laboratory animal use. Figure 5.3 depicts a double-level enrichment cage. Studies have shown that cage complexities have high enrichment value. However, controversy exists regarding which enrichment items provide the greatest benefit and least risk to the animals. This is largely complicated by the significant difficulty of interpreting an animal's response to or desire for an object. It must be noted that rats may interpret an object in a very different way than a human may predict. The use of all enrichment items must be critically evaluated before being routinely provided to a colony. In addition, scientific studies have shown that the physical and mental development of rats can be directly affected by social housing conditions (e.g., singly or group housed) and the absence or provision of enrichment items within their environment. Both consistency and caution must be exercised in the use of environmental enrichment with study animals where the changes may impact research results. For these reasons, formal approval should be secured from the principal investigator, the IACUC, and the facility veterinarian prior to the introduction of any enrichment items.

Feeding and Watering

Rats maintained on a standard commercial rodent diet that contains at least 20%–25% protein and 4% fat grow and reproduce well. Rodent chow is usually provided in the form of firm dry blocks or pellets. Rats eat an average of 5–6 g/100 g of body weight per day. They

tend to become obese if fed *ad libitum* (free choice); restricted feeding is recommended for animals used in long-term studies. Restriction of caloric protein and fat, as well as feeding soy protein rather than casein (present in high levels in dairy products), extends the life span of rats. Food pellets can be placed either on top of the cage lid or in a hopper attached to the cage wall. Food should not be placed directly on the cage floor unless the animals are debilitated or too young to reach the hopper. Supplemental feeding is not necessary or indicated for research animals except in a limited amount as an approved form of enrichment or prescribed supportive care. Like most other rodents, rats are coprophagic, consuming their own feces to conserve important nutrients. Fresh, potable water should be made available *ad libitum*, either from a bottle or an automatic watering system. Adult rats drink an average of 10–12 mL/100 g of body weight of water per day.

Sanitation

Caging should be cleaned as frequently as necessary to limit accumulated feces, moisture, and ammonia levels. The required frequency can vary (most commonly once or twice weekly for group-housed rats in a static MI cage) and is heavily influenced by the number and size of rats, the type of caging (static versus ventilated), and study requirements (e.g., select surgical models, conditions inducing polyuria). As stated in the *Guide*, cages, water bottles, and feed hoppers should be disinfected with chemicals, hot water 61.6°–82.2°C (143°–180°F), or a combination of both. Detergents and chemical disinfectants enhance the effectiveness of hot water, but they must be thoroughly rinsed from all caging surfaces with fresh water. Refer to Chapter 4 for a more detailed description of disinfection methods.

TECHNIQUES

Handling and Restraint

If young rats are handled gently and frequently, they become quite docile. Older rats that have not been handled often may be nervous, or "flighty." Transferring rats from one cage to another is easily accomplished by picking them up by the base of the tail. Rats should never be picked up by the middle or tip of the tail because the skin may be stripped off, creating a painful degloving injury. For routine handling, place a hand under the body and cradle the rat in the crook of the handler's elbow. By placing the opposite hand to cover or snug the animal's body, the rat's preference for physical contact is accommodated.

Rats can be restrained by a number of methods. One method includes placing the index and middle finger behind each ear as shown in Figure 5.4. The ring finger and thumb circle the thorax caudal to the front limbs, and a second hand supports the rat's body weight. Another method involves holding the rat with one hand by the base of the tail and placing the other hand firmly over the back and rib cage, with the thumb and forefinger placed directly behind the rat's elbows to push the legs forward so that they cross under the chin of the rat. Care must be taken to avoid placing too much pressure on the thorax as doing so will impede respiration and could lead to death.

Docile rats can be safely handled while wearing only exam gloves. Properly trained personnel eliminate the need for protective gloves that are difficult to sanitize, often awkward, and can make rats more fearful and difficult to handle. A hand towel can be wrapped

Fig 5.4. Restraint of the rat.

around the body of the rat to facilitate restraint when administering compounds or other procedures.

Clear plastic restraint bags are commercially available or may be fashioned from a durable freezer bag. The bags should form the shape of a cone with an opening at the tapered end. Restraint bags are especially helpful for individuals who are working alone with large or difficult rats. The bag is held open in one hand with the tapered end pointing down. The rat is picked up by the base of the tail and quickly lowered, head first, into the bag, so the nose is at the opening. The rear of the bag is then gathered together, totally enclosing the rat. Injections can be given directly through the bag. Animals should be restrained only for short periods in a plastic bag because they can quickly overheat. Rigid plastic restrainers that have openings to permit injections, blood collection, and other manipulations are also commercially available.

Identification

Cage cards are most often used as a form of general identification. There are several permanent methods to identify rats individually, including ear tagging, ear or tail tattooing, subcutaneous (SC) placement of a microchip for quick electronic identification, and ear punching. Dyes, permanent markers, or clipped fur can be used as temporary identification methods.

Blood Collection

Table 5.2 lists approximate adult rat blood volumes. Assuming the animal is mature, healthy, and on an adequate plane of nutrition, the blood volume of most species averages approximately 7% of the body weight in grams. A typical rule of thumb for blood withdrawal suggests that up to 10% of the circulating blood volume (or 1% of the body weight) can be withdrawn from normal, healthy animals every 2–3 weeks with minimal adverse effect.

The lateral tail veins are good sites for collecting small to moderate amounts of blood. A handler may manually restrain the rat or a clear, plastic restrainer (rigid or bag) that allows the tail to hang through the back works well. Dipping the tail in warm water (40°C) for approximately 10 seconds allows vasodilation of the tail veins. An alarming thermometer should be used to ensure that water bath temperatures do not exceed 40°C because hotter

Table 5.2. Adult rat blood volumes and suggested sample size

	Volume (mL)
Total blood	20–40
Single sample	2–3
Exsanguination	8–12

Source: Adapted from Harkness et al. (2010).
Note: Values are approximate.

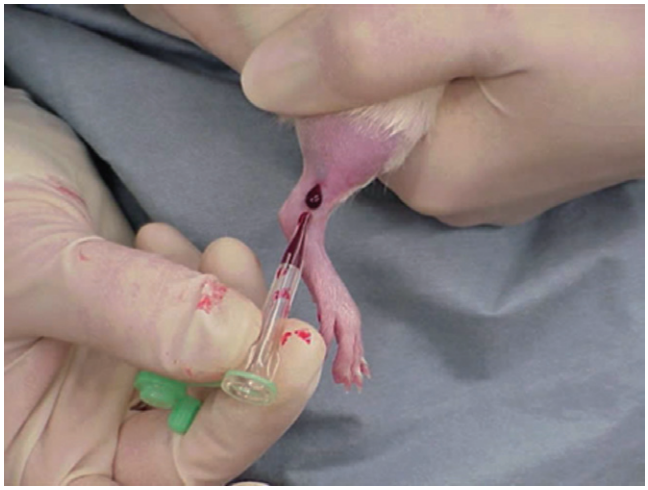

Fig 5.5. Blood collection from the saphenous vein. (Photo courtesy of ULAM, University of Michigan.)

temperatures can scald the skin of the tail. A 23-gauge butterfly needle with the tubing cut off can be used to enter the vein and allow the blood to drip into a collection tube. Hemostasis is achieved by applying direct pressure to the site. A needle and syringe can also be used to collect blood from the tail veins. Alternatively, capillary tube collection from the hub of a needle placed in the lateral tail veins is an effective method. Multiple successive blood samples can be taken by entering the same site or moving toward the tail base. The ventral tail artery can also be used to obtain a small amount of blood.

Up to approximately 1 mL of blood can be collected from the lateral saphenous vein (Figure 5.5). For this commonly used technique, the cranial portion of the conscious rat is restrained. Firm but gentle manual pressure is applied slightly proximal and lateral to the knee to extend the leg and dilate the vessel. Fur over the caudolateral aspect of and slightly proximal to the hock joint is removed by shaving or with use of a depilatory agent. A sterile ointment is applied to the skin to prevent blood from dispersing over the skin surface and seeping into the adjacent fur. A 20-gauge needle is used to puncture the vessel and the blood is collected. Hemostasis is achieved by applying pressure to the collection site. Smaller volumes of blood can be collected from the dorsal pedal vein.

Larger volumes of blood, up to 1 mL, may be obtained from the jugular veins of a conscious or (preferably) anesthetized rat. Special restraining boards are available to restrain a rat in dorsal recumbency and expose the neck for jugular access. In a conscious rat, two people are required for collection: one to restrain the rat and turn its head to the side and the second to collect the sample. The cranial vena cava can also be used to collect larger samples from an anesthetized rat. In this technique, the needle is directed through the thoracic inlet on the right side of the neck and slowly advanced caudally and medially toward the rat's opposite elbow. Slight negative pressure is maintained on the syringe so that when a flash of blood is seen in the needle hub, advancement ceases and the sample is collected. These procedures must be performed with a steady hand because unintended movement of the needle may lacerate the vessels or thoracic tissues and could cause death.

The retroorbital plexus is a site for collecting moderate amounts of blood in rats, although less invasive alternatives are available and may be preferred. This procedure requires anesthesia and the use of a microhematocrit tube to penetrate the venous plexus behind the eye. Refer to Chapter 4 (Figure 4.4) for a more detailed description of the technique. In contrast to the mouse technique, the tube enters slightly dorsal to the medial canthus rather than at the medial canthus. The retroorbital blood collection method should be avoided when possible, and especially when multiple samples are collected, due to the invasiveness of the technique, the potential for trauma to the eye and periorbital tissues, and the development of more refined techniques. If eye injury results, appropriate veterinary care and/or euthanasia must be provided.

Surgically placed jugular vein, femoral vein, or carotid artery catheters may be used for studies that require multiple blood collections. Catheterization is a refined blood collection method as it avoids the stress and discomfort caused by multiple needle sticks although it is both costly and time consuming.

Automated technology advancements continue to produce options that can be of great benefit to both personnel and animals in the research setting. Commercial, automated blood samplers are now available and offer several advantages over standard dosing and blood collection methods. Surgically catheterized rats are placed into a jacket that allows the catheter(s) to exit through a swivel port, and allows the rats to roam freely inside their cages while connected to the computerized unit. Using a series of peristaltic pumps, a computer program simultaneously schedules and controls dosing and blood sample collection for multiple rats. In addition, volumes withdrawn can be automatically replaced with fluids to help maintain physiologic stability. This technology not only reduces handling stress, it also makes research more efficient because it reduces personnel required for both dosing and blood collection. It should be noted, however, that catheters must be handled with strict aseptic technique, regularly flushed, filled with a catheter locking solution when not actively used, and inspected to maintain their patency throughout their anticipated functional time period (commonly 1–5 weeks).

A cardiac puncture can be performed on anesthetized rats as a terminal blood collection. The heart is approached from the abdomen just lateral to the xiphoid process with the needle directed at approximately a 30°–35° angle slightly to the left of midline in the rat's thorax. Alternatively, the cardiac puncture can be approached laterally, just caudal to the rat's elbow. A terminal sample can also be collected from the blood that pools after laceration of the axillary vessels.

Urine Collection

Specially designed metabolism cages facilitate collection of urine and feces over an extended period (hours to weeks). These cages are designed with feed and water containers positioned to prevent contamination of the urine and feces separating units located below the cage floor. Also, rodents frequently urinate and defecate when handled, enabling a small sample to be collected from the table.

Drug Administration

Medications are routinely added to the water or feed for ease of administering to large groups. This method relies on each animal eating its expected quantity of food each day, which may not occur if the food is unpalatable or unfamiliar to the animal, or if the animal's appetite is decreased. A gavage needle can be used for dosing individuals with liquid medication. These gavage needles, available in both metal and flexible plastic, have large, blunted ends (balls) on their tips to help minimize the risk of esophageal perforation or accidental entry into the trachea. Flexible plastic gavage needles may be considered a refinement to the metal type. Maximum dosing volumes for the various routes are listed in Table 5.3.

Smaller gauge needles, such as 23- or 25-gauge needles, should be used for injections. Subcutaneous (SC) injections can be given in the loose skin over the back. Rats have such a small muscle mass that intramuscular (IM) injections should be used only when absolutely necessary (Box 5.3). Irritation of the sciatic nerve from a misplaced injection or from certain drugs, such as ketamine, will often lead to self-mutilation of the limb. Intraperitoneal (IP) injections are preferred over IM injections and should be placed in a rat's lower right abdominal quadrant, just off the midline. This location is recommended to help decrease the likelihood of penetrating the liver, bladder, or cecum. The IP injection technique is described in more detail in Chapter 4. Video tutorials for rat injection techniques are available at www.procedureswithcare.org.uk/.

Table 5.3. Maximum dosing volumes in rats

Route	Amount (mL/100 g)
IP	2.0
SC	2.0
IM	0.1–0.3/site
IV	0.5
PO	2.0 (5 mL max)

Source: Adapted from Harkness and Wagner (1995) and Lukas (1999).

Box 5.3

Rats have such a small muscle mass that intramuscular injections should be used only when absolutely necessary.

Although continuous or repeated intravenous (IV) administration of drugs is not practical, it is often accomplished for research purposes by surgical placement of a vascular access port or an indwelling catheter in the jugular or femoral veins. The free end of the catheter is tunneled through subcutaneous tissues and exteriorized in the dorsal cervical or interscapular region. Alternatively, the tail vein can be used for intravenous administration in rats. Mini–osmotic pumps used in research are designed to deliver a constant infusion of drugs via the SC, IP, or IV route. Their function is based on differences in osmotic pressure between the compartment within the pump and the tissue space in which the pump is implanted. The pumps are aseptically prepared with the drug of choice and then surgically implanted. Drug delivery rates and durations can be selected from a wide range, for example, 0.11–10.0 µL/h and 1 day to 6 weeks, respectively. Pumps can be surgically accessed, refilled, and reimplanted in an individual animal. Use of mini–osmotic pumps is considered a refinement in dosing because it avoids the stress of daily handling; however, it does require surgical intervention.

Anesthesia, Surgery, and Postoperative Care

Agents commonly used for anesthesia and tranquilization of rats and recommended dosages are listed in Table 5.4. Preanesthetic fasting is not recommended in rodents unless an empty stomach is inherent to the success of the procedure. Injectable anesthetic regimens are used mainly for shorter procedures when administration of inhalant anesthesia is difficult due to equipment availability or is not compatible with research needs. A combination of ketamine and xylazine is frequently used to induce a surgical plane of anesthesia to facilitate relatively short-term procedures (e.g., less than 45 minutes). The appropriate dose of this combination may vary between ages and strains of rats. Once mixed, the drug combination will degrade over time and may lose its sterility; therefore, institutions routinely recommend that a new mixture be made at a predetermined interval (e.g., daily or weekly). The acid pH of ketamine has been associated with muscle damage and nerve irritation and should be given preferentially by the intraperitoneal route. A bland ophthalmic ointment should be placed in each eye of the animal to prevent corneal drying during the anesthetic period as the blinking reflex is reduced or absent. Pentobarbital was once the most frequently used injectable anesthetic, but it has been found to have a narrow safety margin and significant depressant effects on the cardiovascular systems causing hypothermia and prolonged anesthetic recovery times. Occasionally, it is still used when the newly obtained experimental results must be compared with historical results. It is safest to start with the lower end of the dose range when using this drug.

Inhalant anesthetics are safe and convenient for the induction and maintenance of brief periods of anesthesia in rats. With the use of appropriate anesthetic and monitoring equipment, inhalation anesthesia can be maintained successfully for prolonged periods (i.e., hours). Intubation and positive pressure ventilation systems are available for use in rats and other small rodents. Of the inhalant anesthetic agents, isoflurane and sevoflurane are preferable and must be used with a precision vaporizer. Ether use is strongly discouraged and disallowed at many institutions; it should only be permitted when absolutely necessary for the research study. Ether is flammable, forms explosive vapor mixtures with air or oxygen, and is an irritant to the respiratory tract. Volatile anesthetics should always be used with appropriate waste gas scavenging systems or in a fume hood.

Table 5.4. Anesthetic agents and tranquilizers used in rats

Drug	Dosage	Route	Reference
Inhalants			
Carbon dioxide	50%–80% CO_2 mixed with 20%–50% O_2	inhalation	Fenwick and Blackshaw (1989)
Isoflurane	1.5–4%	inhalation	Flecknell (2009)
Sevoflurane	3.5–8%	inhalation	Flecknell (2009)
Injectables			
Chloral hydrate (5% conc.)	300–450 mg/kg	IP	Silverman and Muir (1993)
Chloralose (alpha, 5% conc.)	31–65 mg/kg	IP	White and Field (1987)
Diazepam	2.5–5 mg/kg	IP	Flecknell (2009)
Fentanyl–droperidol	0.02–0.06 mL/100 g	IP	Wixson et al. (1987)
Fentanyl/fluanisone	0.3–0.6 mL/kg	IP	Redrobe (2002)
Fentanyl/fluanisone	0.4 mL/kg	IP	Redrobe (2002)
+ diazepam	2.5 mg/kg	IP	
Fentanyl	0.3 mg/kg	IP	Flecknell (2009)
+ medetomidine	0.3 mg/kg	IP	
Ketamine	75 mg/kg	IP	Flecknell (2009)
+ acepromazine	2.5 mg/kg	IP	
Ketamine	75 mg/kg	IP	Wellington et al. (2013)
+ dexmedetomidine	1 mg/kg	IP	
Ketamine	40–80 mg/kg	IP	Wixson et al. (1987)
+ diazepam	5–10 mg/kg	IP	
Ketamine	75 mg/kg	IP	Flecknell (2009)
+ medetomidine	0.5 mg/kg	IP	
Ketamine	75 mg/kg	SC	Harkness et al. (2010)
+ medetomidine (mixed)	0.5 mg/kg	SC	
Ketamine	75 mg/kg	IP	Flecknell (2009)
+ midazolam	5 mg/kg	IP	
Ketamine	40–80 mg/kg	IP	Wixson et al. (1987)
+ xylazine	5–10 mg/kg	IP	
Midazolam	5 mg/kg	IP	Flecknell (2009)
Pentobarbital	30–40 mg/kg	IP	Wixson et al. (1987)
Propofol	10–12 mg/kg	IV	Flecknell (2009)
Tiletamine–zolazepam	40 mg/kg	IP	Flecknell (2009)
Tiletamine–zolazepam	20–40 mg/kg	IP	Wilson et al. (1992)
+ butorphanol	1.25–5 mg/kg	IP	
Tiletamine–zolazepam	20–40 mg/kg	IP	Wilson et al. (1992)
+ xylazine	5–10 mg/kg	IP	
Tribromoethanol	300 mg/kg	IP	Flecknell (1987)
Tribromoethanol	150 mg/kg	IP	Gopalan et al. (2005)
+ medetomidine	0.5 mg/kg	IP	
Urethane	1000 mg/kg	IP	Flecknell (2009)

SC = subcutaneous; IP = intraperitoneal; IV = intravenous.

Common surgical procedures of rats include castrations and mammary tumor excisions. To prevent intestinal entrapment, care should be taken to close the open inguinal canals during castration. Mammary tumors are usually well encapsulated and easy to remove; however, they frequently reoccur. A number of other common surgical procedures are performed on research rats, including hypophysectomy, adrenalectomy, thymectomy, and jugular and femoral vein or carotid artery catheterization.

Surgeries should be performed in an aseptic manner. Although a misconception exists that rodents are inherently resistant to surgical infections, studies have shown that rats do develop significant physiological abnormalities when poor aseptic technique is employed. Care should be taken to avoid hypothermia, blood loss, and dehydration, which can lead to slow anesthetic recoveries and death (Box 5.4). Refer to Chapter 4 for more detailed intraoperative and postoperative management techniques.

Box 5.4

Care should be taken to avoid hypothermia, blood loss, and dehydration, which can lead to slow anesthetic recoveries and death.

Postanesthetic recovery includes placing the animal on a clean paper towel or dry cloth as an interface between the rat and small-particle bedding, which might be aspirated. A circulating-water heating blanket, self-regulating heating pad, or other method of providing heat should be used to promote euthermia. Use of drugstore-quality heating blankets must be avoided as they can cause thermal burns. Administering warmed saline or lactated Ringer's solution subcutaneously (up to 2 mL/100 g) to rats that have undergone a prolonged anesthetic period or surgical procedure helps to prevent hypothermia and dehydration and assists with recovery. To prevent hypostatic lung congestion, the rat should be turned approximately every 30 minutes until it is ambulatory. To avoid cannibalism, an anesthetized rat should not be placed back in a cage with conscious cagemates until it has recovered full ambulation. Placing several anesthetized animals in the same cage to recover does not usually lead to problems although they must still be monitored for appropriate recovery. Provision of moistened chow or critical care supplements can assist in the recovery phase. Select, commercially available supportive and critical care products are listed in Appendix 3.

Analgesics must be provided as a standard of care for alleviating pain associated with surgical or other painful procedures in rats. If a procedure is known to cause pain in humans, it must be assumed to also cause pain in rats unless proven otherwise (Box 5.5). Scientific justification must be provided and approved by the IACUC if there is a need to withhold analgesics for research animals. Research rodents present some unique challenges for pain control, including the need to dose many rodents at a time, the difficulty of assessing postoperative pain, and the concern about effects on research. Animal care and research staff have an ethical responsibility to continue to look for better ways to reduce pain and distress in their research subjects. Novel drug formulations, such as liposome-encapsulated opioids, are being developed to provide a one-dose, slow-release, long-acting analgesic for moderate to severe pain in rats. The use of such drugs could provide consistent and reliable pain relief

Box 5.5

If a procedure is known to cause pain in humans, it must be assumed to also cause pain in rats unless proven otherwise.

without the need for frequent and repeated animal handling and injection. Much effort has been devoted to incorporating analgesics in drinking water or food items (e.g., flavored gelatin) that a rat would freely consume. However, these methods have not yet been successful for multiple reasons, including an instinctual avoidance of novel food items (neophobia), poor palatability, and limited consumption of drug-supplemented food or water.

The provision of analgesics prior to pain induction (preemptive analgesia) affords better pain control than administration after the painful procedure and may decrease required anesthetic dosages. Butorphanol is recommended for mild postoperative discomfort. Buprenorphine causes more sedation but works well to control acute or chronic visceral pain. A sustained-release formulation of buprenorphine (Buprenex SR LAB) is available. Clark and colleagues (Clark et al., 1997) reported that buprenorphine induced pica (consumption of nonfood items such as fur or bedding) at higher dose ranges (0.05–0.3 mg/kg). Nonsteroidal anti-inflammatory agents such as carprofen and meloxicam can be used in combination with opioids or alone to control pain in rats. Both carprofen and meloxicam are good choices for mild to moderate musculoskeletal and visceral pain and have longer duration of action (24 hours) than the opioids. Meloxicam comes in an injectable and oral liquid form that is very palatable. Table 5.5 lists several analgesics that are effective in rats.

Imaging Techniques

Great advances have been made in rodent and small animal imaging over the last decade. Many imaging modalities once used only in humans [e.g., digital x-ray imaging, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), dual-energy x-ray absorptiometry (DEXA) scan, and ultrasound] have been adapted for use with these species, producing images of exquisitely fine detail. In addition, novel imaging techniques have been developed for use in animal research, including *in vivo* optical imaging. Some x-ray units (e.g., Faxitron animal imaging and irradiation systems) have been designed for use specifically with small rodents. These units produce a highly magnified and clear resolution image of the field of interest.

For conventional x-ray imaging, an x-ray machine with high milliamperage, such as a 200- or 300-mA unit with the capability for low settings of peak voltage delivered (kVp) and small incremental changes, is ideal. High-detail film–screen combinations work well. Short exposure times of 1/40 second or less should be used to decrease the chance of motion artifact. Suggested exposure factors are 42–46 kVp, 300 mA, 1/40 second, 40 SID, 7.5 mA s. Alternatively, nonscreen film can be used with standard x-ray or dental units. Nonscreen film produces high-detail radiographs, but longer exposure times are needed. Digital imaging techniques offer substantial flexibility in image enhancement and delivery application. The best method of restraint is to sedate the animal and use adhesive tape to secure it to the

Table 5.5. Analgesic agents used in rats

Drug	Dosage	Route	Reference
Acetaminophen	200 mg/kg	PO	Flecknell (2009)
Acetylsalicylic acid	100–150 mg/kg q4h	PO	Heard (1993)
Buprenorphine	0.01–0.05 mg/kg q8–12h	SC, IV	Flecknell (2009)
	0.1–0.25 mg/kg q8–12h	PO	Flecknell (2009)
Buprenorphine sustained release	1–1.2 mg/kg (one 72-hour injection)	SC	Foley et al. (2011)
Butorphanol	1–2 mg/kg q4h	SC	Flecknell (2009)
Carprofen	5 mg/kg	SC	Flecknell (2009)
Codeine	25–60 mg/kg q4h	SC	Jenkins (1987)
Diclofenac	10 mg/kg	PO	Flecknell (2009)
Flunixin meglumine	2.5 mg/kg q12–24h	SC	Heard (1993)
Ibuprofen	15 mg/kg	PO	Flecknell (2009)
Ketoprofen	5 mg/kg	SC	Flecknell (2009)
Meloxicam	1 mg/kg q24h	PO, SC	Harkness et al. (2010)
Meperidine	20 mg/kg q2–3h	SC	Heard (1993)
Syrup	0.2 mg/mL in drinking water	PO	Huerkamp (1995)
Morphine	2.5 mg/kg q2–4h	SC	Flecknell (2009)
Nalbuphine	4–8 mg/kg q3h	IM	Heard (1993)
Oxymorphone	0.2–0.5 mg/kg q6–12h	SC	Harkness et al. (2010)
Pentazocine	10 mg/kg q2–4h	SC	Heard (1993)
Piroxicam	3 mg/kg q24h	PO	Harkness et al. (2010)
Tramadol	5 mg/kg	SC, IP	Flecknell (2009)

IM = intramuscular; IP = intraperitoneal; PO = per os; SC = subcutaneous.

cassette in the appropriate position. A radiolucent tube or stockinette with the patient positioned inside can be used for restraint of conscious animals.

Euthanasia

The term euthanasia literally translates as "good" (*eu*) and "death" (*thanatos*). Euthanasia should provide a rapid death with little to no pain or distress. Individuals must be appropriately trained to use the various methods of euthanasia to minimize the chances of pain or distress if performed inappropriately. Methods must be consistent with the most current American Veterinary Medical Association (AVMA) *Guidelines for the Euthanasia of Animals* (AVMA, 2013), performed by trained personnel, detailed in the research protocol, and approved by the IACUC. Per AVMA Guidelines, "Carbon dioxide is acceptable with conditions for euthanasia in those species where aversion or distress can be minimized. Carbon dioxide exposure using a gradual fill method is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness; a displacement rate from 10% to 30% of the chamber volume/min is recommended. Whenever gradual displacement methods are used, CO_2 flow should be maintained for at least 1 minute after respiratory

arrest. If animals need to be combined, they should be of the same species and, if needed, restrained so that they will not hurt themselves or others. Oxygen administered together with CO_2 appears to provide little advantage and is not recommended for euthanasia. The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO_2, is unacceptable. Carbon dioxide and CO_2 gas mixtures must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate [author note: e.g., dry ice]. As gas displacement rate is critical to the humane application of CO_2, an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size of the container being utilized is absolutely necessary."

The euthanasia of rat feti and neonates requires special consideration. Neonates are resistant to hypoxia and require greater duration of exposure; for this reason, other methods may be preferred for this age group. Fetal rats (unborn animals who have not breathed) under 15 days of gestation may be euthanized by allowing them to remain *in utero* following routine euthanasia of the dam. Alternatively, euthanasia can be induced by removal of the fetus from the uterus. However, feti removed from the uterus and allowed to breathe air must be promptly euthanized by a physical method of euthanasia (e.g., cervical dislocation or decapitation). Neonatal rats under 10 days of age may show marked resistance to CO_2. CO_2 euthanasia may still be used with these animals although prolonged exposure time may be required. Alternate methods of euthanasia of neonatal rats include injection of anesthetics, decapitation, or cervical dislocation. Death must always be verified prior to carcass disposal.

Other acceptable methods of euthanasia include overdose of an inhalant anesthetic and IP administration of a barbiturate anesthetic at three to four times the anesthetic dose.

Many other euthanasia methods are acceptable when performed only on a fully anesthetized animal, including exsanguination and the use of potassium chloride (KCl) administered intravenously to induce cardiac arrest. Occasionally, it is necessary to euthanize animals without the use of anesthetics or tranquilizers (e.g., decapitation with a guillotine) that have been documented to complicate research results. Investigators must fully justify the need for using such a euthanasia method and the method must be approved by the IACUC.

THERAPEUTIC AGENTS

Suggested rat antimicrobial and antifungal drug dosages are listed in Table 5.6. Antiparasitic agents are listed in Table 5.7, and miscellaneous drugs are listed in Table 5.8.

INTRODUCTION TO DISEASES OF RATS

Most laboratory rodent colonies today are relatively free of the viruses, bacteria, parasites, and fungi that cause clinical disease. However, sporadic outbreaks of disease in the laboratory setting still likely occur partially because of the increase in transporting rodents both

Table 5.6. Antimicrobial and antifungal agents used in rats

Drug	Dosage	Route	Reference
Amikacin	10 mg/kg q12h	SC	Morrisey and Carpenter (2004)
Ampicillin	20–50 mg/kg q12h	PO, SC	Morrisey and Carpenter (2004)
Cephalexin	15 mg/kg q12h	SC	Flecknell (1996)
Cephaloridine	10–25 mg/kg q24h	SC	Anderson (1994)
Chloramphenicol	50–200 mg/kg q8h	PO	Burgmann and Percy (1993)
	30–50 mg/kg q12h	SC	Burgmann and Percy (1993)
Ciprofloxacin	7–20 mg/kg q12h	PO	Harkness et al. (2010)
Doxycycline	5 mg/kg q12h	PO	Harkness et al. (2010)
Enrofloxacin	0.05–0.2 mg/mL drinking water for 14 d		Harkness et al. (2010)
	5–10 mg/kg q12h	PO, SC	Harkness et al. (2010)
Erythromycin	20 mg/kg q12h	PO	Morrisey and Carpenter (2004)
Gentamicin	5–10 mg/kg divided q8–12h	SC	Morrisey and Carpenter (2004)
Griseofulvin	25–50 mg/kg q12h for 14–60 d	PO	Harkness et al. (2010)
	1.5% in DMSO for 5–7 d	Topical	Harkness et al. (2010)
Ketoconazole	10–40 mg/kg q24h for 14 d	PO	Harkness et al. (2010)
Metronidazole	10–40 mg/kg q24h	PO	Burgmann and Percy (1993)
Neomycin	2.6 mg/mL drinking water		Anderson (1994)
	25 mg/kg q12h	PO	Morrisey and Carpenter (2004)
Oxytetracycline	0.4 mg/mL drinking water		Burgmann and Percy (1993)
	10–20 mg/kg q8h	PO	Burgmann and Percy (1993)
Pencillin G	22,000 IU/kg q24h	SC	Morrisey and Carpenter (2004)
Sulfadimethoxine	10–15 mg/kg q12h	PO	Harkness et al. (2010)
Sulfamerazine	1 mg/mL drinking water		Anderson (1994)
Sulfamethazine	1 mg/mL drinking water		Anderson (1994)
Sulfaquinoxaline	0.25–1 mg/mL drinking water		Burgmann and Percy (1993)
Tetracycline	2–5 mg/mL drinking water		Burgmann and Percy (1993)
	10–20 mg/kg q8–12h	PO	Burgmann and Percy (1993)
Trimethoprim–sulfa	15–30 mg/kg q12h	PO, SC	Morrisey and Carpenter (2004)
Tylosin	10 mg/kg q24h	PO, SC	Harkness et al. (2010)
	0.5 mg/mL drinking water		Harkness et al. (2010)

DMSO = dimethyl sulfoxide; IM = intramuscular; PO = per os; SC = subcutaneous.

nationally and internationally. Laboratory personnel who own pet rodents may serve as a possible source of contamination of research animals although the associated degree of risk has not been fully quantified. Gaertner (2004) postulated three reasons infectious agents continue to occur in otherwise clean facilities: some agents are extremely contagious (mouse hepatitis virus, MHV), some are highly resistant to environmental inactivation (parvoviruses), and some are difficult to diagnose or eradicate (pinworms). Basic knowledge of the variety of infectious agents that may infect rats ensures a quick and appropriate response to

Table 5.7. Antiparasitic agents used in rats

Drug	Dosage	Route	Reference
Carbamate (5%)	Twice weekly	Topical	Harkness et al. (2010)
Doramectin	0.2 mg/kg for 5 d	PO	Oge et al. (2000)
Fenbendazole	20 mg/kg q24h for 5 d	PO	Allen et al. (1993)
	150 mg/kg of feed; provide for 7 d on, 7 d off for 3 treatments		Boivin et al. (1996)
Fipronil	7.5 mg/kg q30–60d	Topical	Morrisey and Carpenter (2004)
Ivermectin	0.2–0.4 mg/kg every 7–10 d for 3 treatments	PO, SC	Harkness et al. (2010)
	25 mg/L drinking water for 4 d on, 3 d off for 5 wk		Klement et al. (1996)
Mebendazole	40 mg/kg q7d for 21 d	PO	Adamcak (2000)
Metronidazole	10–40 mg/animal	PO	Morrisey and Carpenter (2004)
	2.5 mg/mL drinking water for 5 d		Adamcak (2000)
Moxidectin	0.2 mg/kg for 4 d	PO	Oge et al. (2000)
Permethrin	0.25% dust in cage		Bauck et al. (1995)
	Cotton ball soaked in 5% solution placed in cage 4–5 wk		Bauck et al. (1995)
Piperazine citrate	2–5 mg/mL drinking water for 7 d, off 7 d, repeat		Anderson (1994)
Praziquantel	6–10 mg/kg	PO	Harkness et al. (2010)
Pyrethrin powder	3 times a week for 3 wk	Topical	Anderson (1994)
Thiabendazole	100–200 mg/kg	PO	Harkness et al. (2010)

PO = per os; SC = subcutaneous.

a disease outbreak if it does occur. Presented here is a general overview of common bacterial, viral, fungal, and parasitic agents of rats and some standard diagnostic and treatment modalities.

Bacterial Diseases

Pneumonia and Respiratory Diseases

Mycoplasma pulmonis is uncommon in barrier-maintained laboratory rats but remains an important pathogen for conventional colonies and pet rats (which may function as a nidus of infection). It causes a chronic respiratory disease syndrome called murine respiratory mycoplasmosis (MRM). It has also been associated with infections of the genital tract. Although *M. pulmonis* is the etiologic agent of MRM, other viruses (e.g., Sendai, sialodacryoadenitis) and bacteria [e.g., cilia-associated respiratory (CAR) bacillus, *Streptococcus pneumoniae*, *Bordetella bronchiseptica*] are often recovered from the lungs of infected animals.

Mycoplasma pulmonis can be transmitted by direct contact between an infected dam and her offspring, by intrauterine or sexual transfer, and by aerosol. The organism has an affinity for the epithelial cells of the respiratory tract, middle ear, and endometrium. The disease is

Table 5.8. Miscellaneous agents used in rats

Drug	Dosage	Route	Reference
Atipamezole	1 mg/kg	SC, IP, IV	Flecknell (1997)
Atropine	0.05–0.1 mg/kg	SC	Harkness and Wagner (1995)
	10 mg/kg q20 min (organophosphate toxicity)	SC	Harkness and Wagner (1995)
Cimetidine	5–10 mg/kg q6–12h	PO	Allen et al. (1993)
Dexamethasone	0.5–2 mg/kg then decreasing dose q12h for 3–14 d	PO, SC	Harkness and Wagner (1995)
Diphenhydramine	1–2 mg/kg q12h	PO, SC	Morrisey and Carpenter (2004)
Doxapram	5–10 mg/kg	IP, IV	Morrisey and Carpenter (2011)
Furosemide	2–10 mg/kg q12h	SC, PO	Morrisey and Carpenter (2004)
Glycopyrrolate	0.01–0.02 mg/kg	SC	Huerkamp (1995)
	0.5 mg/kg	IM	Flecknell (2009)
Naloxone	0.01–0.1 mg/kg	SC, IP	Huerkamp (1995)
Oxytocin	0.2–3 IU/kg	SC, IV	Anderson (1994)
Prednisone	0.5–2.2 mg/kg	SC, PO	Anderson (1994)
Sucralfate	25–50 mg/kg	PO	Morrisey and Carpenter (2004)
Vitamin B complex	0.02–0.2 mL/kg	SC	Anderson (1994)
Vitamin K	1–10 mg/kg q24h for 4–6 d	IM	Harkness and Wagner (1995)
Yohimbine	0.5–1 mg/kg	IV	Harkness and Wagner (1995)

IM = intramuscular; IP = intraperitoneal; IV = intravenous; PO = per os; SC = subcutaneous.

usually subclinical and slowly progressive, with clinical signs becoming evident only when an advanced stage of disease is reached. Factors such as high ammonia levels, other respiratory pathogens, and various forms of stress have a profound effect on the clinical course and severity of the disease.

A serous or catarrhal nasal and ocular discharge may occur, but the sound of snuffling is usually more obvious than other early clinical signs. Animals with extensive pulmonary involvement may exhibit labored breathing, weight loss, lethargy, a hunched posture, and a rough hair coat. Acute deaths are usually the result of secondary bacterial infections. Unilateral or bilateral otitis interna, characterized by the presence of a head tilt, is part of the chronic respiratory disease syndrome. The infection frequently extends up the eustachian tube into the middle ear and then to the inner ear, causing a labyrinthitis. When rats with labyrinthitis are held by the tail in a vertical position, they typically spin, rotating their bodies rapidly.

In the earliest stage, pulmonary lesions are limited to peribronchiolar lymphoid hyperplasia, visible only on microscopic examination of the lung. As the disease progresses, well-demarcated foci of firm, red–gray consolidation appear. Accumulation of inflammatory debris and mucus may result in bronchial distention, which can give the surface of the lung a "cobblestone" appearance. Different strains of rats have different susceptibilities to infection with *M. pulmonis*. Genital disease may be characterized by purulent endometritis, salpingitis, and perioophoritis. Additionally, pregnant rats infected with *M. pulmonis* have

had a variety of adverse outcomes, including low birth weight, small litter size, stillbirths, and fetal resorption.

Diagnosis is based on history, clinical findings, gross and microscopic lesions, and isolation of *M. pulmonis* from the nasopharynx, tympanic bullae, trachea, or lungs. A serum enzyme-linked immunosorbent assay (ELISA) is the most rapid tool for screening colonies for the presence of *M. pulmonis* infection although additional tests are often required to differentiate *M. pulmonis* from the nonpathogenic *M. arthritidis*. A polymerase chain reaction (PCR) test offers a rapid way to screen cell lines, biological agents, and tissues of animals and to confirm the diagnosis of animals detected via serologic screening.

Bacterial culture and sensitivity testing are necessary to determine the most appropriate choice of antibiotics. Antimicrobials added to the drinking water for periods of a week or more will suppress *M. pulmonis* infection but will not eliminate the disease. Oxytetracycline, tetracycline, or doxycycline in the drinking water are commonly used. For treatment of individual animals, enrofloxacin, chloramphenicol, or tylosin may be injected SC for 5 days or more. Labyrinthitis does not usually respond to treatment, but animals with pronounced head tilt will often survive for months. Elimination of this disease requires cesarean rederivation of breeding stock and maintenance of animals under a barrier system. Areas of research most affected by MRM include respiratory, reproductive, and immune system studies.

Streptococcus pneumoniae, once a common cause of respiratory disease in young rats, today is rarely recognized in well-managed facilities. Many rats harbor the organism in the upper respiratory tract without clinical signs, and disease outbreaks can occur following stress. The disease tends to be acute to subacute and primarily affects younger animals. Affected animals exhibit depression, a serosanguinous to mucopurulent nasal discharge, a snuffling respiratory sound, dyspnea, an ocular discharge, and ruffled fur.

Gross lesions often found at necropsy include seropurulent and fibrinopurulent pleuritis, pericarditis, epicarditis, peritonitis, meningitis, otitis media and interna, metritis, and bronchopneumonia. It is not uncommon to have fibrinopurulent peritonitis and pleuritis with minimal involvement of lung parenchyma.

Penicillin administered SC or oxytetracycline in the drinking water are among the more frequently used antibiotics. Many others, including ampicillin, chloramphenicol, and gentamicin, are also generally beneficial.

Naturally occurring infections of the cilia-associated respiratory (CAR) bacillus have been reported in rats, mice, rabbits, cattle, swine, and other species. In most species, it appears to be an opportunistic invader of the respiratory tract. Infection in rats is usually asymptomatic, but infection is lifelong. Transmission is by direct contact; screening of sentinel rats exposed to bedding may miss infections.

The CAR bacillus is found between and parallel to the cilia of the respiratory epithelium. It may be detected histologically by using a modified Steiner silver stain of a scraping from the respiratory tract or a section of lung. An ELISA test can be used to quickly screen colonies. PCR can also be used to confirm the presence of CAR bacillus in a colony. No effective treatment has been described, and elimination or rederivation may be necessary to rid a colony of the organism.

Pasteurella pneumotropica is a Gram-negative coccobacillus that is generally latent in rats, but it is considered an opportunistic pathogen secondary to other agents such as Sendai virus and *M. pulmonis*. It is most often associated with the nasopharynx, cecum, vagina, uterus,

and conjunctiva. Transmission is by direct contact. Most animals are asymptomatic, although infections of the conjunctiva, endometrium, or mammary gland have rarely been reported. Enrofloxacin may be beneficial in controlling clinical manifestations of infection but will not eliminate the carrier state. Cesarean rederivation or embryo transfer may also eliminate the infection.

Tyzzer's Disease

Clostridium piliforme is the causative agent of Tyzzer's disease. Infected adults are usually subclinically affected while overt disease is most likely to occur in weanlings. Transmission is by fecal–oral spread of spores, which are highly resistant in the environment. Disease occasionally manifests as an acute epizootic when stressors such as poor environmental conditions or concurrent infections lead to immunosuppression. Clinical signs may include diarrhea, dehydration, and anorexia. Animals may be found dead in the absence of obvious signs of illness. Gross lesions include miliary, pale foci throughout the liver. Megaloileitis (enlarged and reddened lower intestinal tract) may be present. The heart may also be affected with pale circumscribed areas or streaks. Differential diagnosis includes other bacterial septicemias, *Corynebacterium kutscheri*, and rat virus (RV) infections. A definitive diagnosis may be difficult to establish and requires identification of the filamentous bacilli in hepatocytes, intestinal epithelium, or myocytes with silver, Giemsa, or methylene blue stain. Due to the organism's complexity, serology screens may yield false positives and require further investigations with alternate diagnostic methods. Oxytetracycline and tetracycline have been used to suppress epizootic episodes.

Staphylococcus

Staphylococcus aureus causes a variety of lesions in rats, including ulcerative dermatitis and pododermatitis. Trimming the toenails is beneficial in eliminating the self-mutilation from scratching often associated with ulcerative dermatitis. Good sanitation procedures and, in some cases, parenteral or topical antibiotics should be used to treat staphylococcal infections. Treatment with topical antibiotics is often impractical, as rats will frequently lick the medication from their skin.

Miscellaneous Bacterial Infections

Corynebacterium kutscheri, a Gram-positive rod, usually exists as a latent or subclinical infection but can persist and cause disease in older animals. The organism may be cultured from the submandibular lymph nodes, oropharynx, nasopharynx, middle ears, and preputial glands. Stressful situations often elicit a subacute respiratory disease with abscesses in the lungs. Abscesses may also be found in the liver, kidney, skin, and joints. In chronic cases, the abscess contents in the lungs become caseous, hence the term commonly used for this infection, pseudotuberculosis. Transmission appears to be by direct contact. A differential diagnosis includes streptococcosis, MRM, and CAR bacillus infections. Diagnosis is by culture of the organism (preferably from the submandibular lymph nodes) or through serology screens. Positive ELISA tests should always be confirmed by culture. The organism is sensitive to a wide variety of antibiotics, including ampicillin, chloramphenicol, and tetracycline. If the infection exists in an acute form, the rapid course may render treatment of individual animals ineffective.

Pseudomonas aeruginosa, a Gram-negative bacillus, is usually not pathogenic in immunologically competent animals as it is a common commensal organism of rodents. Animals may, however, become septicemic following experimental manipulations that compromise their immune system, such as radiation exposure. Often drinking water is chlorinated (10–13 ppm) or acidified (2.5–2.8 pH) to reduce introduction of the organism through the water supply. To remove this organism from a colony, the animals must be rederived through cesarean section or embryo transfer.

Salmonella spp. infections are uncommon in well-managed animal facilities. With the possibility of introduction by wild rodents, it should remain on the differential list for diseases of unknown etiology. Infections may exist in a subclinical form or cause acute disease with a high mortality. Salmonellosis is a concern because of its zoonotic disease potential and interspecies transmission. Rats can be asymptomatic carriers and shed the organism in the feces for many months.

Streptobacillus moniliformis is considered a commensal of low pathogenicity for rats but can produce disease in mice, guinea pigs, and humans. The organism is normally found in the nasopharynx of asymptomatic rats and is the etiologic agent for the zoonotic disease called rat-bite fever.

Mycotic Diseases

Pneumocystis Carinii

Pneumocystis carinii is a single-celled, airborne fungal pathogen that is one of the most common diseases in laboratory rats. It causes chronic progressive pneumonia in immunodeficient rats. Recently, it has been found to cause infectious interstitial pneumonia in immunocompetent rats, a condition previously and erroneously attributed to rat respiratory virus. *P. carinii* induces pneumonia of varying severity depending upon the immune status of the animal; lesions are similar but milder in immunocompetent rats. This organism should be excluded from rat colonies used in respiratory research.

Diagnosis of diseased immunodeficient animals is usually obtained through necropsy and histologic exam of lung tissue using silver stain to demonstrate the presence of the organism. Immunocompetent animals are routinely screened by serology or PCR. Trimethoprim–sulfa has been used to suppress disease severity in affected immunocompromised rats. No treatment has been reported to completely eliminate the organism from infected animals. Infected animals should be rederived through embryo transfer or hysterectomy.

Viral Diseases

Sialodacryoadenitis Virus and Rat Coronavirus

A number of coronaviruses have been isolated from rats, but the two prototype viruses are rat coronavirus (RCV) and sialodacryoadenitis virus (SDAV). Although the two viruses were once thought to cause distinctly different diseases, clinical signs or pathology cannot differentiate infection with either virus. These viruses are common in both the pet and conventionally housed rat populations but are uncommon in barrier-maintained colonies. The coronaviruses of rats are highly contagious and cause inflammation of the salivary and lacrimal glands. Transmission is by direct contact, aerosol, fomites, or administration of contaminated biologics. Rat coronaviruses may induce asymptomatic infections. Alternatively, infections may lead to transient clinical disease. Overt disease occurs either as an endemic

infection of breeding colonies or as an explosive outbreak among nonimmune young rats. Tissue tropisms include salivary glands, lacrimal glands including the Harderian gland, and respiratory epithelium. Infections may exacerbate respiratory disease attributable to *M. pulmonis*.

The usual signs of infection are eye squinting, swelling of the ventral cervical region and jaw, and protrusion of the eye. Generally, the swelling subsides in 10–14 days and the rat returns to normal. Keratoconjunctivitis is the only clinical sign of infection in some outbreaks. Affected rats usually remain active and continue to eat. Viral shedding lasts about one week, and then animals become immune and are not persistently infected. Although coronaviruses may spread rapidly in a susceptible colony, mortality is usually very low. Treatment is not indicated unless protrusion of the eyeball leads to corneal ulceration or keratitis, for which a topical ophthalmic preparation is indicated. Rats that are reinfected may show no clinical signs yet shed the virus for several weeks. Infected animals can be detected through serologic testing for the presence of antibodies and PCR of tissues from acutely infected rats. Control of infection may entail colony depopulation or rederivation, especially with immunocompromised animals. Alternatively, the "burnout" technique can be used with fully immunocompetent rat colonies whereby breeding and the introduction to the colony of newly susceptible animals is halted for 6–8 weeks. Current colony animals are allowed to contract and then recover from the virus. Research effects are limited to tissues affected during the active clinical phase as well as an animal's general compromised health during this time.

Parvoviruses

Parvoviruses are small, unenveloped DNA viruses that are resistant to heat, lipid solvents, and acidic environments. There are several parvoviruses that naturally infect rats, including the prototypic (Kilham's) rat virus (RV), the rat parvovirus (RPV), rat minute virus (RMV), and (Toolan's) H-1 virus. These viruses are among the most prevalent infectious agents found in contemporary laboratory rat colonies. Parvoviruses have predilection for actively dividing cells; thus neonates are typically more susceptible than adults to active disease. RV infections are sporadic and usually produce subclinical signs. Young adult rats may have clinical signs, including scrotal cyanosis, dehydration, and abdominal swelling. Pregnant rats with RV may have reduced litter size, runted litters, and neonatal death. Clinical disease is not normally observed with RPV, RMV, or H-1 infections in rats. Transmission is by aerosol, through direct contact, fomites, transplacental, and administration of infected biologics. Some infections are persistent for prolonged periods, but persistence is viral-strain dependent. Infection of athymic rats produces a more severe and persistent infection than in immunocompetent rats. Parvoviruses have been associated with suppression of the immune system. There are differences in strain sensitivities to these infections.

Polymerase chain reaction, ELISA, immunofluorescent assays (IFA), and other serologic methods can be used to diagnose parvovirus. PCR can also be used to screen infected tissues such as mesenteric lymph nodes or the spleen. The exact impact of parvoviruses on research is not fully known, but they are generally unwelcome because of their known effects on the immune system, tumor development, fetal development, and the actively dividing cells of the hematopoietic, neurologic, and gastrointestinal systems. Control of infection generally includes depopulation of infected rats and/or cesarean rederivation or embryo transfer for

valuable rats. Parvoviruses can persist in the environment for months to years. Environmental decontamination is an important step, and the use of a chemical disinfectant such as chlorine dioxide or hydrogen peroxide that can be vaporized to reach all surfaces should be considered.

Hantaviruses

Several hantaviruses have been recognized in wild rats, including the Hantaan virus and the Four Corners virus. Wild rodents serve as the primary reservoir hosts for hantaviruses but show no clinical disease. Most major wild rat populations in the United States are infected with hantaviruses.

The virus is shed in the saliva, urine, and feces of infected rodents for an unknown duration and may, in fact, be persistently shed. Transmission may occur through bites, aerosols, direct contact with contaminated fomites, or ingestion of contaminated food and water. One of the hantaviruses that occurs in humans is hemorrhagic fever with renal syndrome (HFRS). Clinical signs include high fever, malaise, myalgia, headaches, diarrhea, vomiting, proteinuria, oliguria, hemorrhaging, and death. Another syndrome in humans is hantavirus pulmonary syndrome. Transmission of hantavirus from laboratory rats to people has been reported in Japan, Belgium, and the United Kingdom. Rats caught in the wild and used in research should be carefully screened for hantaviruses. Wild rodents should be prevented from colonizing in populated areas.

Infectious Diarrhea of Infant Rats

Infectious diarrhea of infant rats (IDIR), also known as rat rotavirus, is a Group B rotavirus that causes disease in suckling rats. It usually occurs at less than 2 weeks of age and is characterized by the presence of soft yellow feces or an accumulation of dried feces or hemorrhage around the anus. Affected rats usually continue to nurse, as evidenced by the milk spot, but their growth is often stunted. Pathology findings include small intestinal villous atrophy and syncytial cells. The virus can persist for long periods in the environment and is resistant to some disinfectants. Group B rotaviruses can also cause diarrheal disease in humans. Although most Group B rotaviruses are believed to be species-specific, human isolates have been shown to induce diarrhea in infant rats and an identical or very similar virus to IDIR has been associated with a small-scale human outbreak of diarrhea. Thus, the potential for cross-infectivity exists. No treatment exists other than supportive care. Methods to eliminate the virus from rat colonies include depopulation or rederivation.

Miscellaneous Viral Infections

A number of viruses, including cytomegalovirus, adenovirus, pneumonia virus of mice, and Sendai virus, produce naturally occurring infections in rats, but infections are usually subclinical. Latent or indigenous viruses that may be activated by stress or may alter the response of animals to experimental procedures are nevertheless of concern in the research laboratory. Emphasis must be placed on quarantine and serologic screening of incoming rats of unknown origin. Additionally, rat antibody production (RAP) testing or PCR screening of transplantable tumors, cell lines, and other biological materials is an important aspect of preventing viral contaminants from entering a research colony.

Parasitic Diseases

Mites

Several mites can cause disease in rats. *Radfordia ensifera*, the fur mite of rats, can cause pruritus, hair loss, and debilitation with heavy infestations. *Notoedres muris*, a rare mange mite that burrows into the deeper layers of the epidermis, can produce red papules or reddened and thickened skin. *Ornithonyssus bacoti*, the tropical rat mite, is a bloodsucking mite that can cause pruritus and skin irritation. *O. bacoti* drops off of the rat after each feeding and has been associated with pruritus and skin lesions in humans, including animal caretakers. *O. bacoti* presents a human health risk as it can transmit multiple, serious diseases to humans, including Q fever, plague, Hantaan viruses, and western equine encephalitis. *O. bacoti* infections in barrier-maintained rat colonies are very rare, but they are possible if infected rats enter a facility either through purposeful animal transport or on infested, wild rodents. Administration of topical ivermectin, permethrin dusts, or cotton balls embedded with permethrin, moxidectin, and other miticidal preparations effectively treat mite infestations. Caution should be used when treating suckling rats or other rats with incomplete or damaged blood–brain barriers as they are more sensitive to the neurotoxic effects of avermectins.

Pinworms

Syphacia muris, *S. obvelata*, and *Aspiculuris tetraptera* are the pinworms of rats, with *S. muris* being the most common. *S. muris* has a direct life cycle of 11–15 days, with females laying their eggs around the perineum. Transmission is by ingestion of eggs. The eggs hatch in the small intestine, and larvae mature in the cecum in 10–11 days. Pinworms ordinarily do not cause clinical symptoms but have been shown to alter hematologic values and immunological responses, as well as to cause reduced weight gain and growth rate of young rats (Box 5.6). The eggs of *S. muris* and *S. obvelata* may be detected in infected rats by microscopic examination of cellophane tape pressed against the perianal region. *A. tetraptera* infections are diagnosed by the presence of eggs in the feces or adult worms in the intestine. Perianal tape tests and fecal examinations have a high rate of false negative results. Negative tests should be verified through direct examination of cecal and colon contents at necropsy for adult worms.

Sound management practices are required to prevent the introduction of pinworms into a colony. A review by Pritchett and colleagues provides an overview of oxyurid life cycles and treatment modalities (Pritchett and Johnston, 2002). Ivermectin, fenbendazole-medicated feed, and piperazine may all be used in treatment. Although pinworm eggs are thought to

Box 5.6

Pinworms ordinarily do not cause clinical symptoms but have been shown to alter hematologic values and immunological responses, as well as to cause reduced weight gain and growth rate of young rats.

persist in the environment, Barlow and colleagues (Barlow et al., 2005) and Huerkamp and colleagues (Huerkamp et al., 2004) both report that eradication can be achieved by treating rats for a more prolonged period with fenbendazole-medicated feed without environmental decontamination. A paper by Keen and colleagues (Keen et al., 2005) indicates that, unlike ivermectin, fenbendazole treatment does not affect the behavior of rats and allows the animals to remain on study. Oge (Oge et al., 2000) reported doramectin was effective against *S. muris*; however, moxidectin was not. Equipment can be decontaminated with dry heat (100°C for 30 minutes) or ethylene oxide gas. Similarly, hydrogen peroxide and chlorine dioxide can both be used to fumigate a room or lab that has become contaminated to eliminate the infectious eggs.

Lice

Polyplax spinulosa, the spined rat louse, is a bloodsucking louse commonly seen in wild rats. *P. spinulosa* causes skin irritation, anemia, and debilitation. It can transmit the blood parasite *Mycoplasma haemomuris*. Ivermectin has been effectively used to eliminate this louse. Permethrin dust applied to the animals and the bedding is also reported to be effective.

Tapeworms

The rat is a definitive host for *Rodentolepis nana*, the dwarf tapeworm, and *Hymenolepis diminuta*, the rat tapeworm. These worms are pathogenic only with heavy infestations. Diagnosis of infection is usually by fecal flotation, fecal smear, or identification of the adult worms in the small intestine. Typical morphologic features used to differentiate *R. nana* from *H. diminuta* include adult worm size as well as the shape and characteristics of the scolex, eggs, and embryos.

Rodentolepis nana is of concern because of its direct life cycle, interspecies transmission, and zoonotic potential. Prevention of hymenolepid infections requires strict sanitation practices and elimination of vectors. Praziquantel and thiabendazole are reported to be effective in treating rat tapeworms. However, treatment is generally not recommended for *R. nana* infections because of the zoonotic potential. Total eradication in large rat populations is difficult to achieve without repopulation.

Flagellates

Spironucleus muris and *Giardia muris* are pathogenic flagellates occasionally found in the small intestine. Clinical disease is most likely to occur in young or immunosuppressed animals. Control depends on good sanitation practices. Treatment with metronidazole is reported to decrease the parasite load for both flagellates but does not eliminate infection. Mebendazole, albendazole, and fenbendazole are among the most effective agents used to treat giardiasis. Rederivation is the most reliable means to eliminate infection.

Urinary Bladder Worm

Trichosomoides crassicauda is a helminth parasite that may be found in the urinary bladder or renal pelvis of rats. It is usually of no clinical significance but has been associated with urolith formation and bladder cancer. Treatment with ivermectin is effective.

Parasitic Bacterium

Mycoplasma haemomuris (previously classified as *Haemobartonella muris*) was once common in laboratory rats but is now found only in wild rats, where the bacterium attaches to the surface of red blood cells. Infections are almost always inapparent and organisms are not seen in the peripheral blood unless an animal is splenectomized.

Neoplasia

Spontaneous neoplasms involving essentially every organ have been reported. The incidence depends to a large extent on the particular stock or strain and age of animals examined. Environmental influences also play an important role in neoplasia. Studies have shown that diet is important, and a 20% reduction in food intake leads to a significant reduction in overall incidence of tumors in Wistar rats (Box 5.7).

Box 5.7

Reduction in food intake leads to a significant reduction in overall incidence of tumors in some rat strains.

Mammary Tumors

Mammary tumors are very common in most stocks of laboratory rats. Up to 50% of aged female Sprague-Dawley rats develop mammary tumors. Because of the distribution of mammary tissue, the tumors may occur over a wide area of the body and may reach enormous sizes. These tumors are usually classified as benign fibroadenomas, are well encapsulated, and do not metastasize. They are usually freely mobile under the skin. Surgical removal is feasible, and prospects of recovery are favorable. Tumors frequently, however, reoccur.

Testicular Tumors

Interstitial cell tumors of the testes are highly common (~80% incidence) in F344 rats, an inbred strain commonly used in toxicologic studies. Grossly, the tumors are yellow-brown with areas of hemorrhage. They are frequently soft and discrete and may occur unilaterally or bilaterally. Incidence in other rat stocks or strains is quite low, with Wistars having an 11% incidence.

Keratoacanthoma

Keratoacanthomas are benign tumors of the skin that can become quite large. They appear as proliferations of keratin or horny growths with craterlike centers and may occur anywhere on the skin. No treatment is required unless the tumor becomes large and/or inhibits mobility or normal body functions.

Large Granular Lymphocytic Leukemia

Large granular lymphocytic leukemia is a major cause of death (10%–16%) in aging F344 rats. This neoplasia is not associated with a retrovirus as it is in mice. A leukocytosis of up

to 180,000/μL may occur. Clinical signs include weight loss, anemia, jaundice, and depression. Gross findings include marked splenomegaly with moderate hepatomegaly and lymphadenopathy. Histologically, a diffuse infiltration of large malignant lymphocytes throughout many organs, including the spleen, liver, lymph nodes, and lungs, is seen.

Pituitary Adenomas

Pituitary adenomas occur frequently in old rats, particularly in Sprague-Dawley (75%) and Wistar (>35%) rats. They are histologically classified as chromophobe adenomas. These tumors are frequently slow-growing but can grow very large (up to 0.5 cm) and compress adjacent central nervous system tissue and may induce hydrocephalus. Grossly, the tumors are often hemorrhagic and appear dark red and soft. Clinical signs include severe depression, incoordination, torticollis, and death.

Zymbal's Gland Tumors

Tumors of Zymbal's gland, which is located at the base of the ear, occur with some frequency in rats. The tumor tends to be locally invasive but not metastatic.

Miscellaneous Conditions

Chromodacryorrhea

Aged, clinically ill, or stressed rats may have obvious signs of chromodacryorrhea (Box 5.8; *chromo* meaning "colored", *dacryo* "tears", and *rrhea* "many"). The Harderian gland in the rat orbit is a lacrimal gland that secretes porphyrins. When secretion becomes excessive, tears containing porphyrin form a red crust around the eyes and may run down the nasolacrimal duct to form crusts around the nose (Figure 5.6). This dark, reddish-brown material resembles blood. The amount of secretion increases with age and during stressful conditions such as shipping. Treatment specifically for chromodacryorrhea is not indicated, although cleaning away the crusts may keep the rat more comfortable. The presence of excess secretions may suggest the possibility of some other underlying condition.

Box 5.8

Aged rats and sick or stressed rats may have obvious signs of chromodacryorrhea.

Chronic Progressive Glomerulonephropathy

Chronic progressive glomerulonephropathy (CPN) is the most important kidney disease of aged rats. In long-term rat studies, CPN is one of the most common causes of death. Several factors may play a role in the development of CPN, including age, sex, strain, and diet. Some rat stocks and strains, such as the Sprague-Dawley and F344, have a higher incidence while others, such as the Wistar and Long-Evans, have a low incidence. Males have a higher incidence, earlier onset, and more severe lesions than females. Moderate diet restriction (80%

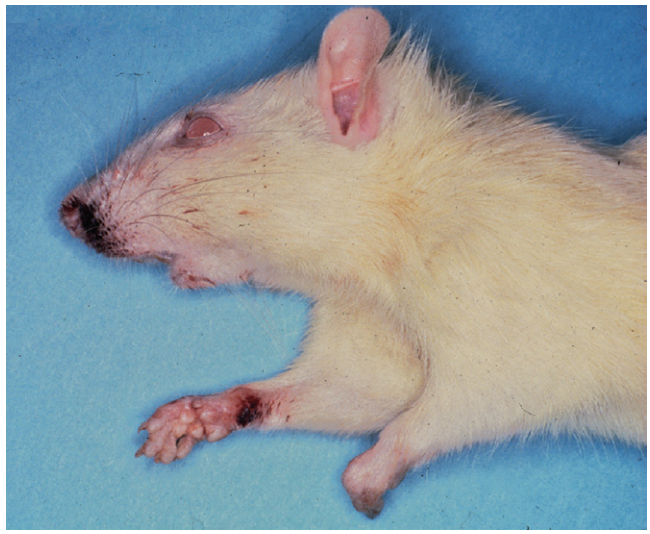

Fig 5.6. Chromodacryorrhea. (Photo courtesy of ACLAM.)

of *ad libitum*–fed body weight) will greatly reduce the incidence and severity of CPN compared with rats that are fed *ad libitum*. Clinical signs may include weight loss, unkempt hair coat, and acute death from renal failure. There is marked proteinuria, and secondary hyperparathyroidism may develop with resultant widespread tissue mineralization. Grossly, the renal cortices are pitted and irregular.

Chronic Myocardial Disease

Some strains of rats, such as Sprague-Dawley, have a high incidence of chronic heart disease. Similar to CPN, this is more common in the aged male and in *ad libitum*–fed animals. Grossly, the heart is enlarged.

Dehydration

Dehydration should not be overlooked as a possible cause of acute death in rats. Water may be unavailable because of an air lock in the sipper tube even when a water bottle is full. Malfunction of drinking valves in automatic watering systems can also result in a lack of water. Pain often results in a decrease in both food and water consumption. To check for dehydration, simply lift the skin over the back between the shoulder blades. A normally hydrated animal's skin slides quickly and smoothly back into place, whereas a dehydrated animal has a tenting of the skin with a slow return to normal position. If tenting is observed, the rat is at least 10% dehydrated and requires fluid supplementation. Sunken eyes are another sign of dehydration.

Dermatophytosis (Ringworm)

Ringworm in rats is usually caused by *Trichophyton mentagrophytes*. Infection may cause widespread skin lesions in a colony or may produce asymptomatic carriers. Systemic mycoses

Fig 5.7. Malocclusion. (Photo courtesy of ACLAM.)

are extremely rare in rats. Human ringworm infections have been associated with rats infected with this organism.

Malocclusion

Tooth overgrowth can occur when the jaw is malaligned or the diet is too soft (Figure 5.7). If severe and undetected, overgrown incisors can lead to emaciation and death due to an inability to eat. Rodent incisors can be trimmed with a dental bur or sharp suture removal scissors. The bur is less likely to fracture the teeth.

Polyarteritis Nodosa

Polyarteritis nodosa is a chronic degenerative disease of aging rats, especially in Sprague-Dawley and spontaneously hypertensive rats (SHRs). At necropsy, gross findings include segmental thickening and marked tortuosity of the medium-sized arteries of the mesentery, testes, and pancreas, as well as of the pancreaticoduodenal artery. The cause of these lesions has not been determined but may be an immunologically mediated response.

Ringtail

Ringtail is a syndrome caused by housing suckling or preweaned rats at low (<20%) ambient relative humidity (Figure 5.8). It is usually seen in the winter months when heating systems are in use. Ringtail appears as one or more annular constrictions of the tail. Distal to the constriction, the tail may become edematous or necrotic. If the tail is sloughed, the stump

Fig 5.8. Ringtail. (Photo courtesy of Wayne State University.)

will usually heal without complication. Providing solid-bottomed cages with adequate bedding and maintaining the relative humidity at approximately 50% can help to prevent ringtail.

Spontaneous Radiculoneuropathy

Radiculoneuropathy is a spontaneous aging disease of rats that involves degeneration of the spinal roots and concurrent atrophy of the skeletal muscles of the lumbar region and hind limbs. Affected animals exhibit posterior weakness or paresis.

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CHAPTER 5 REVIEW

Multiple Choice

1. Hooded outbred stock of rat
   A. Sprague-Dawley
   B. Buffalo
   C. Wistar
   D. Long-Evans
2. Control inbred strain for the spontaneously hypertensive rat
   A. WKY
   B. F344
   C. BUF
   D. ZDF
3. False statement regarding rats
   A. Pancreas is diffuse.
   B. Rats have gallbladders.
   C. Rats possess paired auditory sebaceous glands.
   D. A limiting ridge prevents them from vomiting.
4. True statement regarding breeding and reproduction of rats
   A. Only monogamous system is used.
   B. Vaginal plug can be used to confirm breeding.
   C. Pheromones play a very important role in behavior.
   D. Pups are weaned at 30 days of age.
5. Up to _____% of the circulating blood volume can be withdrawn every 2–3 weeks from normal, healthy animals with minimal effect.
   A. 5
   B. 7
   C. 10
   D. 12
6. A preferred site used to collect up to 1 mL of blood in an unanesthetized rat
   A. lateral saphenous vein
   B. heart
   C. retroorbital plexus
   D. jugular vein
7. Recommended site for administration of intraperitoneal injections in rats
   A. upper right abdominal quadrant
   B. lower left abdominal quadrant
   C. upper left abdominal quadrant
   D. lower right abdominal quadrant

(*Continued*)

8. The rat should be turned every 30–60 minutes during postanesthesia recovery to prevent
   A. hypothermia
   B. inhalation of bedding material
   C. hypostatic lung congestion
   D. dehydration
9. Opioid analgesic that can induce pica at high doses
   A. buprenorphine
   B. meloxicam
   C. butorphanol
   D. carprofen
10. False statement regarding euthanasia of rats
   A. Barbiturate solution can be given intraperitoneally.
   B. Neonates require a shorter duration of CO_2 exposure.
   C. Dry ice should not be used to generate CO_2 gas.
   D. KCl must be used in an anesthetized animal.

Match Up

Match the following with their respective descriptions:

A. IDIR
B. *Radfordia ensifera*
C. *Aspiculuris*
D. *Ornithonyssus bacoti*
E. *Mycoplasma pulmonis*
F. SDAV
G. Hantavirus
H. CAR bacillus
I. Zymbal's gland tumor
J. Ringtail
K. Pituitary adenoma
L. *Syphacia*
M. Testicular interstitial cell tumor
N. RV

11. _____ Fur mite of rat
12. _____ Caused by low housing humidity
13. _____ Rotavirus that causes clinical disease in suckling rats
14. _____ Pinworm species for which the perianal tape test may be diagnostic
15. _____ Highly contagious coronavirus
16. _____ Neoplasia common in F344 rats
17. _____ Causative agent of MRM
18. _____ Parvovirus
19. _____ Zoonotic agent endemic in some wild rodent populations
20. _____ Tumor occurs commonly in old rats

6

Gerbils

The Mongolian gerbil is a rodent of the family Cricetidae and is a relative of the hamster. Its scientific name is *Meriones unguiculatus*, which translates into "mammal having claws." The Mongolian gerbil is also known as the jird, sand rat, or desert rat. It is native to desert regions of China and Mongolia. Gerbils are intermediate in size between mice and hamsters. Adults typically weigh 50–130 g, with males slightly larger than females. The most common coat color is agouti, which has a light buff to white ventrum and mixed white, yellow, and black hairs dorsally, giving it an overall brown color. Gerbils may also be black, gray, white, cinnamon, dove, or piebald (patches of two or more colors, often including white).

USES

Gerbils have many attributes that contribute to their usefulness as a research animal. They are clean, friendly, easy to handle, and tame; they rarely bite and are nearly odor free. In addition, they tend to be very curious and quite active.

Gerbils are useful in a number of research areas, including stroke, behavior, parasitology, epilepsy, radiobiology, hearing, lipid metabolism, and infectious disease research. They are an important animal model for stroke research because they have an incomplete circle of Willis, which is the major arterial vascular supply to the base of the brain. This unique anatomic feature makes it possible to create ipsilateral cerebral ischemia by unilateral carotid ligation. Gerbils can be infected with a wide variety of parasites. They are particularly useful in studying diseases caused by filarial nematodes. Gerbils are an important model for the study of epilepsy because they have a high incidence of spontaneous epileptiform seizures.

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BEHAVIOR

Gerbils are social animals and, under normal circumstances, live peacefully in same-sex or mixed-sex groups. They may fight if crowded or if grouped as adults. Gerbils are active burrowers and, in their natural desert environment, construct elaborate tunnels with multiple entrances, nesting rooms, and food chambers. They tend to be most active in the evening, with short periods of activity during the day. Gerbils frequently sit upright on their hind limbs in an inquisitive stance. When caged, they may commonly scratch a bottom corner repeatedly with their front paws while pushing bedding behind them with their hind limbs. Gerbils attract attention or express aggression by thumping their hind limbs to create a drumming sound. They generally mate for life, forming stable breeding pairs. Both sexes nest build and take an active role in parenting.

ANATOMIC AND PHYSIOLOGIC FEATURES

Gerbil biologic and reproductive data is listed in Table 6.1. The gerbil has a longer and more slender body conformation than that of the hamster. Gerbils are characterized by a long tail that is fully furred with a tuft of longer hairs on the end. They have prominent furred ears; large, slightly protruding eyes; furred foot pads and strong claws for burrowing; and elongated hind limbs used for jumping or maintaining a semierect posture. Gerbils have a Harderian gland located posterior to each globe within the ocular orbit. These exocrine glands secrete lipid- and porphyrin-rich material that lubricates the eyes and conjunctival

Table 6.1. Biologic and reproductive data for gerbils

Adult body weight: Male	80–130 g
Adult body weight: Female	55–85 g
Life span	3–4 y
Body temperature	37°–38.5°C (98.6°–101.3°F)
Heart rate	360 beats per minute
Respiratory rate	90 breaths per minute
Food consumption	5–8 g/100 g per day
Water consumption	4–7 mL/100 g per day
Breeding onset: Male	70–85 d
Breeding onset: Female	65–85 d
Estrous cycle length	4–6 d
Gestation period	24–26 d
Postpartum estrus	Fertile
Litter size	3–7
Weaning age	20–26 d
Breeding duration	12–17 mo
Chromosome number (diploid)	44

Source: Adapted from Harkness et al. (2010).

space. The secretions drain through an opening in the nictitating membrane, down the nasolacrimal duct, to the external nares. Harderian gland secretions appear to play a role in pheromone-mediated behavior, temperature regulation, and protection and lubrication of the eye, and they may influence the retinal–pineal axis. Both sexes have an elliptical, mid-ventral abdominal sebaceous gland covered with coarse hair. The ventral sebaceous gland, used in territorial marking, is nearly twice as large in the male and produces an orange, oily, and musky-scented secretion. The gerbil's dental formula is typical of rodents: 2(I 1/1, C 0/0, P 0/0, M 3/3). Only the incisors continuously grow. The stomach consists of a nonglandular forestomach and a glandular stomach. The adrenal glands are very large relative to the total body weight and are approximately three times larger than those of the rat. The thymus persists into adulthood. Gerbils have a great capacity for temperature regulation and for water and electrolyte conservation.

Sexing gerbils is fairly easy because the male has a prominent, darkly pigmented scrotum and a substantially greater anogenital distance than the female. The female has three body openings in the anogenital region: the urinary papilla, vagina, and anus. Newborn gerbils can be sexed by anogenital distance and by noting the male's larger genital papilla. Females have four pairs of teats.

There are several distinctive characteristics of the hematologic and urinary profiles of gerbils. Gerbils frequently exhibit lipemia and hypercholesterolemia even when fed standard rodent diets. The lipemia can be accentuated by feeding high-fat chows or sunflower seeds. The life span of their red blood cells is relatively short, approximately 9–10 days. It is common to see large numbers of reticulocytes and erythrocytes with basophilic stippling in the peripheral blood, especially in young animals. Lymphocytes are the predominant leukocyte. Influenced by their desert ecology, gerbils produce small quantities of highly concentrated urine. Selected hematologic and biochemical values are listed in Appendix 1, "Normal Values."

BREEDING AND REPRODUCTION

Gerbils breed readily throughout the year, but they are not as prolific as mice. They are inclined to periods of nonbreeding, especially during the winter. Controlling light cycles to supply 14 hours of light per day, providing tunnels or huts for hideaways, changing the bedding type, or housing in an opaque cage may encourage nonbreeding gerbils to resume breeding. Pairs should be formed at 8 weeks of age or earlier and should not be separated unless they are incompatible. Breeding pairs are usually monogamous; if one mate is lost, the survivor generally will not accept another (Box 6.1). Compared with other rodents,

Box 6.1

Breeding pairs are monogamous; if one mate is lost, the survivor generally will not accept another.

puberty occurs later in gerbils. They should be bred when they are 10–12 weeks of age. Gerbils are polyestrous, spontaneous ovulators, with an estrous cycle length of 4–6 days. They have a fertile postpartum estrus. A female in estrus acts restless and may have a congested vulva. Observation of both male and female behavior is a better guide to the presence of estrus than are vaginal smears because the changes in the vaginal epithelium are not obvious as in the mouse or rat. Breeding usually occurs in the late evening. The female is actively pursued by the excited male until she stands to allow mounting. Copulation is characterized by multiple intromissions. A vaginal plug is formed during mating, but it is not readily detectable as it is small and lies deep in the vagina. Duration of gestation is 24–26 days in a nonlactating gerbil. If the female is bred while nursing a large litter or during periods of stress, implantation may be delayed and gestation may be as long as 42 days. The birthing process takes approximately one hour and the average litter size is five. Neonates are altricial, born hairless and blind. Their ears open at 3–7 days, hair grows at 7–10 days, and eyes open at 14–20 days of age. Newborns are rarely abandoned or cannibalized, except if the litter is small. Both males and females participate in nest building and care of the young. Development of young is somewhat slower in gerbils than in mice or rats. Young gerbils start eating solid foods around 2 weeks of age. Care should be taken to ensure that they can reach the food hopper and water supply. Food pellets can be placed on the cage floor or moistened and placed in a container on the cage floor. Gerbils are typically weaned at 20–26 days of age.

HUSBANDRY

Housing and Environment

Gerbils are best housed in rigid plastic rodent cages with a solid floor and deep bedding. The enclosure should be at least 15 cm (6 in.) in height to provide the animals adequate space to sit upright. Each adult gerbil should be provided with 230 cm^2 (36 $in.^2$) floor area while breeding pairs with litters should be provided 1300 cm^2 (180 $in.^2$). The cage needs to have a secure lid and be designed to prevent escape. Gerbils are active gnawers and burrowers and, although they do not climb well, they can jump. An escaped gerbil will generally return to its cage or move to the middle of a room rather than hide. Ground corncob, hardwood chips, composite recycled paper pellets, and sand are satisfactory for bedding. Cedar and pine shavings are not recommended as they are irritating to the skin and mucous membranes (Box 6.2) and may induce hepatic enzymes that may alter normal drug metabolism. Bedding depth should be at least 2 cm (1 in.) to facilitate nest building and burrowing activity. The recommended temperature range for gerbils in a research facility is 20°–26°C (68°–79°F)

Box 6.2

Cedar and pine shavings are not recommended bedding materials because they are irritating to the skin and mucous membranes.

with a relative humidity between 30% and 50%. Humidity in excess of 50% causes the gerbil's haircoat to become matted and wet. Providing a container of sand in the cage has been reported to be helpful in preventing oily coats. Ventilation should provide 10–15 air changes per hour. The light cycle is usually controlled by a timing device to provide 12 hours of light per day although 14 hours of daylight may improve breeding. Housing gerbils with other species should be avoided because interspecies transmission of diseases (e.g., *Salmonella* spp., *Bordetella bronchiseptica*, and *Syphacia*) can occur.

Sanitation

Gerbil caging requires less frequent cleaning than do the cages of other rodents because gerbils produce only a few drops of urine daily and their fecal pellets are small, dry, and hard. The *Guide for the Care and Use of Laboratory Animals* (ILAR, 2011) recommends cages and cage accessories be sanitized at least once every two weeks. Cages, bottles, and feed hoppers can be disinfected with chemicals, hot water 61.6°–82.2°C (143°–180°F), or a combination of both. Detergents and chemical disinfectants enhance the effectiveness of hot water, but they must be thoroughly rinsed from the surfaces of the item. Refer to Chapter 4, "Mice," for a more detailed description of disinfection methods.

Environmental Enrichment and Social Housing

An animal's social needs should be carefully considered when housing animals in the laboratory environment. Ideally, enrichment should increase the normal behavior for the species and decrease abnormal or stereotypic behaviors. Exercise wheels, items for hiding (e.g., PVC piping, huts, and tunnels), sufficient bedding for burrowing, and shreddable materials for nesting can be used to provide environmental enrichment. Commercial, synthetic small-rodent nesting fiber should not be used, as it can wind around the feet or teeth or cause impactions if ingested. All enrichment items must be reviewed and approved by the IACUC, veterinarian, and principal investigator prior to their use.

Feeding and Watering

Gerbils should be fed a pelleted rodent chow that contains 16%–22% protein and they will consume an average of 5–8 g/100 g of body weight per day. Diet should be provided *ad libitum* in a food hopper. Although gerbils prefer sunflower seeds to pelleted chows, these are not satisfactory as a balanced food source. Sunflower seeds are low in calcium and high in fat and do not meet the nutritional needs of the species. Feeding excessive amounts of seeds can cause obesity, which can shorten the gerbil's life span. Gerbils, especially females, typically hoard food. Fresh, potable water should be supplied *ad libitum* by bottle, sipper sack, or automatic watering system. Adults drink an average of 4–7 mL/100 g of body weight per day.

TECHNIQUES

Handling and Restraint

Although not inclined to bite, gerbils are quick and can leap great distances in an effort to escape. A gerbil may be lifted from its cage by grasping the base of the tail. Care must be

Fig 6.1. Two-hand method of gerbil restraint. (Photo courtesy of M. Tear.)

taken not to grasp the tip of the tail as a degloving injury may result, in which the tail skin is pulled off to expose the underlying vertebrae (Box 6.3). A gerbil can also be scooped up in the palm of the hand; however, it should be held firmly. To restrain for examination or manipulation, first hold the base of the tail with one hand, then grasp the scruff of the neck with the other hand, as shown in Figure 6.1. Alternatively, the gerbil may be grasped by placing a hand over the gerbil's back to enclose the body firmly, as shown in Figure 6.2. Gerbils resist being placed on their back and tend to struggle. Injections or manipulations are more readily accomplished when animals are held in an upright position.

Box 6.3

Care must be taken not to grasp the tip of the tail as a degloving injury may result, in which the tail skin is pulled off to expose the underlying vertebrae.

Identification

In the research setting, cage cards are most often used for general identification. Methods to permanently identify individual gerbils include ear punching, ear tagging, or subcutaneous (SC) placement of a microchip for quick electronic identification. Dyes, permanent markers, and fur clipping can be used as temporary identification methods.

Blood Collection

Assuming the animal is mature, healthy, and on an adequate plane of nutrition, the blood volume of most species averages 7% of the body weight in grams. Table 6.2 lists approximate

Fig 6.2. One-hand method of gerbil restraint. (Photo courtesy of M. Tear.)

Table 6.2. Adult gerbil blood volumes and suggested sample size

	Volume (mL)
Total blood	4.4–8.0
Single sample	0.5–1.0
Exsanguination	2–4

Source: Adapted from Harkness et al. (2010).

Note: Values are approximate.

adult gerbil blood volumes. A typical rule of thumb for blood withdrawal suggests that up to 10% of the circulating blood volume can be withdrawn from normal, healthy animals every 2–3 weeks with minimal adverse effect. Small amounts of blood (0.1–0.3 mL) are best obtained from the lateral saphenous vein. If greater than 10% total blood volume is removed during a single collection, the blood volume should be replaced with lactated Ringer's or other physiologic solution. Larger blood samples can be obtained by retroorbital puncture, jugular venous puncture, or cardiac puncture, in an anesthetized animal. Cardiac puncture is risky and normally is performed only if the animal will be euthanized prior to anesthetic recovery.

Urine Collection

Collecting urine from a gerbil can be a challenge because of the small quantities that are produced daily. Handling stress commonly causes gerbils to urinate.

Drug Administration

Small scales are useful for obtaining accurate body weights to calculate a medication dose. Dosage accuracy is increased through dilution of drugs to create a more manageable volume and by use of a tuberculin syringe. Most drug administration techniques used in small animal practice can be used with gerbils when modified to fit their small size. Medication can be administered orally by mixing the medication in the water or feed. This method should only be used if the animal's consumption of the medicated food or water can be assured. If more accurate dosing is necessary, a syringe may be used to place the medication in the oral cavity or a small, ball-tipped gavage needle can be used to administer the medication directly into the stomach. Small-gauge hypodermic needles, such as 23 or 25 gauge, should be used for parenteral injections. Medication can be administered by subcutaneous injection using the skin over the shoulders, provided that the administered agent is not excessively irritating. A maximum volume of 3–4 mL is suggested. Because of the risk of accidental sciatic nerve injury, small muscle mass, and stress of restraint from extending the leg, the intraperitoneal (IP) route of drug administration is recommended over the intramuscular route. If intramuscular injections are required, volumes should be no greater than 0.1 mL. For the gerbil, the IP procedure is modified slightly from that of the mouse or rat as gerbils detest being placed on their backs. The gerbil should be restrained and held vertically to prevent it from struggling during the injection. Intraperitoneal injections, with a maximum volume of 2–3 mL, should be given in the lower quadrant of the abdomen, lateral to the midline. Although technically challenging, the lateral saphenous vein can be used for intravenous injections. The maximum dosing volumes are summarized in Table 6.3.

Anesthesia, Surgery, and Postoperative Care

A variety of agents that can be used to tranquilize and anesthetize gerbils are listed in Table 6.4. Preanesthetic fasting is not recommended because the gerbil has a high metabolic rate and there is potential for inducing hypoglycemia and hypothermia. The use of acepromazine in this species is not recommended because it lowers the seizure threshold. Single-injection anesthesia techniques are generally used to minimize handling stress. Mixtures of ketamine with xylazine administered by the IP route produce fairly consistent anesthesia. Gerbils can be placed in an induction chamber or masked down with an inhalation agent delivered from a precision vaporizer. When using inhalation agents, care must be used to scavenge anesthetic gases to ensure the safety of personnel working with the animal or in the general area. Iso-

Table 6.3. Maximum adult gerbil dosing volumes

Route	Volume (mL)
IP	2–3
SC	3–4
IM	0.1
IV	0.3
PO	1.5

Source: Adapted from Harkness and Wagner (1995) and Lukas (1999).

Table 6.4. Anesthetic agents and tranquilizers used in gerbils

Drug	Dosage	Route	Reference
Inhalants			
Isoflurane	1%–4% to effect	Inhalation	Wixson (1994)
Sevoflurane	To effect	Inhalation	Morrisey and Carpenter (2004)
Injectables			
Acepromazine	Not recommended, lowers seizure threshold		
Alphaxalone—alphadolone	80–120 mg/kg	IP	Flecknell (2009)
Diazepam	5 mg/kg	IP	Flecknell (2009)
Fentanyl–fluanisone	0.5–1 mg/kg	IP	Flecknell (2009)
Fentanyl–fluanisone	0.3 mL/kg	IP	Flecknell (2009)
+ diazepam	5 mg/kg	IP	
Fentanyl	0.05 mg/kg	SC	Flecknell (2009)
+ metomidate	50 mg/kg	SC	
Ketamine	50 mg/kg	IP	Harkness and Wagner (1995)
+ diazepam	5–10 mg/kg	IP	
Ketamine	75 mg/kg	IP	Flecknell (2009)
+ medetomidine	0.5 mg/kg	IP	
Ketamine	75 mg/kg	SC	Harkness et al. (2010)
+ medetomidine (mixed)	0.5 mg/kg	SC	
Ketamine	50 mg/kg	IP	Harkness et al. (2010)
+ xylazine	2 mg/kg	IP	
Medetomidine	0.1–0.2 mg/kg	SC	Johnson-Delaney (1999)
Midazolam	5 mg/kg	IP	Flecknell (2009)
Pentobarbital	60 mg/kg up to 6 mg maximum	IP	Norris (1987)
Tiletamine–zolazepam	20 mg/kg	IP	Huerkamp (1995)
+ xylazine	10 mg/kg	IP	
Tribromoethanol	250–300 mg/kg	IP	Flecknell (2009)

IP = intraperitoneal; SC = subcutaneous.

flurane and sevoflurane should be administered via a precision vaporizer with a mask or nose-cone apparatus in conjunction with a nonrebreathing system. Alternatively, very brief (approximately 1–2 minutes) anesthesia can be produced through use of a simple anesthetic chamber. See Chapter 4 for additional information.

Gerbils may show an idiosyncratic writhing before entering a surgical plane of anesthesia. This should not be confused with light anesthesia. The depth of anesthesia can be determined by using a combination of reflexes such as the righting reflex, pedal withdrawal reflex, and the abdominal pinch reflex. Muscle tone and purposeful movement in response to painful

stimuli may also be used as indicators of anesthetic depth. Ocular position and the palpebral reflex are inconsistent and unreliable indicators of anesthetic depth in the gerbil.

While anesthetized, gerbils should have a sterile, bland ophthalmic ointment placed in their normally protruding eyes to prevent exposure keratitis. Use of a circulating water heating blanket or other suitable heating source designed for use with animals promotes euthermia and is recommended. Generic, electric heating pads should never be placed in direct contact with the animal as they may cause thermal burns.

Gerbils may require a simple castration, tail amputation, or growth removal. Care should be taken to close the inguinal canals during castration to prevent intestinal entrapment. In research, one of the most common surgical procedures is to produce cerebral infarction. Suture lines are carefully placed around a carotid artery and the gerbil is allowed to recover. Several days postoperatively when the animal has stabilized, a stroke can be produced by applying tension to the suture thereby causing unilateral ligation of the common carotid artery. Surgeries should be performed in an aseptic manner. Prevention of blood loss is an important consideration.

The anesthetized gerbil should be placed on a clean, dry paper or cloth towel and recovered in an escape-proof incubator. Small-particle bedding materials are contraindicated in recovery cages because they can stick to the eyes, nose, and mouth of the recovering animal and may be aspirated. Postoperative care should include hydration of the anesthetized animal (e.g., SC or IP injection of a warmed solution such as saline, saline–dextrose, lactated Ringer's), and support of normal body temperature. Hypostatic pulmonary congestion should be prevented by turning the animal at least every 30 minutes. It is important to maintain hydration and nutrition levels in the postoperative period to support normal metabolic processes and gut physiology. Select, commercially available supportive and critical care products for laboratory animals are provided in Appendix 3.

Table 6.5 lists analgesics than can be used. Gerbils that are in pain may have piloerection, a hunched posture, a reluctance to move, and may exhibit decreased interactions with their cagemates. Butorphanol is recommended for mild postoperative discomfort. Buprenorphine works well to control acute or chronic visceral pain but causes more sedation.

Imaging Techniques

Great advances have been made in rodent and small animal imaging over the last decade. Many imaging modalities once used only in humans [e.g., digital x-ray imaging, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), dual-energy x-ray absorptiometry (DEXA) scan, and ultrasound] have been adapted for use with these species, producing images of exquisitely fine detail. In addition, novel imaging techniques have been developed for use in animal research, including *in vivo* optical imaging.

For conventional x-ray imaging, an x-ray machine with high milliamperage, such as a 200- or 300-mA unit with the capability for low settings of peak voltage delivered (kVp) and small incremental changes, is ideal. High-detail film–screen combinations work well. Short exposure times of 1/40 second or less should be used to decrease the chance of motion artifact. Suggested exposure factors are 42–46 kVp, 300 mA, 1/40 second, 40 SID, 7.5 mA·s. Alternatively, nonscreen film can be used with standard x-ray or dental units. Nonscreen film produces high-detail radiographs, but longer exposure times are needed. Digital imaging

Table 6.5. Analgesic agents used in gerbils

Drug	Dosage	Route	Reference
Acetylsalicylic acid	100–150 mg/kg q4h	PO	Heard (1993)
Buprenorphine	0.01–0.05 mg/kg q8h	SC, IP	Flecknell (2009)
Butorphanol	1–5 mg/kg q4h	SC	Heard (1993)
Carprofen	5 mg/kg q24h	SC	Pollack (2002)
Flunixin meglumine	2.5 mg/kg q12–24h	SC	Heard (1993)
Ketoprofen	5 mg/kg	SC	Pollack (2002)
Meperidine	20 mg/kg q2–3h	SC	Heard (1993)
Syrup	0.2 mg/mL drinking water		Huerkamp (1995)
Meloxicam	1 mg/kg q24h	SC	Flecknell (2009)
Morphine	2–5 mg/kg q2–4h	SC	Heard (1993)
Nalbuphine	4–8 mg/kg	SC	Mason (1997)
Oxymorphone	0.2–0.5 mg/kg q6–12h	SC	Heard (1993)
Pentazocine	10 mg/kg q2–4h	SC	Heard (1993)
Piroxicam	3 mg/kg q24h	PO	Harkness et al. (2010)
Tramadol	5–10 mg/kg q12–24h	PO	Morrisey and Carpenter (2011)

IM = intramuscular; PO = per os; SC = subcutaneous.

techniques offer substantial flexibility in image enhancement and delivery application. The best method of restraint is to sedate the animal and use adhesive tape to secure it to the cassette in the appropriate position. A radiolucent tube or stockinette with the patient positioned inside can be used for restraint of conscious animals.

Euthanasia

Methods of euthanasia must be consistent with the most current American Veterinary Medical Association (AVMA) *Guidelines for the Euthanasia of Animals* (AVMA, 2013) and performed by trained personnel. Per AVMA Guidelines, "Carbon dioxide is acceptable with conditions for euthanasia in those species where aversion or distress can be minimized. Carbon dioxide exposure using a gradual fill method is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness; a displacement rate from 10% to 30% of the chamber volume/min is recommended. Whenever gradual displacement methods are used, CO_2 flow should be maintained for at least 1 minute after respiratory arrest. If animals need to be combined, they should be of the same species and, if needed, restrained so that they will not hurt themselves or others. Oxygen administered together with CO_2 appears to provide little advantage and is not recommended for euthanasia. The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO_2, is unacceptable. Carbon dioxide and CO_2 gas mixtures must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate [author's note: e.g., dry ice]. As gas displacement rate is critical to the

humane application of CO_2, an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size of the container being utilized is absolutely necessary." Neonates are resistant to hypoxia and require greater duration of exposure. For this reason, other methods may be preferred for this age group. Studies have shown that the method of CO_2 euthanasia can result in significant differences in immunologic and hematologic variables; thus consistency of method may be important in accurate interpretation of research data. Other acceptable methods of euthanasia include overdose of an inhalant anesthetic and IP administration of a barbiturate anesthetic at three to four times the anesthetic dose.

THERAPEUTIC AGENTS

Suggested gerbil antimicrobial and antifungal drug doses are listed in Table 6.6. Antiparasitic agents are listed in Table 6.7, and miscellaneous drugs are listed in Table 6.8.

INTRODUCTION TO DISEASES OF GERBILS

Most laboratory rodent colonies today are relatively free of the viruses, bacteria, parasites, and fungi that cause clinical disease. However, sporadic outbreaks may still occur, and it is important for individuals to have a basic knowledge of the most common infectious agents and how to respond to them. Presented here is a general overview of common bacterial, viral, fungal, and parasitic agents of gerbils and some standard diagnostic and treatment modalities.

A sick gerbil typically presents with similar clinical signs regardless of the cause. General signs of illness include weight loss, porphyrin staining of the eyes or nose, loss of body and coat condition, and reduced activity. Hypothermia, anorexia, and dehydration are common and need to be treated aggressively. Supportive care in the form of warmed fluids given IP or SC and dietary supplementation are important. Unlike the hamster, guinea pig, and rabbit, whose normal intestinal microflora is highly susceptible to antibiotics with a Gram-positive spectrum, the gerbil can be safely treated with a wide variety of antibiotics. One notable exception is the gerbil's sensitivity to dihydrostreptomycin, which can induce an ascending paralysis and death due its toxic effects in this species.

Bacterial Diseases

Tyzzer's Disease

Gerbils are highly susceptible to *Clostridium piliforme*, the causative agent of Tyzzer's disease. The disease is most frequently seen at weaning age, but adults can be affected. Mortality is high in young animals 3–7 weeks of age. Poor sanitation and stress are important contributing factors of disease. Affected animals typically have a rough hair coat, are lethargic and anorexic, and die within 1–3 days of infection. Diarrhea may be absent or mild in gerbils, in contrast to other species, which show a profuse watery diarrhea. Transmission is thought to be fecal–oral. Characteristically, infected animals have hepatomegaly with numerous gray, white, or yellow foci measuring 1–2 mm in diameter. Edema and hemorrhage of

Table 6.6. Antimicrobial and antifungal agents used in gerbils

Drug	Dosage	Route	Reference
Amikacin	2–5 mg/kg q8–12h	SC	Harkness et al. (2010)
Ampicillin	6–30 mg/kg q8h	PO	Anderson (1994)
Cephalexin	25 mg/kg q24h	SC	Flecknell (1996)
Cephaloridine	30 mg/kg q12h	IM	Flecknell (1987)
Chloramphenicol	50–200 mg/kg q8h	PO	Burgmann and Percy (1993)
	30–50 mg/kg q12h	SC	Burgmann and Percy (1993)
Ciprofloxacin	7–20 mg/kg q12h	PO	Harkness et al. (2010)
Doxycycline	2.5 mg/kg q12h	PO	Allen et al. (1993)
Enrofloxacin	0.05–0.2 mg/mL drinking water for 14 d		Harkness et al. (2010)
	5–10 mg/kg q12h	PO, SC	Harkness et al. (2010)
Gentamicin	2–4 mg/kg q8h	SC	Harkness et al. (2010)
Griseofulvin	25–50 mg/kg q12h for 14–60 d	PO	Harkness et al. (2010)
	1.5% in DMSO for 5–7 d	Topical	Harkness et al. (2010)
Ketoconazole	10–40 mg/kg q24h for 14 d	PO	Rollin and Kesel (1995)
Metronidazole	7.5 mg/70–90 g animal q8h	PO	Collins (1995)
Neomycin	2.6 mg/mL drinking water		Anderson (1994)
	100 mg/kg q24h	PO	Burgmann and Percy (1993)
Oxytetracycline	0.8 mg/mL drinking water		Burgmann and Percy (1993)
	10 mg/kg q8h	PO	Burgmann and Percy (1993)
	20 mg/kg q24h	SC	McKellar (1989)
Sulfadimethoxine	10–15 mg/kg q12h	PO	Harkness et al. (2010)
Sulfamerazine	0.8 mg/mL drinking water		Anderson (1994)
Sulfamethazine	0.8 mg/mL drinking water		Anderson (1994)
Sulfaquinoxaline	1 mg/mL drinking water		Collins (1995)
Tetracycline	2–5 mg/mL drinking water		Burgmann and Percy (1993)
	10–20 mg/kg q8–12h	PO	Burgmann and Percy (1993)
Trimethoprim/sulfa	30 mg/kg q12–24h	PO, SC	Harkness et al. (2010)
Tylosin	0.5 mg/mL drinking water		Collins (1995)
	10 mg/kg q24h	PO, SC	Collins (1995)

DMSO = dimethyl sulfoxide; IM = intramuscular; PO = per os; SC = subcutaneous.

the intestines, particularly in the ileocecal area, may also be present. Oxytetracycline or tetracycline can be used to suppress, but not eliminate, infection. Supplemental fluid therapy can be provided if indicated. In an outbreak, it is best to isolate ill animals and cull them. Infectious spores can remain in the environment for prolonged periods of time and are resistant to numerous disinfectants. All cages, feeding, and watering equipment should be sterilized. Microisolation cages can be used to prevent cage-to-cage transmission.

Table 6.7. Antiparasitic agents used in gerbils

Drug	Dosage	Route	Reference
Amitraz	1.4 mL/L water using cotton-ball application q14d for 3–6 treatments	Topical	Harkness et al. (2010)
Fenbendazole	20 mg/kg q24h for 5d	PO	Harkness et al. (2010)
Ivermectin	0.2 mg/kg q7d for 3wk	PO, SC	Anderson (1994)
Piperazine citrate	2–5 mg/mL drinking water for 7d, off 7d, repeat		Anderson (1994)
	20–60 mg/100g	PO	Russell et al. (1981)
Pyrantel pamoate	50 mg/kg	PO	Rollin and Kesel (1995)
Praziquantel	30 mg/kg q14d for 3 treatments	PO	Burke (1995)
Pyrethrin powder	3 times per week for 3wk	Topical	Anderson (1994)
Thiabendazole	100 mg/kg q24h for 5d	PO	Allen et al. (1993)

PO = per os; SC = subcutaneous.

Table 6.8. Miscellaneous agents used in gerbils

Drug	Dosage	Route	Reference
Atipamezole	1 mg/kg	SC, IP, IV	Flecknell (1997)
Atropine	0.05–0.1 mg/kg	SC	Harkness and Wagner (1995)
Cimetidine	5–10 mg/kg q6–12h	PO	Allen et al. (1993)
Dexamethasone	0.5–2 mg/kg then decreasing dose q12h for 3–14d	PO, SC	Harkness and Wagner (1995)
Diphenylhydantoin	25–50 mg/kg q12h	PO	Laber-Laird (1996)
Doxapram	5–10 mg/kg	IV, IP	Harkness (1993)
Furosemide	5–10 mg/kg q12h	SC	Allen et al. (1993)
Glycopyrrolate	0.01–0.02 mg/kg	SC	Huerkamp (1995)
Naloxone	0.01–0.1 mg/kg	SC, IP	Huerkamp (1995)
Oxytocin	0.2–3 IU/kg	SC	Anderson (1994)
Phenobarbital	10–20 mg/kg q12h	PO	Laber-Laird (1996)
Vitamin K	1–10 mg/kg q24h for 4–6d	IM	Harkness and Wagner (1995)
Yohimbine	0.5–1 mg/kg	IV	Harkness and Wagner (1995)

IM = intramuscular; IP = intraperitoneal; IV = intravenous; PO = per os; SC = subcutaneous.

Salmonellosis

Adult gerbils are fairly resistant to *Salmonella* spp.; however, disease has been observed in young gerbils. Clinical signs are generally nonspecific and include dehydration, depression, emaciation, and rough hair coat. The abdomen is frequently distended and diarrhea may or may not be present. Males have been reported to have testicular enlargement. Sudden death may occur. Animals that recover may become asymptomatic carriers and transmit infection to other animals or humans. Animals that are positive on bacteriological culture should be

culled from the colony. Treatment is contraindicated due to the zoonotic potential of the organism.

Staphylococcal and Streptococcal Infections

Beta-hemolytic *Staphylococcus aureus* has been associated with a diffuse dermatitis in young gerbils. The face, feet, legs, and ventral body surface are affected. Bite wounds, nose abrasions secondary to burrowing, and the ventral sebaceous gland can become infected with *Staphylococcus* spp., *Streptococcus* spp., and other bacteria. Topical treatment with an antibacterial preparation applied daily is often beneficial. Tetracycline, sulfonamides, or chloramphenicol can be used for parenteral treatment.

Viral Diseases

No clinically significant and naturally occurring viral infections of gerbils have yet been identified.

Parasitic Diseases

Demodex

Alopecia and dermatitis associated with *Demodex* spp. have been reported in gerbils. Demodex mite infestations are not noted to be a problem in clinically healthy animals. Old age and debilitation are important predisposing factors to disease development. Gerbils can be treated similarly to hamsters, with a dilute solution of amitraz applied topically with a cotton ball.

Pinworms

Gerbils can become infected with several species of oxyurids, including *Syphacia muris* and *S. obvelata*. *Syphacia* spp. live in the distal region of the intestinal tract and can occasionally be diagnosed by applying clear adhesive tape to the perineum and then examining it microscopically for the presence of ova. Both *S. muris and S. obvelata* can be transmitted between gerbils as well as between other species (*S. muris*: mice, rats, hamsters; *S. obvelata*: mice). Gerbils are the primary natural host of *Dentostomella translucid*, which resides in the proximal small intestine. *Dentostomella* infection is detected by the fecal flotation method. None of the oxyurids cause clinical problems. Ivermectin, piperazine, fenbendazole, or thiabendazole are effective treatments.

Tapeworms

Cestodiasis in gerbils is normally subclinical; however, severe infections with the dwarf tapeworm, *Rodentolepis nana*, have been reported. Infections have been associated with debilitation and diarrhea. *R. nana* is a concern because of its direct life cycle, transmission to other rodents, and zoonotic potential. Infected animals may be culled to eliminate the public health risk, although praziquantel and thiabendazole are effective treatments.

Neoplasia

The incidence of spontaneous tumors is relatively low in the gerbil. Neoplasms occur primarily in aged gerbils over 2 years old. The most commonly recognized neoplasms in gerbils are squamous cell carcinoma of the ventral marking gland in males, ovarian granulosa cell

tumors in females, adrenocortical tumors, cutaneous tumors such as squamous cell carcinoma and melanoma of the ear and foot, and renal and splenic hemangiomas.

Miscellaneous Conditions

Nasal Dermatitis (Sore Nose)

Nasal dermatitis, also known as facial eczema or sore nose, is most commonly encountered in recently weaned and adult gerbils. Affected animals initially exhibit alopecia around the external nares and upper labial region with a varying degree of red-brown, moist dermatitis and ulceration that may extend over the head, forelimbs, and torso. Chemical irritation of the skin by porphyrin-containing Harderian gland secretions is thought to be the primary cause. Mechanical trauma due to excessive burrowing may be an important contributing factor. Secondary infections with *Staphylococcus* spp. are common. Using sand as bedding material, providing access to a sand bath, cleaning the face, application of a topical antibacterial ointment, and removing stress factors may be helpful in treating the condition.

Tail Degloving (Tail Slip)

The gerbil tail is especially sensitive to degloving wounds (Figure 6.3), particularly if it is handled roughly or by the tip. The skin of the distal tail is pulled off, exposing the underlying vertebral tissue. If a tail degloving injury occurs, the tail should be amputated at the level of the skin break and the remaining skin closed over the tail tip.

Hair Loss (Barbering and Bald Nose)

Gerbils may barber each other if housed in overcrowded conditions or as a stereotypy. The hair of an animal is chewed off around the base of the tail or along the tail in a closely shaved pattern.

Bald nose can occur in gerbils housed in cages with wire top feeders. Gerbils can exhibit an aggressive feeding behavior by pushing their noses through the wire to obtain food.

Fig 6.3. Degloved tail. (Photo courtesy of M. Tear.)

Selecting an alternative feeding method resolves the problem. A similar condition can occur in gerbils that have vigorous burrowing habits. Providing cage enrichment in the form of tunnels or nesting materials usually diminishes the behavior.

Epileptiform Seizures

Gerbils exhibit spontaneous epileptiform seizures that vary from hypnotic and cataleptic to grand mal. The seizures may be initiated by handling, stress, loud noises, or novel environment. Typically, the episodes are brief, lasting up to a few minutes. Animals resume normal activity soon afterward and show no ill effects. Frequent handling of young animals has been reported to decrease episodes. Treatment is generally not recommended.

Chromodacryorrhea

The Harderian gland in the orbit produces porphyrin-rich secretions. The porphyrins are normally removed by grooming; however, when there is excessive secretion, a red crust appears in the medial canthus of the eye or on the end of the nose. Animals that are stressed or ill commonly exhibit these "red tears."

Malocclusion

Malocclusion is rarely a problem in the gerbil but may occur as a result of upper incisor loss with overgrowth of the lower teeth. Trimming the teeth with a dental bur may be necessary to prevent overgrowth.

Cystic Ovaries

Cystic ovaries are very common in females over 2 years of age and can be associated with reduced litter size, infertility, symmetrical alopecia, abdominal swelling, dyspnea, lethargy, and anorexia.

Chronic Interstitial Nephritis

Aged gerbils are susceptible to chronic interstitial nephritis. Clinical signs include polyuria, polydipsia, and weight loss.

Aural Cholesteatoma

Spontaneous aural cholesteatomas frequently occur in gerbils over 2 years of age. Clinical signs include head tilt and keratin mass accumulation in the external ear canal.

Ocular Proptosis

Aged gerbils can develop ocular proptosis, including protrusion of the nictitating membrane.

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CHAPTER 6 REVIEW

Multiple Choice

1. Scientific name of the Mongolian gerbil
   A. *Meriones unguiculatis*
   B. *Mus musculus*
   C. *Cricetulus griseus*
   D. *Oryctolagus cuniculus*
2. Compared with mice and rats, this is a very large organ relative to total body weight.
   A. thymus
   B. nonglandular forestomach
   C. kidney
   D. adrenal gland
3. Gestation length in a nonlactating gerbil is
   A. 30 days
   B. 24 days
   C. 42 days
   D. 21 days
4. Preferred orientation of gerbil to facilitate parenteral injection is
   A. horizontal
   B. vertical
   C. dorsal recumbent
   D. lateral recumbent

5. Gerbils are an important model for stroke research due to their
   A. high cholesterol levels
   B. large brain relative to body size
   C. incomplete circle of Willis
   D. lipemia
6. False statement regarding gerbils
   A. Neutrophil is predominant leukocyte.
   B. Life span of red blood cells is short.
   C. Reticulocytes are commonly seen in peripheral blood.
   D. Urine is highly concentrated.
7. To attract attention or express agitation, gerbils
   A. make a whistling sound
   B. sit upright on their hind limbs
   C. repeatedly scratch cage corner
   D. thump their hind limbs
8. Most common coat color of gerbil is
   A. albino
   B. piebald
   C. agouti
   D. black
9. Gerbils have a high incidence of spontaneous
   A. Addison's disease
   B. epileptiform seizures
   C. Cushing's disease
   D. kidney tumors
10. False statement regarding gerbil housing environment
   A. Bedding depth should be at least 1 inch.
   B. Cage height should be at least 6 inches.
   C. 12 hours of light should be provided.
   D. Humidity should be 60%–70%.
11. A degloving injury during handling usually involves
   A. front paw
   B. tail
   C. rear paw
   D. ear
12. Gerbils are highly susceptible to
   A. *Salmonella* spp.
   B. *Clostridium piliforme*
   C. *Staphylococcus aureus*
   D. Sendai virus

(*Continued*)

13. Barbering in the gerbil usually affects the
    A. tail
    B. flank
    C. ear
    D. head
14. Common problem in females over 2 years of age
    A. cystic ovaries
    B. pyometra
    C. endometriosis
    D. uterine adenocarcinoma
15. This gland is associated with chromodacryorrhea.
    A. ventral sebaceous
    B. adrenal
    C. Harderian
    D. submandibular salivary
16. Can be used to encourage a nonbreeding gerbil to breed except
    A. increase light to 14 hours per day
    B. provide a new mate
    C. house in an opaque cage
    D. add tunnels or huts to cage
17. Gauge of needle suggested for parenteral injections
    A. 20
    B. 25
    C. 22
    D. 18
18. Not recommended to use in the gerbil as it lowers seizure threshold
    A. ketamine
    B. xylazine
    C. isoflurane
    D. acepromazine
19. Antibiotic that is toxic in the gerbil
    A. penicillin
    B. tetracycline
    C. dihydrostreptomycin
    D. enrofloxacin
20. Gerbils are the primary host of this pinworm.
    A. *Dentostomella translucid*
    B. *Syphacia obveleta*
    C. *Aspiculuris tetraptera*
    D. *Syphacia muris*

7 Hamsters

Hamsters are rodents of the family Cricetidae. There are over 50 species, subspecies, and varieties of hamsters. Only two are found in any significant number in laboratories: the golden or Syrian hamster (*Mesocricetus auratus*) and the Chinese or striped hamster (*Cricetulus griseus*). The Syrian is by far the most frequently used, and the specific information provided in this chapter refers primarily to this strain. The adult Syrian weighs approximately 114–140 g and comes in a variety of colors, including reddish–golden brown with gray ventrum (wild-type), cinnamon, cream, white, piebald (patches of two or more colors, often including white), and albino. A long-haired variety also exists called a teddy bear; however, this type is not usually seen in research. Their life span is approximately 18–24 months. Nearly all Syrian hamsters supplied by U.S. commercial vendors are derived from three or four littermates captured in Syria in 1930. The Chinese hamster is much smaller than the Syrian, weighs approximately 30 g, and is gray–brown with a dark stripe down the back as shown in Figure 7.1. The Siberian or Djungarian hamster (*Phodopus sungorus*), also known as the dwarf or hairy-footed hamster, is infrequently found in research facilities. The Siberian hamster is mouse-sized, weighing 30–50 g. This hamster has a short furred tail, grayish dorsal surface with a black dorsal stripe, and white ventrum.

USES

Hamsters are important research animals although their use has decreased from the 1970s when the largest numbers of hamsters were used in the United States. Their small size, ease of handling, low care requirements, and relative freedom from spontaneous diseases are

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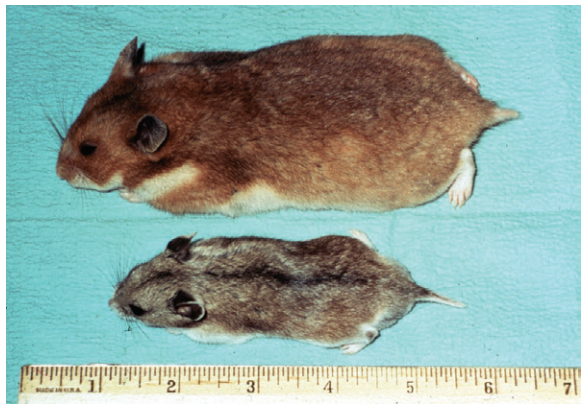

Fig 7.1. Size comparison of Syrian and Chinese hamsters. (Photo courtesy of ACLAM.)

positive attributes. Syrian hamsters have proven valuable in a number of areas of biomedical research, particularly in studies of infectious disease, cancer, immunology, hypothermia, dental caries, reproductive physiology, cardiomyopathy, thrombosis, and circadian rhythm. Chinese hamsters are used as a model of type 1 juvenile-onset diabetes and in cytogenetic studies (the study of chromosomes and their role in heredity), which take advantage of their low diploid chromosome number of 22. Both Syrian and Chinese hamsters have been used in radiobiology research as they are radioresistant (resistant to the effects of radiation).

Multiple inbred strains of hamsters have been developed as animal models of human and animal disease conditions and other research uses. These include the following: BIO 1.50 (muscular dystrophy, carcinogenicity, and dental caries); BIO 2.4 (cystic prostatic hypertrophy); BIO 4.24 (obesity and adrenal cortical tumors); BIO 12.14 (paralysis and muscular dystrophy); BIO 14.6 (cardiomyopathy and muscular dystrophy); BIO 15.16 (carcinogenicity studies); BIO 87.20 (cystic prostatic hypertrophy and carcinogenicity studies); BIO F1D (carcinogenicity studies); and CHF148 (cardiomyopathy).

BEHAVIOR

Hamsters tend to be solitary rather than social animals. Studies have shown that group housing is stressful and can lead to aggression and obesity. Hamsters are generally aggressive and territorial toward unfamiliar hamsters of either sex, but are less apt to fight if housed together at the time of weaning or before they are sexually mature. Females, in particular, are prone to fight or kill other hamsters, especially when pregnant or lactating. Hamsters frequently exhibit agonistic postures but are not naturally aggressive to their handlers. They, however, will often bite if suddenly disturbed or if handled roughly. They are easily tamed if handled gently and repeatedly.

In their natural desert environment, hamsters live in deep tunnels that provide cooler temperatures and higher humidity. Like most rodents, they are nocturnal although they do

exhibit periods of activity during the day. They are sound sleepers and, upon casual observation, may appear dead. Healthy hamsters are active and spend time storing and transporting food in their distensible cheek pouches. When exercise wheels are provided, they readily run on them and are reported to run up to 8 km (5 mi) per day. They make very little noise except to chatter or screech when hurt, frightened, or fighting.

The hamster is considered a permissive hibernator; it may or may not hibernate, depending upon environmental conditions. Low light intensity, short day lengths, quietness, and cooler temperatures of approximately 8°C (46°F) induce hibernation. During this period, the hamster has a decreased body temperature with extremely slow respirations and heart rate, yet it remains sensitive to touch. Hibernation is not continuous but consists of 2–3 days of inactivity alternating with short periods of arousal to normal alertness.

ANATOMIC AND PHYSIOLOGIC FEATURES

General biologic and reproductive data for the Syrian hamster are listed in Table 7.1. The hamster has a stout body with short legs; large, bright, black eyes; prominent dark ears; and a stubby tail. It has a large quantity of very loose skin that is covered with dense, soft, short fur. Hamsters have well-developed cheek (buccal) pouches that are located along the lateral neck and head and extend back to the shoulder region. Animals may fill the pouches with

Table 7.1. Biologic and reproductive data for Syrian hamsters

Adult body weight: Male	85–130 g
Adult body weight: Female	95–150 g
Life span	18–24 mo
Body temperature	37°–38°C (98.6°–100.4°F)
Heart rate	250–500 beats per minute
Respiratory rate	35–135 breaths per minute
Tidal volume	0.6–1.4 mL
Food consumption	8–12 g/100 g per day
Water consumption: Male	4.5–5 mL/100 g per day
Water consumption: Female	13.6–14 mL/100 g per day
Breeding onset: Male	10–14 wk
Breeding onset: Female	6–10 wk
Estrous cycle length	4 d
Gestation period	15–16 d
Postpartum estrus	Infertile
Litter size	5–9
Weaning age	20–25 d
Breeding duration	10–12 mo
Chromosome number (diploid)	44

Source: Adapted from Harkness et al. (2010) and Mulder (2012).

Fig 7.2. Compartmentalized stomach—forestomach and glandular stomach. (Photo courtesy of ACLAM.)

food. Females may place newborn pups in their pouches if stressed. When full, the check pouches can be mistaken as abnormal masses of the thorax. The cheek pouches are easily everted in an anesthetized animal, are devoid of glands, and are immunologically privileged sites as they largely lack lymphatic drainage. Transplantation of foreign tissue to the cheek pouch does not cause immunological rejection. Hamsters have a Harderian gland located posterior to each globe in the ocular orbit. These exocrine glands secrete lipid- and porphyrin-rich material that lubricates the eyes and conjunctiva.

Typical of rodents, hamsters have a dental formula of 2(I 1/1, C 0/0, P 0/0, and M 3/3) with fixed roots). The teeth turn yellow-orange with age due to the composition of the dental enamel and, secondary to their shape, tend to retain bits of food near the crowns, predisposing hamsters to dental caries. The mandibular symphysis may not fuse even in adulthood. The stomach is distinctly compartmentalized into a forestomach and a glandular stomach as shown in Figure 7.2. The nonglandular forestomach acts like a rumen and has a higher pH than the glandular stomach. The digestive tract suggests an adaptation for water conservation, having a long duodenum and jejunum, short ileum, large cecum, and long colon. The lungs consist of a large, single left lobe and five smaller lobes on the right side. The hamster kidney has an extremely long papilla that extends out into the ureter, which allows *in vivo* collection of urine samples from single collecting tubules.

Adult hamsters have prominent deposits of brown adipose tissue, the majority of which are located ventral to and between the scapulae. This tissue plays a thermogenic role, particularly in hibernating hamsters and in newborns. Two flank glands, also called hip or costovertebral glands, are present in both sexes but are much more developed in the male. These sebaceous glands are present in each flank region in areas of darkly pigmented and roughened skin that is covered with coarse hairs. The overlying fur may appear wet due to gland excretions. The glands are used to mark territory and are involved in mating behavior. In males, they also play a role in converting testosterone to dihydrotestosterone. They are frequently mistaken as an abnormal feature (e.g., tumor) by those unfamiliar with hamster anatomy.

Differentiation between adult males and females is rather simple and can be determined without handling the animal. When viewed from above, the male's hindquarters appear more

pointed. The tail is elevated due to the presence of the underlying large scrotal sac. The female's hindquarters are broader and more rounded. Adult males have prominent testicles that can retract through the open inguinal canals. Applying gentle pressure to the male's caudal abdomen will cause the testicles to descend into the scrotal sac. Male hamsters have an os penis as well as large seminal vesicles that are located on each side of the bladder and resemble a ram's horns. Adult females are larger than males and have on average seven pairs (range = 6–11 pairs) of mammary glands with mammae visible on their ventral surface; males lack mammae. Two cervical canals lead to a duplex uterus. The female has three orifices in the anogenital area—the urethral, vaginal, and anal—and displays a shorter anogenital distance than the male. Newborns can be sexed by comparing the anogenital distance. The anogenital distance is greater in the male, and the genital papilla of the male is more prominent than that of the female.

There are several distinctive characteristics of the hematologic and urinary profiles of hamsters. Leukocyte counts are 5,000–10,000/μL, with the lymphocyte being the most dominant leukocyte. Polychromasia is relatively common in hamster erythrocytes. Hamster urine has a basic pH and appears turbid and milky due to the presence of calcium carbonate crystals. Hamsters excrete approximately 7 mL of urine daily. Select hematologic and biochemical values are listed in Appendix 1, "Normal Values."

BREEDING AND REPRODUCTION

Males and females reach sexual maturity at approximately 12 weeks of age and 90 g (males) or 90–100 g (females) of body weight. The female hamster is continuously polyestrous, cycling every 4 days. There is a normal seasonal breeding quiescence, however, in the winter months. Ovulation is spontaneous and follows lordosis onset by approximately 8 hours. A white, stringy, opaque, and odiferous postovulatory discharge is present on the second day of the cycle. This normal discharge indicates that the hamster reached peak estrus the day before and its timing can be used to predict optimal breeding time. The female can be successfully mated in the evening of the third day after the observance of the postovulatory discharge.

Except during estrus, the female will usually attack a newly introduced male. Because of this behavior, it is best to observe the pair closely for compatibility and to leave them together for only 30 minutes although a pair may be left together overnight if mating activity is observed within the first approximately 10 minutes they are paired. When a receptive female is placed with a male, after a short familiarization period, she will assume the lordosis position, an arching of the lower back with a wide rear leg stance that facilitates copulation. Mating is characterized by repeated intromissions and ejaculations. Signs of pregnancy include a distended abdomen and rapid weight gain at 10 days postmating. Pregnancy can be confirmed by the absence of a postovulatory discharge on days 5 and 9 after mating. The postpartum estrus is usually anovulatory. Pseudopregnancies do occur.

The gestation period, the shortest of any of the common laboratory animals, averages 16 days. The female is frequently restless before parturition. A bloody vaginal discharge may be seen prior to delivery of the first fetus. Litter size is usually five to nine pups. At birth, pups are hairless, have closed eyes and ears, but have fully erupted incisors. Ears open at

4–5 days of age, hair growth is noticeable at 9 days, and eyes open at 14–16 days. Young start eating food and drinking water when they are 7–10 days of age. Care should be taken to ensure the young can reach the water supply and are provided with food on the floor of the cage. Young are weaned at 3 weeks of age. A fertile estrus follows weaning by 2–18 days.

Cannibalism of the young and litter abandonment are common during the first pregnancy, the first week postpartum, and anytime a dam is stressed. To reduce this risk, the female should be placed in a clean cage with clean bedding, food, and nesting material at 14 days after mating to minimize the need to change the cage immediately before or soon after parturition. Housing in opaque or tinted cages and in a quiet area is also helpful. To minimize maternal stress, the dam and pups should be disturbed as little as possible during the most critical 7–10 day postdelivery period. If the cage must be disturbed during this period, it may be helpful to first provide the female with preferred food items. The food may be ingested to fill the cheek pouches, thereby deterring her from cannibalizing the young. The reproductive efficiency of both sexes decreases by 14 months of age.

HUSBANDRY

Housing and Environment

A variety of caging used for other laboratory rodents is satisfactory. Hamsters prefer solid-bottom cages with contact bedding. An adult hamster over 100 g requires a floor area of at least 123 cm^2 (19 $in.^2$) and a cage height of at least 15 cm (6 in.). Caging materials should be carefully selected to preclude destruction and escape because hamsters are noted escape artists. They frequently will dislodge cage lids and/or gnaw on caging materials, escape, and evade capture. They do not return to their cages as will rats and gerbils. Rigid plastic and stainless steel are acceptable caging materials. Ground corn cobs, paper products, and hardwood chips can be used as bedding materials. The use of cedar or untreated softwood bedding is not recommended as they emit aromatic hydrocarbons that can adversely affect liver metabolism. Additional items may be added to the cage to increase cage complexity (e.g., huts or tubes) or for the animal to gnaw or manipulate.

Environmental parameters for hamsters, recommended in the *Guide for the Care and Use of Laboratory Animals* (ILAR, 2011), include a temperature between 20° and 26°C (68° and 79°F), relative humidity between 30% and 70%, and 10–15 air changes per hour. Typically, 12–14 hours of light per day is provided (14 hours for breeders), most frequently controlled by automatic timers.

Sanitation

Hamsters are fastidious in that they will use different locations of the cage in which to store food, urinate, and defecate. Weekly cleaning of the cage usually suffices because hamsters produce little waste or odor. Cages, bottles, and feed hoppers should be disinfected with chemicals, hot water 61.6°–82.2°C (143°–180°F), or a combination of both. Detergents and chemical disinfectants enhance the effectiveness of hot water, but they must be thoroughly rinsed from all caging surfaces with fresh water. Soaking or rinsing the cages with an acid solution may help to remove urine scale (mineral) buildup. Refer to Chapter 4, "Mice," for a more detailed description of disinfection methods.

Environmental Enrichment and Social Housing

An animal's social needs should be carefully considered when housing animals in the laboratory environment. Enrichment should increase the normal behavior for the species and decrease abnormal or stereotypic behaviors. Paper or plastic huts as well as tunnels (e.g., PVC piping) can provide hiding places and may, if appropriately designed, help to decrease cagemate aggression. Animals enjoy burrowing in deep bedding. Running wheels can provide a source of exercise; hamsters enjoy running on exercise wheels and have been reported to run up to 5 miles a day, mostly during dark hours. Items that can be directly manipulated by the animal may also be beneficial. Both males and females build nests, with nest-building activity increasing in females during pregnancy and lactation. Commercially prepared nesting material, such as Nestlets, may be provided; however, synthetic small-rodent stringlike nesting fiber should not be used as it can wind around the feet or teeth or cause a gastrointestinal impaction. All enrichment items must be reviewed and approved by the IACUC, veterinarian, and principal investigator prior to their use.

Feeding and Watering

Fresh, properly stored commercial pelleted rodent diets that contain 15%–25% protein, 35%–40% carbohydrate, 4%–5% fat, and 5% crude fiber are adequate for hamsters. Mixed seed diets are not appropriate as hamsters are often selective when fed mixed seed diets, eating favorite items and not receiving a balanced diet. Examples of potential consequences include a nutritional myopathy caused by a vitamin deficiency and alopecia precipitated by a low-protein diet. These conditions are uncommon in modern research facilities in which animals are fed appropriate diets, but they are seen in pet hamsters. Hamsters eat an average of 8–12 g/100 g of body weight per day. Young start to consume solid food at 7–10 days of age. Diet can be provided in a slotted, sheet metal hopper or a wire mesh feeder. Slot widths of less than 11 mm in food hoppers, as may be used with mice or rats, should be avoided as hamsters have difficulty reaching food because of their broad noses. Hamsters hoard food and will frequently remove pellets from the feeder and store them on the cage floor or in a corner of the cage. It is permissible, according to the Animal Welfare Act, to place pelleted feed on the floor of a primary enclosure for this species. Although hamsters enjoy fruits and vegetables, it is neither necessary nor suggested to supplement their diets. Fresh, potable water should be available *ad libitum*. Water can be provided by bottle, sipper sack, or automatic watering system. On average, hamsters drink approximately 8–10 mL of water per 100 g of body weight per day although the range does differ between sexes (males, 4.5–5 mL/100 g per day; females, 13.6–14 mL/100 g per day). Hamsters are coprophagic and may consume their own feces as they are excreted from their rectum. Coprophagy serves to recycle B vitamins and other nutrients.

TECHNIQUES

Handling and Restraint

To avoid an aggressive startle response, it is important to ensure the animal is awake and expects to be handled. A hamster can be transferred from one cage to another by cupping the hands under the animal, using a small cup or tube, or grasping a pinch of skin at the

Fig 7.3. Restraint of the hamster using whole-hand grip. (Photo courtesy of M. Tear.)

nape of the neck. Restraint may be accomplished by grasping all of the loose skin at the nape of the neck and back. It is important to immobilize the hamster fully to prevent handler injury (Box 7.1). This restraint method is commonly referred to as scruffing, shown in Figure 7.3. Protective gloves and forceps are not recommended because they may cause aggressive behavior.

Box 7.1

It is important to immobilize the hamster fully to prevent handler injury.

Identification

Cage cards are most often used as a form of general identification in a research setting. Ear punching, ear tagging, or subcutaneous microchip implantation may be used to permanently identify individual animals. Dyes, permanent markers, or fur clipping can be used as temporary identification methods.

Blood Collection

Assuming the animal is mature, healthy, and on an adequate plane of nutrition, the total blood volume of most species averages 7% of the body weight in grams. Table 7.2 lists approximate adult hamster blood volumes. A typical rule of thumb for blood withdrawal suggests that up to 10% of the circulating blood volume can be withdrawn from normal, healthy animals every 2–3 weeks with minimal adverse effect. Small amounts of blood can be obtained by retroorbital bleeding of the orbital venous sinus under anesthesia, by capillary tube collection from limb veins (e.g., lateral metatarsal vein), and from the jugular (anesthesia required). The retroorbital bleeding technique is similar to the technique described in Chapter 4; as with the mouse, the retroorbital sinus may be accessed from both the lateral

Table 7.2. Adult hamster blood volumes and suggested sample size

	Volume (mL)
Total blood	6.8–12
Single sample	0.5–1.2
Exsanguination	3–5

Source: Adapted from Harkness et al. (2010).
Note: Values are approximate.

and the medial canthus of the eye. When using the lateral canthus technique, the capillary tube should be directed medially. Use of a topical ophthalmic anesthetic is highly recommended to help alleviate postprocedural discomfort. Larger blood samples can be obtained via cardiac puncture in an anesthetized animal. Cardiac sampling is associated with significant mortality and should be used only as a terminal blood collection method. Hamster blood quickly coagulates; therefore care must be taken to use appropriate anticoagulants.

Urine Collection

Urine may be collected using a metabolic cage that separates the urine and fecal pellets. Other methods to collect 1–2 drops of urine include briefly restraining the hamster, placing it on a cold metal surface, or placing it in a plastic bag, each of which may stimulate urination.

Drug Administration

Most drug administration techniques used in small animal practice can be used with hamsters when modified to fit their smaller size. To avoid over- or under-dosing medications, small scales are useful for obtaining accurate body weights for drug dose calculations. To increase dosing accuracy of small drug volumes, substances may be diluted to larger, more easily measured volumes and syringes used that are designed for small volume administration (e.g., tuberculin syringe).

In some situations, medications can be administered orally by mixing them in the water or feed. Many factors may affect a hamster's willingness to drink or eat medicated water or food. Therefore, administration of all substances, especially analgesics, in feed or drinking water is discouraged unless it can be shown that all animals will consume required amounts. A tuberculin syringe can be used to administer a liquid into the oral cavity. For greater accuracy or for administering unpalatable liquids, a specialized gavage needle can be used to deliver a substance directly into the stomach. Both rigid metal and flexible plastic gavage needles are available. The ball at the end of the gavage needle helps ensure that the needle does not pass into the trachea. A maximum oral volume of 2 mL is suggested.

Small-gauge hypodermic needles, such as 23 or 25 gauge, should be used for injections. Medications can also be administered by subcutaneous injection, with a maximum volume of 3–4 mL, using the loose skin over the shoulders. Intramuscular injections are not frequently used because hamsters have such small muscle mass. Another drawback to the intramuscular route is that certain drugs such as ketamine can cause muscle necrosis and

Table 7.3. Maximum adult hamster dosing volumes

Route	Amount (mL)
IP	3–4
SC	3–4
IM	0.1
IV	0.3
PO	2.0

Source: Adapted from Harkness and Wagner (1995) and Lukas (1999).

nerve irritation that can lead to self-mutilation of the affected area. If the intramuscular route must be used, a maximum volume of 0.1 mL is recommended. In the hamster, as with other small rodents, medications and injectable anesthetics are most commonly administered by the intraperitoneal route. A maximum volume of 3–4 mL is suggested. When receiving an intraperitoneal injection, some recommend that the animal's head be tilted downward in an effort to avoid puncture of the intestines. The injection is made immediately to the right or left of the midline in the lower half of the abdomen. Intravenous injections are most frequently made into the cephalic vein, the jugular vein, or the lateral metatarsal vein. A maximum volume of 0.3 mL is recommended for the intravenous route. Suggested maximum dosing volumes are listed in Table 7.3.

Anesthesia, Surgery, and Postoperative Care

A variety of agents that can be used to tranquilize and anesthetize hamsters are listed in Table 7.4. Preanesthetic fasting is not recommended as the hamster has a high metabolic rate and, as such, is prone to hypoglycemia and hypothermia. Single-injection anesthesia techniques are generally used to minimize handling stress. Fairly consistent anesthesia can be induced by the intraperitoneal administration of a ketamine–xylazine mixture. Ketamine can also be combined with an inhalant anesthetic such as isoflurane.

Hamsters can be placed in an induction chamber or masked down with an inhalation anesthetic delivered from a precision vaporizer. Isoflurane and sevoflurane should be administered via a precision vaporizer with a mask or nose-cone apparatus in conjunction with a nonrebreathing system. Alternatively, very brief (approximately 1–2 minutes) anesthesia can be produced through use of a simple anesthetic chamber. See Chapter 4 for additional information.

While anesthetized, hamsters should have bland, sterile ophthalmic ointment placed in their eyes to prevent exposure keratitis. The depth of anesthesia in hamsters can be estimated by observation of the righting reflex, muscle tone, pedal withdrawal reflex, abdominal skin pinch reflex, respiratory rate and depth, and color of mucous membranes and skin.

Surgery should be done in an aseptic manner. Prevention of blood loss is an important consideration. Surgical procedures are most frequently performed for experimental rather than for therapeutic purposes. Nephrectomy, adrenalectomy, thymectomy, and ovariectomy are a few of the surgeries most commonly performed in research. The hamster oocyte is the only rodent ovum that will accept sperm from any other species. As a consequence, female

Table 7.4. Anesthetic agents and tranquilizers used in hamsters

Drug	Dosage	Route	Reference
Inhalants			
Isoflurane	1%–4% to effect	Inhalation	Wixson (1994)
Sevoflurane	To effect	Inhalation	Morrisey and Carpenter (2004)
Injectables			
Acepromazine	2.5 mg/kg	IP	Flecknell (2009)
Alphadolone—alphaxalone	150 mg/kg	IP	Flecknell (2009)
Chloral hydrate	270–360 mg/kg	IP	Hughes (1981)
Diazepam	5 mg/kg	IP	Flecknell (2009)
Fentanyl–droperidol	Not recommended because of induction of CNS abnormalities		Thayer et al. (1972)
Fentanyl–fluanisone	0.3–0.6 mL/kg	IP	Flecknell (2009)
Fentanyl–fluanisone	1 mL/kg	IP	Flecknell (2009)
+ diazepam	5 mg/kg	IP	
Ketamine	200 mg/kg	IP	White and Field (1987)
Ketamine	150 mg/kg	IP	Flecknell (2009)
+ acepromazine	5 mg/kg	IP	
Ketamine	100 mg/kg	IP	Harkness et al. (2010)
+ diazepam	5 mg/kg	IP	
Ketamine	100 mg/kg	IP	Flecknell (2009)
+ medetomidine	0.25 mg/kg	IP	
Ketamine	50–200 mg/kg	IP	Curl and Peters (1983)
+ xylazine	10 mg/kg	IP	
Ketamine	200 mg/kg	IP	Flecknell (2009)
+ xylazine	10 mg/kg	IP	
Medetomidine	0.03–0.1 mg/kg	SC, IP	Flecknell (2009)
Midazolam	5 mg/kg	IP	Flecknell (2009)
Pentobarbital	70–90 mg/kg	IP	White and Field (1987)
Tiletamine–zolazepam	30 mg/kg	IP	Forsythe et al. (1992)
+ xylazine	10 mg/kg	IP	
Urethane (50% w/v)	150 mg/100g	IP	Reid et al. (1989)
Xylazine	1–5 mg/kg	IP	Flecknell (2009)

IP = intraperitoneal; SC = subcutaneous.

hamsters may be ovariectomized and their oocytes utilized in human male fertility testing and research. When castrating a hamster, it is important to close the normally open inguinal canals.

The anesthetized hamster should be placed on a clean, dry paper or cloth towel and recovered in an escape-proof incubator. Use of a circulating water heating blanket or self-regulating heating pad promotes euthermia and is recommended. Generic, electric heating pads should never be placed in direct contact with the animal as they may cause thermal burns. Small-particle bedding materials are contraindicated in recovery cages because they

can stick to the eyes, nose, and mouth of the recovering animal and may be aspirated. Postoperative care should include hydration of the anesthetized animal (e.g., subcutaneous or intraperitoneal injection of a warmed solution such as saline, saline–dextrose, lactated Ringer's), and support of normal body temperature. Hypostatic pulmonary congestion should be prevented by turning the animal at least every 30 minutes. It is important to maintain hydration and nutrition levels in the postoperative period to support normal metabolic processes and gut physiology. Select, commercially available supportive and critical care products for laboratory animals are provided in Appendix 3.

When in pain, hamsters are often reluctant to move, have a hunched body appearance, and are more aggressive. Butorphanol is recommended for mild postoperative discomfort. Buprenorphine works well to control acute or chronic visceral pain but causes more sedation. Hamsters are markedly resistant to morphine's hypnotic and sedative effects, and require higher doses than usually required in other species. The nonsteroidal anti-inflammatory drug carprofen is a useful analgesic that requires only once-daily dosing and is effective against mild to moderate incisional, inflammatory, and visceral pain. Analgesic agents that can be used in hamsters are listed in Table 7.5.

Imaging Techniques

Great advances have been made in rodent and small animal imaging over the last decade. Many imaging modalities once used only in humans [e.g., digital x-ray imaging, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), dual-energy x-ray absorptiometry (DEXA) scan, and ultrasound] have been adapted for use with these species, producing images of exquisitely fine detail. In addition, novel imaging techniques have been developed for use in animal research, including *in vivo* optical imaging.

Table 7.5. Analgesic agents used in hamsters

Drug	Dosage	Route	Reference
Acetaminophen	1–2 mg/mL drinking water		Huerkamp (1995)
Acetylsalicylic acid	240 mg/kg q24h	PO	Harkness et al. (2010)
Buprenorphine	0.1–0.5 mg/kg q8h	SC	Harkness et al. (2010)
Butorphanol	1–5 mg/kg q4h	SC	Harkness et al. (2010)
Carprofen	5 mg/kg q24h	SC	Pollack (2002)
Flunixin meglumine	2.5 mg/kg q12–24h	SC	Heard (1993)
Ketoprofen	5 mg/kg	SC	Pollack (2002)
Meperidine	20 mg/kg q2–3h	SC	Heard (1993)
Syrup	0.2 mg/mL drinking water		Huerkamp (1995)
Morphine	2–5 mg/kg q2–4h	SC	Heard (1993)
Nalbuphine	4–8 mg/kg	SC	Mason (1997)
Oxymorphone	0.2–0.5 mg/kg q6–12h	SC	Heard (1993)
Pentazocine	10 mg/kg q2–4h	SC	Heard (1993)
Piroxicam	3 mg/kg q24h	PO	Harkness et al. (2010)
Tramadol	5–10 mg/kg q12–24h	PO	Morrisey and Carpenter (2011)

IM = intramuscular; PO = per os; SC = subcutaneous.

For conventional x-ray imaging, an x-ray machine with high milliamperage, such as a 200- or 300-mA unit with the capability for low settings of peak voltage delivered (kVp) and small incremental changes, is ideal. High-detail film–screen combinations work well. Short exposure times of 1/40 second or less should be used to decrease the chance of motion artifact. Suggested exposure factors are 42–46 kVp, 300 mA, 1/40 second, 40 SID, 7.5 mA·s. Alternatively, nonscreen film can be used with standard x-ray or dental units. Nonscreen film produces high-detail radiographs, but longer exposure times are needed. Digital imaging techniques offer substantial flexibility in image enhancement and delivery application. The best method of restraint is to sedate the animal and use adhesive tape to secure it to the cassette in the appropriate position. A radiolucent tube or stockinette with the patient positioned inside can be used for restraint of conscious animals.

Euthanasia

Methods of euthanasia must be consistent with the most current American Veterinary Medical Association (AVMA) *Guidelines for the Euthanasia of Animals* (AVMA, 2013) and performed by trained personnel. Per AVMA Guidelines, "Carbon dioxide is acceptable with conditions for euthanasia in those species where aversion or distress can be minimized. Carbon dioxide exposure using a gradual fill method is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness; a displacement rate from 10% to 30% of the chamber volume/min is recommended. Whenever gradual displacement methods are used, CO_2 flow should be maintained for at least 1 minute after respiratory arrest. If animals need to be combined, they should be of the same species and, if needed, restrained so that they will not hurt themselves or others. Oxygen administered together with CO_2 appears to provide little advantage and is not recommended for euthanasia. The practice of immersion, where conscious animals are placed directly into a container prefilled with 100% CO_2, is unacceptable. Carbon dioxide and CO_2 gas mixtures must be supplied in a precisely regulated and purified form without contaminants or adulterants, typically from a commercially supplied cylinder or tank. The direct application of products of combustion or sublimation is not acceptable due to unreliable or undesirable composition and/or displacement rate [author's note: e.g., dry ice]. As gas displacement rate is critical to the humane application of CO_2, an appropriate pressure-reducing regulator and flow meter or equivalent equipment with demonstrated capability for generating the recommended displacement rates for the size of the container being utilized is absolutely necessary." Neonates are resistant to hypoxia and require greater duration of exposure. For this reason, other methods may be preferred for this age group. Studies have shown that the method of CO_2 euthanasia can result in significant differences in immunologic and hematologic variables; thus consistency of method may be important in accurate interpretation of research data. Other acceptable methods of euthanasia include overdose of an inhalant anesthetic and intraperitoneal administration of a barbiturate anesthetic at three to four times the anesthetic dose.

THERAPEUTIC AGENTS

Suggested hamster antimicrobial and antifungal drug dosages are listed in Table 7.6. Antiparasitic agents are listed in Table 7.7, and miscellaneous drugs are listed in Table 7.8.

Table 7.6. Antimicrobial and antifungal agents used in hamsters

Drug	Dosage	Route	Reference
Amikacin	2–5 mg/kg q8–12h	SC	Harkness et al. (2010)
Amoxicillin	Do not use—toxic		Flecknell (1987)
Ampicillin	Do not use—toxic		Flecknell (1987)
Amphotericin B	1 mg/animal q12h 5d/wk for 3wk	SC	Laber-Laird et al. (1996)
Cephaloridine	10–25 mg/kg q24h	SC	Harkness et al. (2010)
Chloramphenicol	30–50 mg/kg q12h	SC, PO	Burgmann and Percy (1993)
Chlortetracycline	20 mg/kg q12h	PO, SC	Allen et al. (1993)
Ciprofloxacin	7–20 mg/kg q12h	PO	Harkness et al. (2010)
Doxycycline	2.5 mg/kg q12h	PO	Allen et al. (1993)
Enrofloxacin	5–10 mg/kg q12h	PO, SC	Harkness et al. (2010)
	0.05–0.2 mg/mL drinking water for 14d		Harkness et al. (2010)
Erythromycin	0.13 mg/mL drinking water continuously; use with caution, can cause enterotoxemia		Burke (1995)
Gentamicin	5 mg/kg q24h	SC	Anderson (1994)
Griseofulvin	25–50 mg/kg q12h for 14–60 d	PO	Harkness et al. (2010)
	1.5% in DMSO for 5–7d	Topical	Harkness et al. (2010)
Ketoconazole	10–40 mg/kg q24h for 14d	PO	Rollin and Kesel (1995)
Metronidazole	7.5 mg/70–90g animal q8h	PO	Collins (1995)
Neomycin	100 mg/kg q24h	PO	Burgmann and Percy (1993)
	0.5 mg/mL drinking water		Anderson (1994)
Oxytetracycline	0.25–1 mg/mL drinking water		Burgmann and Percy (1993)
	16 mg/kg q24h	SC	Collins (1995)
Sulfadimethoxine	10–15 mg/kg q12h	PO	Harkness et al. (2010)
Sulfamerazine	1 mg/mL drinking water		Anderson (1994)
Sulfamethazine	1 mg/mL drinking water		Anderson (1994)
Sulfaquinoxaline	1 mg/mL drinking water		Burgmann and Percy (1993)
Tetracycline	10–20 mg/kg q8–12h	PO	Burgmann and Percy (1993)
	0.4 mg/mL drinking water		Burgmann and Percy (1993)
Trimethoprim–sulfa	15–30 mg/kg q12–24h	PO, SC	Harkness et al. (2010)
Tylosin	2–8 mg/kg q12h	SC, PO	Burgmann and Percy (1993)
	0.5 mg/mL drinking water		Burgmann and Percy (1993)
Vancomycin	20 mg/kg for 3+ months	PO	Boss et al. (1994)

DMSO = dimethyl sulfoxide; IM = intramuscular; PO = per os; SC = subcutaneous.

Table 7.7. Antiparasitic agents used in hamsters

Drug	Dosage	Route	Reference
Amitraz	1.4 mL/L water using cotton-ball application q14d for 3–6 treatments	Topical	Harkness et al. (2010)
Carbamate (5%)	Twice weekly	Topical	Harkness et al. (2010)
Fenbendazole	20 mg/kg q24h for 5d	PO	Allen et al. (1993)
Fipronil	7.5 mg/kg q30–60d	Topical	Richardson (1997)
Ivermectin	0.2–0.4 mg/kg q7–10d	PO, SC	Harkness et al. (2010)
Lime sulfur	Dilute 1:40 with water, dip q7d for 6wk		Anderson (1994)
Piperazine citrate	2–5 mg/mL drinking water for 7d, off 7d, then repeat		Anderson (1994)
Pyrantel pamoate	50 mg/kg	PO	Rollin and Kesel (1995)
Praziquantel	6–10 mg/kg	PO	Harkness et al. (2010)
Pyrethrin powder	Three times a week for 3wk	Topical	Anderson (1994)
Thiabendazole	100–200 mg/kg	PO	Harkness et al. (2010)

PO = per os; SC = subcutaneous.

Table 7.8. Miscellaneous agents used in hamsters

Drug	Dosage	Route	Reference
Atipamezole	1 mg/kg	SC, IP, IV	Flecknell (1997)
Atropine	0.05–0.1 mg/kg	SC	Harkness and Wagner (1995)
	10 mg/kg q20min for organophosphate overdose	SC	Harkness and Wagner (1995)
Cimetidine	5–10 mg/kg q6–12h	PO	Allen et al. (1993)
Dexamethasone	0.5–2.0 mg/kg then decreasing dose q12h for 3–14d	PO, SC	Harkness and Wagner (1995)
	0.6 mg/kg	IM	Anderson (1994)
Digoxin	0.05–0.1 mg/kg q12–24h	PO	Morrisey and Carpenter (2004)
Diphenhydramine	1–2 mg/kg q12h	PO, SC	Morrisey and Carpenter (2004)
Doxapram	5–10 mg/kg	IP, IV	Harkness (1993)
Furosemide	1–4 mg/kg q4–6h	SC	Harrenstien (1994)
Glycopyrrolate	0.01–0.02 mg/kg	SC	Huerkamp (1995)
Insulin	2 U/animal	SC	Laber-Laird et al. (1996)
Lactobacilli	Administer during antibiotic treatment period, then 5–7d beyond cessation	PO	Collins (1995)
Lactulose syrup	0.5 mL/kg q12h	PO	Morrisey and Carpenter (2011)
Loperamide hydrochloride (Imodium A–D)	0.1 mg/kg q8h for 3d then q24h for 2d, given in 1 mL of water	PO	Harkness and Wagner (1995)
Naloxone	0.01–0.10 mg/kg	SC, IP	Huerkamp (1995)
Oxytocin	0.2–3 IU/kg	SC	Anderson (1994)
Prednisone	0.5–2.2 mg/kg	SC, PO	Anderson (1994)
Sulfacrate	25–50 mg/kg	PO	Morrisey and Carpenter (2004)
Vitamin A	50–500 IU/kg	IM	Laber-Laird et al. (1996)
Vitamin B complex	0.02–0.2 mL/kg	SC	Anderson (1994)
Vitamin D	200–400 IU/kg	SC	Anderson (1994)
Vitamin E–selenium (Bo-Se)	0.1 mL/100–250g	SC	Anderson (1994)
Vitamin K	1–10 mg/kg q24h for 4–6d	IM	Harkness and Wagner (1995)
Yohimbine	0.5–1 mg/kg	IV	Harkness and Wagner (1995)

IM = intramuscular; IP = intraperitoneal; IV = intravenous; PO = per os; SC = subcutaneous.

INTRODUCTION TO DISEASES OF HAMSTERS

Most laboratory rodent colonies today are relatively free of the viruses, bacteria, parasites, and fungi that cause clinical disease. However, sporadic outbreaks may still occur, and it is important for individuals to have a basic knowledge of the most common infectious agents and how to respond to them. Presented here is a general overview of common bacterial, viral, fungal, and parasitic agents of hamsters as well as standard diagnostic and treatment modalities.

General signs of illness include loss of coat and body condition, hunched posture, ocular discharge, weight loss, and extended periods of daytime inactivity. Hypothermia, dehydration, and anorexia are common and need to be treated aggressively with supportive care.

Bacterial Diseases

Antibiotic-Associated Enterocolitis

The potential value of treating a hamster with a microbial agent must be carefully weighed against the risk of inducing antibiotic-associated enterocolitis (Box 7.2). Care must be used in selecting the antibiotic and length of therapy. Discrepancies exist among reports of antimicrobial use in hamsters. Many antimicrobial agents are highly toxic for hamsters, even when the agent is given in a single, low dose. Lincomycin, clindamycin, ampicillin, vancomycin, erythromycin, cephalosporins, gentamicin, and penicillin have all been associated with enterocolitis.

Box 7.2

The potential value of treating a hamster with a microbial agent must be carefully weighed against the risk of inducing antibiotic-associated enterocolitis.

Hamster intestinal microbial flora is predominantly of the bacterial genera *Lactobacillus* and *Bacteroides*, with few coliforms. Without external disruption, the normal flora controls the proliferation of other organisms and any toxins they may produce. Antibiotic-associated enterocolitis may develop when antibiotics (such as those listed in the preceding paragraph) are administered that disrupt the normal intestinal microbial flora, facilitating the proliferation of *Clostridium difficile* organisms and the toxins A and B that they produce. Clinical signs are similar regardless of the antimicrobial agent involved and include anorexia, ruffled fur, dehydration, and profuse diarrhea. Most affected hamsters die within 4–10 days of antibiotic administration. Some animals do not develop clinical signs but are found dead. The characteristic gross lesion on necropsy is a hemorrhagic ileocolitis. Treatment may consist of discontinuing antibiotic administration, providing a *Lactobacillus* supplement to help reestablish normal intestinal flora diversity, and providing supportive care. Gram-negative antibiotics should be continued if overgrowth of a specific Gram-negative organism (e.g., *C. difficile*) is confirmed through culture. It has been reported that hamsters could be protected against fatal *C. difficile* enteritis by long-term, daily oral administration of vancomycin or metronidazole although disease relapse may occur following antibiotic withdrawal due to the persistence of *C. difficile* spores, continuing environmental contamination, and lack of appropriate immunity. Administration of rifalazil, a new benzoxazinorifamycin, was

not associated with disease relapse following withdrawal and therefore may be a better treatment for this condition.

It is important to note that *C. difficile* toxin production with subsequent morbidity and mortality can occur, even when no antibiotics have been administered. In these situations, it is suspected that another stressful event has resulted in disruption of the intestinal flora. *C. difficile* can also induce a fatal disease in humans.

Proliferative Ileitis—Transmissible Ileal Hyperplasia, Hamster Enteritis, and Wet Tail

Proliferative ileitis is the most common spontaneous disease of hamsters (Box 7.3). The causative agent is the Gram-negative bacteria, *Lawsonia intracellularis*, the same agent that induces proliferative enteritis in swine, ferrets, and rabbits. The organism is predominantly transmitted by the fecal–oral route and disease is usually confined to young animals, 3–8 weeks of age. Mortality is often high (50%–90%); death usually occurs within 24–48 hours of symptom development although death may occur in the absence of clinical signs. Stress plays an important role in susceptibility to clinical disease, with weaning, improper diet, transportation, crowding, and poor sanitation serving as significant predisposing factors. Clinical signs include unkempt hair coat; anorexia; a moistened perineal area; foul-smelling, watery diarrhea; and dehydration. Figure 7.4 shows a typical presentation of the perineal area. However, no clinical signs may be noted. On abdominal palpation, the animal frequently seems uncomfortable, and distended bowel loops are noted. Intussusceptions and rectal prolapses can occur. Typical gross necropsy lesions include gas and yellow diarrhea in the distal intestinal tract as well as a segmentally thickened and edematous ileum.

Prognosis is grave. To be effective, antibiotics must be administered early in the course of disease. Tetracycline, metronidazole, enrofloxacin, and trimethoprim–sulfa combinations

Box 7.3

Proliferative ileitis is the most common spontaneous disease of hamsters.

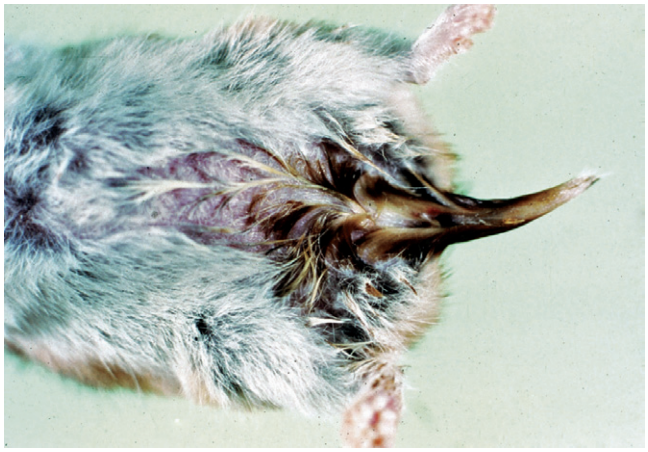

Fig 7.4. Typical presentation of the perineal area in proliferative ileitis. (Photo courtesy of ACLAM.)

are recommended. Chloramphenicol has also been used with some success. Administering warmed fluids intraperitoneally or subcutaneously and increasing the room temperature may be helpful. Quarantine of affected hamsters and good sanitation practices are useful in controlling proliferative ileitis. The importance of correcting any management problems and eliminating stress factors should not be overlooked.

Tyzzer's Disease

Hamsters do not appear to be as susceptible to *Clostridium piliforme*, the causative agent of Tyzzer's disease, as are gerbils. Disease is most often seen in weanlings, with resultant high morbidity and mortality. Clinical signs may include a profuse watery diarrhea as well as nonspecific signs of illness including ruffled hair coat, lethargy, anorexia, and dehydration. Transmission is by the oral route. Characteristically, predominant lesions involve the liver, but enterocolitis without liver disease or heart lesions is sometimes seen. Hepatomegaly with numerous white or yellow pinpoint foci measuring 1–2 mm in diameter is typically observed at necropsy. Edema and hemorrhage of the intestines, particularly in the ileocecal area, may also be present. Oxytetracycline or tetracycline can be used to suppress infection. Supplemental fluid therapy can be provided if indicated, but in disease outbreaks, it is recommended that infected animals be culled. Infectious spores can remain in the environment for prolonged periods of time. To eliminate spores, the environment should be cleaned and all cages and feeding and watering equipment should be sterilized.

Salmonellosis

Salmonellosis is rarely seen in laboratory hamsters although human outbreaks have been traced to infected pet and laboratory hamsters. *Salmonella* is transmitted by the fecal–oral route primarily through contaminated food and bedding material. Clinical signs are those of general illness as enteritis or diarrhea is frequently not observed in infected rodents. Pathologic findings may include multifocal necrosis of the liver, hepatosplenomegaly, and septic pulmonary thrombophlebitis. Small white foci may be seen on the liver and spleen at necropsy. Treatment is not recommended as it does not reliably eliminate the organism from infected animals but can result in an asymptomatic carrier state. Infected colonies are most frequently culled and contaminated equipment and environments sanitized.

Campylobacteriosis

There is a relatively high prevalence (nearly 100%) of *Campylobacter jejuni* colonization of hamsters. The organism is believed to be a normal commensal of the hamster intestinal tract, becoming an opportunistic pathogen presumably during periods of stress or intestinal flora disruption. Transmission is fecal–oral. Most infected hamsters are asymptomatic although a small proportion may develop diarrhea. Diagnosis is by culture or polymerase chain reaction (PCR). Hamsters should be considered a potential source for human infections. Erythromycin administered orally is the drug of choice for treatment of both humans and animals with *C. jejuni* diarrhea.

Helicobacter

Many strains of helicobacter normally inhabit the hamster intestine and rarely cause disease in the hamster. *Helicobacter* strains of most note are *H. cinaedi*, a significant pathogen of immunocompromised humans, and *H. aurati*, a strain previously shown to induce chronic

weight loss and poor body condition in 18- to 24-month-old hamsters. In addition, another strain of helicobacter was recently detected in the livers of aged hamsters and is being characterized.

Abscesses and Skin Ulceration

Abscesses and skin ulceration are most frequently caused by *Staphylococcus aureus*. Other less common organisms include *Streptococcus* spp. and *Pasteurella pneumotropica*. Systemic enrofloxacin, tetracycline, or ciprofloxacin can be used with local treatment of the area.

Pneumonia

Streptococcus spp. and *Pasteurella pneumotropica* infections may cause pneumonia. The illness is usually acute, with affected animals exhibiting sneezing, dyspnea, ocular discharge, and depression. Enrofloxacin, tetracycline, and chloramphenicol are useful antibiotics to treat pneumonia.

Viral Diseases

Few viral pathogens are recognized in the hamster. Two viruses, hamster polyomavirus and hamster parvovirus, may induce a clinical disease state. One virus, lymphocytic choriomeningitis virus, rarely induces clinical signs in hamsters in natural infections but does present a significant public health concern. In addition, hamsters are susceptible to infection by a number of other murine viruses such as Sendai virus and pneumonia virus of mice. Clinical disease is rarely recognized in adult animals. However, infections may significantly affect research results and hamsters can serve as a reservoir of infection for other rodent species housed in the same facility.

Hamster Polyomavirus

Animals infected with hamster polyomavirus typically develop one of two disease conditions. Animals that naturally contract the virus through exposure to contaminated environments or fomites may develop trichoepitheliomas on the face, feet, neck, back, flanks, or abdomen. These lesions are pathognomonic for the disease. Multiple masses may develop but then regress over time. Hamsters injected with the virus may develop lymphoma and clinical signs that pertain to the affected organ(s). The intestines, mesentery, and liver are most frequently affected. Infection can be diagnosed by PCR, histology, and immunohistochemistry. Treatment is not recommended. Colony depopulation, environmental decontamination, and repopulation with animals free of the disease are most commonly performed.

Hamster Parvovirus

Hamster parvovirus (HaPV) was first reported in 1982 in a hamster colony with high mortality in suckling and weaning animals. It has been hypothesized that the hamster is not the natural viral host and that the virus may have originated in a species in which it does not cause clinical disease. In fact, HaPV is nearly genetically identical to mouse parvovirus-3. Virus transmission is believed to be through ingestion or inhalation. Neonates and weanlings are clinically affected with runting, incisor abnormalities, domed craniums, small testicles, and a potbellied appearance. Diarrhea, ataxia, and hemorrhagic disease may also develop. Infected adults rarely develop clinical signs. Infections may persist for multiple weeks. Diagnosis is by PCR or serology. Colony depopulation followed by environmental decontamination is recommended.

Lymphocytic Choriomeningitis Virus

The natural host and reservoir of this virus is the wild house mouse (*Mus musculus*). Infection, however, can be transmitted readily to other species—including the hamster, which is especially susceptible to infection. Lymphocytic choriomeningitis (LCMV) is an important public health concern because the virus may be transmitted from infected animals to humans through contact with urine, feces, saliva, or infected tissues and cell lines. Aerosol transmission has been noted. Both pet and laboratory-reared hamsters have been the source of several hundred human cases in the United States. Currently, most research institutions actively test for and exclude this virus from their rodent colonies. However, animal infections are occasionally identified, most frequently in association with the exchange of infected animals or biologics from foreign institutions or pet suppliers that do not actively exclude the virus. Once infected, animals may transmit the virus both horizontally and vertically. Adult hamsters that contract the disease rarely develop clinical signs. Animals infected prenatally may remain subclinical for multiple months, but then develop a progressive wasting disease. Infected humans may display no clinical signs or a mild febrile illness or, less commonly, may develop aseptic meningitis, encephalitis, or meningoencephalitis. Human infection can be fatal. The virus may not be readily transmitted through dirty bedding exposure. Therefore, a dirty bedding sentinel system may not detect infected colonies. Contact sentinels or other diagnostics are recommended. Serologic testing results have been unreliable, but PCR can be diagnostic. Due to the public health concern, treatment of infected animals is not recommended; rather colony depopulation is routine. It is important that the virus be excluded from colonies, such as by the quarantine and testing of new animals and testing of all biologics. Animal facilities must preclude the unintended entry of wild rodents as they may also serve as a source of infection.

Parasitic Diseases

Demodex

Two species of mites, *Demodex criceti* and *D. aurati*, occur in hamsters. Demodex mites are very common in both research and commercial vendor colonies. The presence of demodex mites is not thought to constitute a disease state as a low number of mites is normally present on immunocompetent hamsters with no apparent ill effects. Disease is most frequently seen in older animals, particularly males, and in immunocompromised individuals. Affected hamsters may have a rough hair coat; proliferative or ulcerative dermatitis; and areas of alopecia over the back, neck, and rump. Demodex mites can be difficult to detect; a deep skin scraping is suggested to visualize the mite. Affected animals may be culled from the colony. If treatment is preferred, it is best to bathe the hamster in a mild soap and towel-dry prior to treating it with a dilute solution of amitraz applied with a cotton ball or other applicator and allowed to dry. Dipping the animal in an amitraz solution is not suggested. Oral ivermectin treatment may be efficacious.

Notoedres

The hamster ear mite, *Notoedres notoedres*, can cause exudative crusting lesions not only on the ears, but also on the snout, extremities, anus, and genitalia. Infestations are treated with ivermectin although infections are extremely unusual in the modern laboratory animal facility.

Pinworms

Hamsters can be infected by *Syphacia* spp., *Aspiculuris* spp., and *Dentostomella* spp. *Syphacia mesocriceti* is specific to the hamster, but many of the other pinworms that infect hamsters infect other species as well. *Syphacia obvelata* can be transmitted between mice and hamsters and *S. muris* between rats and hamsters through contact with the parasite's eggs. Immunocompetent hamsters rarely display clinical signs of infection; however, they may affect certain research projects. Diagnostic methods vary based upon the infecting species but may include perianal cellophane tape tests, fecal flotation, direct examination of intestinal contents, and PCR. Avermectins and benzimidazoles are reported to be effective treatments.

Tapeworms

Several species of cestodes readily infect hamsters. In small numbers, tapeworms are relatively nonpathogenic although heavy burdens may result in emaciation or mechanical obstruction of the intestines. Heavy infestations can cause diarrhea. The cestode *Rodentolepis nana*, the dwarf tapeworm, has been frequently identified in hamster colonies. Infections with *R. nana* are a concern because of its direct life cycle, transmission to other rodents, and zoonotic potential. Recommended treatments include praziquantel or thiabendazole.

Protozoa

Numerous protozoa, including *Spironucleus* spp., *Trichomonas* spp., and *Giardia* spp., are found in the intestines but are of no known clinical significance. Eradication of these parasites from a colony is difficult. Treatments known to be effective in mice are frequently ineffective in hamsters.

Neoplasia

The incidence of spontaneous tumors in hamsters is relatively low although they occur more frequently in females. The most common primary tumors are lymphomas as well as endocrine tumors (especially adrenal gland tumors) and intestinal tumors. The incidence and severity of tumors varies between colonies.

Miscellaneous Conditions

Malocclusion

Malocclusion and overgrowth of incisors occasionally occur in hamsters secondary to trauma or due to a genetic predisposition. If severe and undetected, overgrown incisors can lead to emaciation and death due to an inability to eat. Incisors can be trimmed with suture removal scissors or toenail clippers, but this practice may lead to fractures of the teeth. Use of a dental bur is less likely to cause tooth fractures.

Bedding-Associated Dermatitis

A dermatitis that primarily affects the footpads and digits has been reported in hamsters housed on wood shavings. Lesions may spread to the legs and shoulders. Disease etiology is thought to relate to a contact dermatitis and/or a physical irritation as fine wood pieces have been observed lodged in the dermis or subcutis of affected animals. A granulomatous inflammation with digit degeneration and atrophy may develop.

Nutritional Myopathy

A nutritional myopathy caused by a vitamin deficiency can occur in hamsters fed a nutritionally incomplete diet (e.g., an all-seed diet). In mild cases, the hamster is able to move the hind legs but cannot support its weight. In more advanced cases, the hamster may have posterior paresis. Nutritional improvement, supplementation with vitamin E, and an opportunity to exercise are usually curative.

Trauma

Hamsters are prone to aggression and may inflict serious injuries upon each other. Cannibalism is common in stressed and young animals. Paralysis is most frequently the result of traumatic injuries such as a fall onto a hard surface. Serious trauma, including fractures, may occur with inappropriate handling and restraint. External splinting is often not satisfactory for fracture repair. Stainless steel hypodermic needles used as intramedullary pins may be preferable for repair of fractured long bones. Fine stainless steel wire or staples are preferable for laceration repairs as they may resist an animal's attempt at self-removal.

Osteoarthritis

Osteoarthritis is frequently observed in hamsters older than 2 years. The femoral–tibial joint is most commonly involved. As the characteristics of the disease are similar to those in humans, the hamster is a recognized animal model of the human condition.

Glomerulonephropathy

Progressive and degenerative renal disease is a common problem in aged hamsters and is an important cause of mortality. Females are more commonly affected. Uremia, proteinuria, and polyuria are seen in affected animals. Amyloid deposition frequently occurs concurrently.

Amyloidosis

Amyloidosis is a metabolic condition characterized by excessive, extracellular deposition of inert protein fibrils in a variety of organs. The incidence of the condition varies between animal colonies, but it is most common in aged animals, especially females. The most commonly affected organs are the liver, kidney, stomach, adrenal gland, thyroid, and spleen. Animals may be asymptomatic; clinical signs that develop frequently result from the loss of function of the affected organ. No treatment exists.

Atrial Thrombosis

Both male and female geriatric hamsters frequently suffer from atrial thrombosis although the incidence varies across animal colonies. Most thromboses occur in the left atrium and are secondary to degenerative cardiomyopathy and amyloidosis. Clinical signs are suggestive of heart failure and may include hyperpnea, severe dyspnea, tachycardia, and cyanosis. Temporary clinical improvement has been reported in some hamsters treated with digitalis and furosemide.

Polycystic Disease

Aged hamsters frequently develop cysts, most frequently in the liver. Less frequently, cysts are found in the pancreas, epididymis, and seminal vesicles. Usually, no clinical signs are

associated with the cysts and their cause is unknown, although there appears to be a genetic component.

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CHAPTER 7 REVIEW

Multiple Choice

1. Gestation period in the hamster is
   A. 16 days
   B. 21 days
   C. 24 days
   D. 30 days
2. Hamsters have a
   A. short duodenum and jejunum
   B. small cecum
   C. distinctly compartmentalized stomach
   D. long ileum

3. Brown adipose tissue is located
   A. mid-ventral abdomen
   B. posterior to globe of eye
   C. between the scapulae
   D. flank region
4. False statement regarding effects of hibernation
   A. decreased body temperature
   B. respiration slows
   C. sensitive to touch
   D. increased heart rate
5. Hamster that is smallest
   A. Siberian
   B. Chinese
   C. Syrian
   D. Golden
6. An immunologically privileged site
   A. stomach
   B. cheek pouch
   C. liver
   D. ileum
7. All are a zoonotic potential except
   A. lymphocytic choriomeningitis
   B. *Campylobacter*
   C. *Rodentolepis nana*
   D. *Syphacia obvelata*
8. False statement regarding hamster pups
   A. Eyes open at 5 days of age.
   B. Incisors are fully erupted at birth.
   C. Pups are weaned at 3 weeks of age.
   D. Pups are hairless at birth.
9. The most common spontaneous disease of hamsters is
   A. proliferative ileitis
   B. cardiomyopathy
   C. antibiotic-associated enterocolitis
   D. salmonellosis
10. Females can be successfully mated in the evening of the ___ day after observance of the postovulatory discharge.
    A. 1st
    B. 2nd
    C. 3rd
    D. 4th

(*Continued*)

Fill In the Blank

11. _____ is a branch of genetics that is concerned with the structure and function of chromosomes.
12. Litter abandonment and _____ of young are common during the first pregnancy.
13. The _____ hamster is gray-brown with a dark dorsal stripe.
14. _____, a tendency for cells to have an affinity for acid, basic, or neutral stains, is relatively common in hamster red blood cells.
15. Antibiotic-associated _____ may develop when antibiotics that disrupt the normal intestinal microbial flora are given.
16. _____ glands are sebaceous glands that are used to mark territory and are involved in mating behavior.
17. _____ _____ is the genus species name of the Syrian hamster.
18. The position the female hamster assumes during breeding is called _____.
19. Proliferative ileitis is commonly called _____.
20. _____ is a condition characterized by excessive, extracellular deposition of inert protein fibrils in organs such as the liver, kidney, and spleen.

Critical Thinking

21. You have been recently assigned responsibility for a hamster breeding colony in a biomedical research facility. The number of viable births has decreased over the past 4 months. In addition, there is a high percentage of litter abandonment and cannibalism by the dams. You visit the animal room and observe the following: all hamsters are housed in clear microisolation shoebox cages with ½ inch of corncob bedding; the room temperature is 70°F and humidity is 45%, with 12 air changes per hour; and light timers are set to deliver 12 hours of light per day. You check a number of the cage cards and discover many of the breeders are 16–18 months of age. Make suggestions to assist the researcher with her hamster breeding colony.

8

Guinea Pigs

Guinea pigs are caviomorph (New World hystricognath) rodents native to South America. Although they belong to the order Rodentia, guinea pigs are more closely related to porcupines and chinchillas than to mice and rats. The guinea pig is known as a cavy, derived from its scientific name, *Cavia porcellus*. Guinea pigs historically have been, and continue to be, used as a common human food source in some South American countries. Three primary breeds of guinea pigs are recognized: English, Abyssinian, and Peruvian, and each can be distinguished by the length, texture, and direction of hair growth. English guinea pigs have uniformly short, straight hair, and are the breed seen most frequently in the research setting. Multiple genetic classifications used in research have been derived from the English breed, including outbred stocks (e.g., Dunkin-Hartley and Hartley), inbred strains (e.g., Strain 2 and Strain 13), and mutant strains (e.g., the IAF hairless strain developed following a spontaneously occurring genetic mutation of an outbred Hartley colony). Figure 8.1 depicts a Dunkin-Hartley outbred stock. Abyssinian guinea pigs have short, coarse hair arranged in whorls or rosettes, which gives them an untidy appearance. Peruvian guinea pigs have long, fine, silky hair. Guinea pigs may be monocolored, bicolored, or tricolored.

USES

In the English language, the term guinea pig is synonymous with the term research subject. The number of guinea pigs used in the laboratory peaked in the 1960s largely due to their central role in tuberculosis research. Although their use has significantly decreased, they are still a very valuable and unique resource in many areas of research.

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Fig 8.1. Dunkin-Hartley guinea pig, outbred stock. (Photo courtesy of Harlan.)

Attributes that contribute to their usefulness in research include a body size conducive to surgical manipulations, wide commercial availability, a tractable disposition, and broad historical use as a research model. They share several features with humans, such as the need for dietary vitamin C and susceptibility to tuberculosis. This species is used in studies involving immunology, otology, infectious disease, asthma, delayed hypersensitivity, nutrition, and toxicology. They are a well-established model of anaphylaxis. Sensitized animals have a high incidence of anaphylaxis when injected with a small amount of antigen and subsequently develop lethal bronchiolar constriction in response to histamine release. Guinea pigs are also frequently used as a source of complement, which is used in complement fixation tests to diagnose infectious diseases.

BEHAVIOR

Guinea pigs are communal animals that live together amicably. When group housed, they tend to huddle together as a single unit. Like other rodents, they are thigmotactic, preferring to maintain contact with the cage periphery while avoiding the "open field" of the cage center. Guinea pigs establish male-dominated social hierarchies. Once the hierarchy is formed, the group is usually stable. Introduction of a new male into a group, however, can provoke fighting. Dominant animals will frequently chew and barber the hair near the hip or rump of subordinate cagemates. Barbering is also associated with boredom and overcrowding. Adults, especially males, will chew the ears of the young.

Unlike the mouse and rat, guinea pigs are active during daylight hours and eat frequently during both light and dark hours. They are generally calm but can be easily excited by sudden noise or changes in their environment, tending to freeze at unfamiliar sounds and scatter when exposed to sudden movements. The immobility reaction can last from several seconds to 20 minutes. The scatter reaction can involve stampeding, jumping, or rapid circling of their cage, which can result in the trampling and serious injury or death of young within the cage. Guinea pigs exhibit the Preyer or pinna reflex, in which they cock their ears

in response to sound. Vocalization appears to be an important component of their behavioral repertoire; at least 11 different vocalizations have been recorded, some of which are inaudible to humans. The whistle is used in anticipation of feeding, a purr during social interactions, and a squeal or scream is elicited when injured or fearful. Guinea pigs develop rigid habits and have an aversion to change in diet and environment. Developing strong food preferences early in life, animals may refuse to consume novel food items or previously accepted, modified food or drink (e.g., medication-supplemented food items) to the point of starvation. Indeed, feeding habits can be so strongly entrenched that they may refuse to eat or drink from novel food or water receptacles.

ANATOMIC AND PHYSIOLOGIC FEATURES

Guinea pig biologic and reproductive data are listed in Table 8.1. Guinea pigs have a compact body with short legs and a vestigial tail. Unlike other common laboratory rodents, these animals possess four digits on the forefeet and three on the hind feet. Guinea pigs have large tympanic bullae, which facilitates access to the internal structures of the ear. Hearing, olfaction, and color discrimination are well developed. Their dental formula, 2(I 1/1, C 0/0, P 1/1, and M 3/3), is unique among rodents, as most lack premolars. The opening of the oral cavity is small and a diastema, or gap, is present between the incisor and premolar teeth. All of the teeth are open rooted, erupting continuously. The chisel-like incisors are white, unlike

Table 8.1. Biologic and reproductive data for guinea pigs

Adult body weight: Male	900–1200 g
Adult body weight: Female	700–900 g
Life span	5–7 y
Body temperature	37.2°–39.5°C (99°–103.1°F)
Heart rate	230–380 beats per minute
Respiratory rate	40–130 breaths per minute
Tidal volume	2.3–5.3 mL/kg
Food consumption	6 g/100 g per day
Water consumption	10 mL/100 g per day
Breeding onset: Male	3–4 mo (600–700 g)
Breeding onset: Female	2–3 mo (350–450 g)
Estrous cycle length	15–17 d
Gestation period	59–72 d
Postpartum estrus	Fertile
Litter size	1–6
Weaning age	14–21 d
Breeding duration	18–48 mo
Chromosome number (diploid)	64

Source: Adapted from Harkness et al. (2010).

those of other rodents, and the upper pair of incisors is shorter than the lower pair. The anatomic structure of their pharynx is unique. The soft palate is continuous with the base of the tongue and possesses an opening called the palatal ostium: this arrangement forms the sole passage from the oropharynx to the distal pharynx, rendering this species difficult to intubate. The lung has four lobes on the right and three lobes on the left. The thymus of the guinea pig lies in the ventral cervical region surrounding the trachea. This location facilitates its surgical removal in support of select immunological studies requiring thymectomy; however, it should be noted that accessory thymic tissue may be present in surrounding tissues. This differs from most other rodents in which the thymus is located in the thoracic cavity overlying the heart. The adrenal glands are bilobed and large. The guinea pig is monogastric and, unlike many other rodents, has an undivided stomach lined entirely with glandular epithelium. The cecum is large and holds up to 65% of the gastrointestinal capacity, occupying most of the left midabdominal cavity. Guinea pigs are vigorously coprophagic, regularly consuming feces directly from the anus or from the cage floor. They have sebaceous marking glands located circumanally and on the rump and will often walk or sit while pressing the glands against a surface.

The guinea pig is the only common laboratory animal, other than nonhuman primates, that resembles the human in its dietary requirement of vitamin C. Vitamin C plays a significant role as an antioxidant, in collagen synthesis, and in support of the cardiovascular and immune systems; it must be provided in the diet as guinea pigs lack the enzyme L-gulonolactone oxidase required for endogenous synthesis (Box 8.1). This species (as well as ferrets and nonhuman primates) is steroid resistant; steroid administration does not induce as significant a reduction in lymphocyte numbers and function as occurs in steroid-sensitive species.

Box 8.1

Vitamin C must be provided in the diet as guinea pigs lack the enzyme L-gulonolactone oxidase required for endogenous synthesis.

Sexing is done by manipulation and visualization. Male guinea pigs are larger than females and possess large testes and an os penis. The penis has two prongs at the tip and, with slight manual pressure, is easily extruded from the preputial sheath. The inguinal canals remain open in the male. Males have several accessory sex glands, including large, transparent, smooth seminiferous vesicles that extend 10 cm into the abdomen from the pubis and are commonly mistaken as uterine horns by those unfamiliar with guinea pig anatomy. Females have a bicornuate uterus with a single cervical os. Both sexes possess one pair of mammary glands in the inguinal region; however, the male's nipples are smaller. The female has a vaginal closure membrane that is open only during estrus and parturition. Females have a Y-shaped genital–anal opening with the rectal opening positioned at the base of the Y, the urethra positioned within the top branches of the Y, and the vaginal opening in the center. This is in contrast to the male's slit-shaped rectal opening as depicted in Figure 8.2.

Compared with other rodents, the packed cell volume, erythrocytes, and hemoglobin levels of the guinea pig are relatively low. Typical of rodents, the lymphocyte is the dominant

Fig 8.2. Genitalia of female (Y-shaped) and male (slit-shaped). (Photo courtesy of ACLAM.)

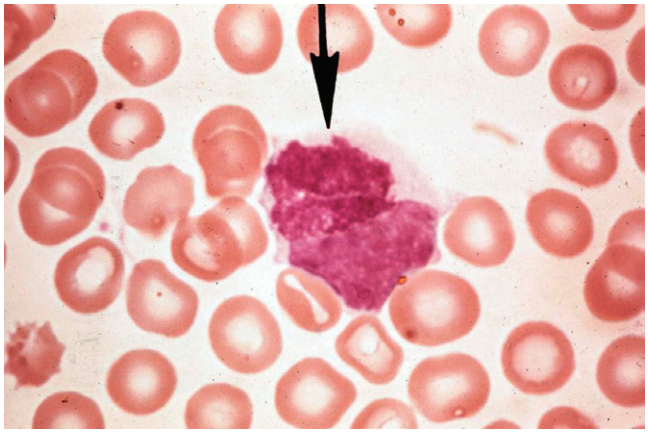

Fig 8.3. Kurloff cell, mononuclear lymphocyte with a large intracytoplasmic inclusion. (Photo courtesy of ACLAM.)

leukocyte. Heterophils (neutrophils) are present with distinct eosinophilic cytoplasmic granules like those of the rabbit. The guinea pig has a unique leukocyte, the Foa Kurloff or Kurloff cell, a mononuclear lymphocyte with a large intracytoplasmic inclusion (Figure 8.3). Kurloff cells proliferate with estrogenic stimulation and exhibit some natural killer cell activity. Although they can be found in the peripheral circulation of both males and nonpregnant females, elevated numbers are found in the circulation of pregnant females, with highest numbers in the placenta. Guinea pigs, especially mature females, are an excellent source of highly active complement, a protein component of the innate immune system that "complements" the body's ability to remove pathogens. Urine is normally alkaline (pH 8–9), opaque, and creamy yellow due to high concentrations of calcium crystals. Selected hematologic and biochemical values are listed in Appendix 1, "Normal Values."

BREEDING AND REPRODUCTION

The sow, or female, is usually bred at 2–3 months of age, which corresponds to a weight of 350–450 g. It is important that breeding occur prior to 7 months of age to prevent permanent fusion of the pelvic symphysis, which often results in dystocia (Box 8.2). The boar, or male, is bred at 3–4 months of age or 600–700 g body weight. The sow is a nonseasonal, continuously polyestrous breeder with a 15- to 17-day estrous cycle. Detection of estrus is not necessary unless timed matings are desired as guinea pigs are normally pair housed for mating. When in estrus, the sow displays a swollen, congested vulva and exhibits lordosis, characterized by extension of all four legs, straightening and arching of the back, and elevation of the pubic area. The male will purr, circle the sow, and sniff and lick her anogenital area prior to mounting. Mating can be confirmed by the presence of a vaginal plug, a solid mass formed from ejaculate fluids that may be retained in the vagina for several hours after copulation. Pregnancy can be confirmed by palpating the fetal mass at 14–21 days of gestation. The fetal skeletons can be visualized radiographically at 6 weeks. A fertile postpartum estrus does occur from 2 to 10 hours after parturition.

Box 8.2

It is important that breeding occur prior to 7 months of age to prevent permanent fusion of the pelvic symphysis, which often results in dystocia.

It is important for the sow to remain active during pregnancy to maintain body condition and prevent obesity. Due to the normally large total fetal mass, a sow's body weight may nearly double during pregnancy. Guinea pigs have a labyrinthine hemomonochorionic placentation, as do humans. The gestation period ranges from 59 to 72 days, with an average of 68 days. The gestation length varies inversely with the size of the litter: longer for small litters and shorter for large litters. During the last week of gestation, the pelvic symphysis relaxes and separates due to the effects of the hormone relaxin. Guinea pigs do not build nests, nor do they retrieve their young; young instead approach the sow. The sow farrows in a squatting position. Farrowing normally takes approximately 30 minutes, with 5–10 minutes between pups. If a sow strains unsuccessfully for 20 minutes, strains intermittently over the course of 2 hours, or if a pup is not delivered within 10–15 minutes of an oxytocin injection, a cesarean section is indicated. Both the sow and the boar may consume the placenta. Unlike most other rodents, young are precocious: they are born fully haired, with teeth, and with their eyes and ears open. The pup receives all maternal antibodies from the placenta, none from the colostrum. The young do not nurse for the first 12–24 hours following birth. They begin eating solid food and drinking water during the first week of life and are nearly self-sufficient by 4–5 days of age. However, an approximate 50% mortality rate is observed in young that do not nurse within the first 3–5 days of life. Guinea pigs can be weaned at 14–21 days or when they weigh approximately 180 g. Following weaning, growth is rapid.

HUSBANDRY

Housing and Environment

Guinea pigs may be housed individually or in groups. Cages may be solid plastic or metal with solid or metal-slat flooring. To reduce limb injuries, solid-bottom cages with bedding are preferred. Wire flooring should be avoided as the feet of smaller animals often fall through the mesh, lacerating or fracturing their limbs. Because guinea pigs are not inclined to climb or jump, most can be kept in open-top cages provided the sides are at least 25 cm (10 in.) high. Rectangular cages are preferred over square as they tend to diminish the stampede response. Adult animals (greater than 350 g) should be provided with at least 652 cm^2 (101 $in.^2$) of floor space per animal with a cage height of at least 7 inches. The bedding material should be as dust free as possible. Ground corncobs, paper products, or other material of plant origin can be used. Although wood shavings are occasionally used, they can adhere to the moist perineal region, causing irritation or obstructions. Housing temperatures range between 20° and 26°C (68° and 79°F), with 21°C (70°F) recommended. Having a compact body, low temperatures are better tolerated than high temperatures, especially if the humidity is high. The environmental humidity should be maintained between 30% and 70%, with at least 10–15 air changes per hour. 12 hours of light per day are provided, controlled through automatic timers. Guinea pigs are susceptible to *Bordetella bronchiseptica* and thus should be serviced before and ideally physically distanced from species such as rabbits, cats, and dogs that may be subclinically infected.

Sanitation

Cages should be cleaned at least two to three times weekly as these animals produce a significant volume of waste. Cages, water bottles, and feed hoppers should be disinfected with chemicals, hot water 61.6°–82.2°C (143°–180°F), or a combination of both. An acid rinse prior to cage washing facilitates removal of scale that develops from an accumulation of urine minerals. Detergents and chemical disinfectants enhance the effectiveness of hot water, but they must be thoroughly rinsed from all caging surfaces with fresh water. Refer to Chapter 4, "Mice," for a more detailed description of disinfection methods.

Environmental Enrichment and Social Housing

Over its successive editions, the *Guide for the Care and Use of Laboratory Animals* (the *Guide*; ILAR, 2011) has placed an increased emphasis on the appropriate provision of environmental enrichment and use of social housing to help meet the psychological and social needs of all species. The *Guide* states that social animals should be housed in physical contact with conspecifics when it is appropriate and compatible with the protocol. Guinea pigs are social animals and do best when housed in small, compatible groups. The provision of enrichment items may benefit this species; objects should be nontoxic, nondigestible, sanitizable, and safe. The types of enrichment items most frequently provided to guinea pigs are those items that the animal can manually manipulate or gnaw on, items that provide increased cage complexity (e.g., partially enclosed structures or huts), or treat food items commercially prepared for laboratory animal use. However, controversy exists regarding which enrichment items provide the greatest benefit and least risk to the animals. This is largely complicated by the significant difficulty of interpreting an animal's response to or desire for an

object. It must be noted that animals may interpret an object in a very different way than a human may predict. Enrichment items may also induce unintended effects such as increased territoriality and aggression or physical trauma. Therefore, the use of all enrichment items must be critically evaluated before being routinely provided to a colony. In addition, scientific studies have shown that the physical and mental development of some species can be directly affected by social housing conditions (e.g., singly or group housed) and the absence or provision of enrichment items within their environment. Both consistency and caution must be exercised in the use of environmental enrichment with study animals where the changes may impact research results. For these reasons, formal approval should be secured from the principal investigator, the IACUC, and the facility veterinarian prior to the introduction of any enrichment items.

Feeding and Watering

Commercial pelleted diets manufactured specifically for laboratory guinea pigs should be fed *ad libitum*. It is important that the food be properly stored and used prior to the manufacturer's determined expiration date (usually within 6 months of milling), to help ensure adequate vitamin C content. Diets with questionable or inadequate levels of vitamin C should never be provided, regardless of any attempts to provide the vitamin via supplemental food items or vitamin C tablets. Rabbit chow should not be fed to guinea pigs as it lacks vitamin C and has excess levels of vitamin D. Guinea pigs eat an average of 6 g/100 g of body weight per day. Feed is best provided in self-feeders, such as a J-feeder, mounted on the cage wall, as animals tend to sit, sleep, and defecate in bowls placed on the cage floor. Good-quality hay can be provided as a supplement to reduce boredom and decrease barbering. Kale, parsley, beet greens, kiwi fruit, broccoli, oranges, and cabbage are good sources of vitamin C and can be fed in small amounts not to exceed 10%–15% by weight of the pelleted diet. Any fresh fruit or vegetable treats provided should be of human quality and thoroughly cleaned prior to feeding to reduce potential of contamination with pathogenic organisms. Potential bacterial, viral, and parasitic contaminants of hay must be considered. To decrease this risk, some institutions provide autoclaved or sterilized hay. Uneaten items should be removed from cages daily. Fresh potable water should be available *ad libitum* by bottle or automatic watering system. Adult guinea pigs drink an average of 10 mL/100 g of body weight per day. Water bowls or crocks placed on the cage floor should not be used because they readily become contaminated with feces and bedding. Guinea pigs are untidy water drinkers; rather than lick, they often chew on and play with sipper tubes and valves and will waste a significant amount of water. Animals may inadvertently flood their own cages through the manipulation of water lixits whose ends are positioned within their cage. Guinea pigs will also blow food up the sipper tube of water bottles and foul the water supply. The use of automatic water systems with valves located outside the cage is therefore more advantageous.

TECHNIQUES

Handling and Restraint

Guinea pigs should be handled gently but firmly. They should be lifted using two hands, grasping under the trunk with one hand while supporting the rear quarters with the other

Table 8.2. Adult guinea pig blood volumes and suggested sample size

	Volume (mL)
Total blood	40–80
Single sample	4–8
Exsanguination	15–30

Source: Adapted from Harkness et al. (2010).
Note: Values are approximate.

hand. Support is particularly important with adults and pregnant animals. Grasping a guinea pig too firmly around the thorax or abdomen can impede breathing and cause injury to the lungs, diaphragm, or liver.

Identification

Cage cards or color pattern records can be used to identify guinea pigs. Permanent individual animal identification can be accomplished by ear notching, ear tagging, tattooing, or microchip placement. Dyes, shaving, and markers can be used for temporary identification.

Blood Collection

Assuming the animal is mature, healthy, and on an adequate plane of nutrition, the blood volume (in milliliters) of most species averages 7% of the body weight in grams. Table 8.2 lists approximate adult guinea pig blood volumes. A typical rule of thumb for blood withdrawal suggests that up to 10% of the circulating blood volume can be withdrawn every 2–3 weeks from normal, healthy animals with minimal adverse effect.

Guinea pigs lack readily accessible peripheral veins. Small amounts of blood (<1 mL) can be obtained from the saphenous vein, cephalic vein, or a toenail bed. Larger quantities of blood can be obtained from the cranial vena cava or jugular vein, femoral artery or vein, or directly from the heart. The guinea pig must be sedated to obtain blood from the cranial vena cava or femoral vessels and must be anesthetized for cardiac puncture. Venipuncture of the cranial vena cava can cause bleeding into the thoracic cavity. Cardiac puncture involves a significant element of risk and is not recommended except as a terminal procedure. Alternatively, a catheter can be surgically implanted into the jugular vein to collect larger quantities or repeated blood samples.

Urine Collection

Urine may be collected by using a metabolic cage, gentle digital pressure on the bladder, cystocentesis, or placing the animal on a cold, clean surface.

Drug Administration

Palatable drugs can be incorporated into the food or water, but guinea pigs often refuse food or water with an unfamiliar taste. Liquids can be administered orally using a small syringe. Orogastric gavage with a metal dosing needle, which is easily accomplished in mice and rats, is not an option with guinea pigs due to the unique anatomy of the palate. Passing a 5- or 6-French infant feeding tube through the palatal ostium of an unanesthetized guinea pig is

Table 8.3. Maximum guinea pig dosing volumes

Route	Volume (mL/kg)
IP	20
SC	20
IM	0.3/site with maximum of two injections
IV	5 (bolus)/20 (infusion)
ID	0.05/site
PO	20

Source: Huerkamp et al. (1996) and Lukas (1999).

an option for administering drugs directly into the stomach; it requires the use of a plastic bite block to protect the tube. Subcutaneous (SC) injections require some force because guinea pig skin is tough, especially over the neck and back. A maximum volume of 20 mL/kg is recommended. Intramuscular (IM) injections with a maximum volume of 0.3 mL may be given in the lumbar muscle or quadriceps muscle to avoid the sciatic nerve. Intraperitoneal (IP) injections are made lateral to the midline in the lower right quadrant of the abdomen. Some recommend tilting the guinea pig's head toward the floor with the objective of shifting the abdominal organs forward, helping to prevent inadvertent injection into the intestine. A maximum volume of 20 mL/kg can be given by the IP route. Intravenous (IV) injections can be made using the saphenous or the cephalic vein. Suggested maximum dosing volumes are summarized in Table 8.3.

Anesthesia, Surgery, and Postoperative Care

Guinea pigs should be fasted for 3–6 hours prior to anesthetic induction to decrease the amount of ingesta in the cecum and stomach that could otherwise restrict movement of the diaphragm and interfere with respiration. These animals frequently retain a considerable amount of feed in their buccal cavities, which can contribute to airway obstruction when they are anesthetized. The mouth can be gently rinsed with 10–20 mL of tap water to remove the pasty material before induction. A variety of agents used to tranquilize and anesthetize guinea pigs are listed in Table 8.4. Anesthesia in the guinea pig is difficult for a number of reasons. This species has a variable response to drugs; thus there is a wide range of dosages for various agents reported in the literature. Care must be used when determining an appropriate dose of injectable agents, as the true or metabolically active body weight can be overestimated due to the proportionally high quantity and weight of cecal and intestinal contents and fecal mass. Under some anesthetics, such as pentobarbital and volatile agents, guinea pigs exhibit a peculiar squirming muscle movement that does not signify return to consciousness. Agents such as ketamine–xylazine injected by the intramuscular route have been noted to cause muscle necrosis and/or self-mutilation of the injected limb. When using this combination of agents, use the lowest dose possible and dilute and/or reduce the amount injected per site. Use of the intraperitoneal route of drug administration is suggested.

Face masks are often used to administer inhalation agents as intubation is difficult due to the small size of the oral opening and trachea as well as the anatomy of the pharynx. Care must be taken during intubation attempts to not traumatize the soft tissue at the base of the

Table 8.4. Anesthetic agents and tranquilizers used in guinea pigs

Drug	Dosage	Route	Reference
Inhalants			
Isoflurane	2%–5% to effect	Inhalation	Harkness and Wagner (1995)
Sevoflurane	To effect	Inhalation	Morrisey and Carpenter (2004)
Injectables			
Acepromazine	0.5–1 mg/kg	IM	Harkness and Wagner (1995)
Alphadolone–alphaxalone	40 mg/kg	IP	Flecknell (1996)
Chloral hydrate	400 mg/kg	IP	White and Field (1987)
Diazepam	2.5 mg/kg	IM, IP	Flecknell (2009)
Fentanyl–droperidol	0.44–0.8 mL/kg	IM	Flecknell (2009)
Fentanyl–fluanisone	1 mL/kg	IM, IP	Flecknell (2009)
Fentanyl–fluanisone	1 mL/kg	IM, IP	Flecknell (2009)
+ diazepam	2.5 mg/kg	IP	
Ketamine	100 mg/kg	IM, IP	Flecknell (2009)
Ketamine	100 mg/kg	IM	Flecknell (2009)
+ acepromazine	5 mg/kg	IM	
Ketamine	40 mg/kg	IM	Clemens and Seeman (2011)
+ dexmedetomidine	0.15 mg/kg	IM	
Ketamine	60–100 mg/kg	IM	Gilroy and Varga (1980)
+ diazepam	5–8 mg/kg	IM	
Ketamine	40 mg/kg	IP	Flecknell (1997)
+ medetomidine	0.5 mg/kg	IP	
Ketamine	40–60 mg/kg	IP	Harkness et al. (2010)
+ xylazine	2–5 mg/kg	IP	
Ketamine	22–64 mg/kg	IM	Anderson (1994)
+ xylazine	2–5 mg/kg	IM	
+ acepromazine	0.75 mg/kg	IM	
Midazolam	5 mg/kg	IM, IP	Flecknell (2009)
Pentobarbital	25–35 mg/kg	IP	Harkness et al. (2010)
Tiletamine–zolazepam	40–60 mg/kg	IM	Laber-Laird et al. (1996)
Tiletamine–zolazepam	40 mg/kg	IM	Buchanan et al. (1998)
+ medetomidine	0.5 mg/kg	IM	
Tiletamine–zolazepam	40 mg/kg	IM	Buchanan et al. (1998)
+ xylazine	5 mg/kg	IM	
Tiletamine–zolazepam	60 mg/kg	IP	Jacobson (2001)
+ xylazine	5 mg/kg	IP	
+ butorphanol	0.1 mg/kg	IM	
Urethane	1500 mg/kg	IV, IP	Flecknell (2009)

IM = intramuscular; IP = intraperitoneal; IV = intravenous.

tongue. Entry into the glottis requires passage through a small opening, the palatal ostium. A 14- to 16-gauge intravenous catheter can be used as an endotracheal tube. Isoflurane and sevoflurane should be used only in precision vaporizers, not by an open-drop method, because lethal concentrations of gases can be rapidly reached at room temperature. Inhalant anesthetics should always be used with appropriate waste gas scavenging systems.

The ear pinch, respiratory rate, and heart rate can be used to monitor the depth of anesthesia. Palpebral reflexes, eyeball position, and pedal reflexes are not reliable indicators of depth of anesthesia in this species. Bland ophthalmic ointment should be instilled in the eyes of anesthetized animals to prevent exposure keratitis. Profuse salivary secretions are common and can be controlled by administering atropine or glycopyrrolate. Use of a circulating water heating pad or other precisely controlled heat source promotes euthermia and is recommended.

Box 8.3

It is important to maintain hydration and nutrition levels in the postoperative period to support normal metabolic processes and gut physiology.

Surgery should be performed in an aseptic manner as guinea pigs, as well as other rodents, are susceptible to infection. Postoperative care should include hydration of the anesthetized animal (e.g., SC or IP injection of a warmed solution such as saline, saline–dextrose, lactated Ringer's), and support of normal body temperature. Hypostatic pulmonary congestion should be prevented by turning the animal at least every 30 minutes. It is important to maintain hydration and nutrition levels in the postoperative period to support normal metabolic processes and gut physiology (Box 8.3). Select, commercially available supportive and critical care products for laboratory animals are provided in Appendix 3. Analgesics must be provided as a standard of care for alleviating pain associated with surgical or other painful procedures. If a procedure is known to cause pain in humans, it must be assumed to also cause pain in other animals unless proven otherwise. Scientific justification must be provided and approved by the IACUC if there is a need to withhold the use of analgesics. Research rodents present some unique challenges for pain control, including the need to dose many rodents at a time, the difficulty of assessing postoperative pain, and the concern about effects on research. Animal care and research staff have an ethical responsibility to continue to look for better ways to reduce pain and distress in their research subjects. Providing analgesia prior to a painful event (preemptive analgesia) affords superior pain control over attempts to control pain once it develops. Guinea pigs that are in pain may have an abnormal posture, appearing hunched, may salivate excessively, and be reluctant to move. Table 8.5 lists analgesics commonly used in this species. Butorphanol is recommended for mild postoperative discomfort. Buprenorphine works well to control acute or chronic visceral pain. Administration of nonsteroidal analgesics such as carprofen, ketoprofen, and flunixin, are alternatives to the use of opioids. To produce a more balanced analgesia, nonsteroidal agents can be used in combination with opioids. Analgesics formulated for sustained release (extended duration of analgesia) are under development for use in this species. Animals would benefit from their

Table 8.5. Analgesic agents used in guinea pigs

Drug	Dosage	Route	Reference
Acetylsalicylic acid	50–100 mg/kg q4h	PO	Heard (1993)
Buprenorphine	0.05 mg/kg q8–12h	SC	Flecknell (2009)
Butorphanol	2 mg/kg q4h	SC	Flecknell (2009)
Carprofen	1–2 mg/kg q12–24h	PO	Morrisey and Carpenter (2004)
	4 mg/kg ~q24h	SC	Flecknell (2009)
Dexmedetomidine	0.15 mg/kg	IM	Clemens and Seeman (2011)
Diclofenac	2.1 mg/kg	PO	Flecknell (2009)
Flunixin meglumine	2.5–5 mg/kg q12–24h	SC	Heard (1993)
Ibuprofen	10 mg/kg q4h	PO	Harkness et al. (2010)
Ketoprofen	1 mg/kg q12–24h	SC, IM	Harkness et al. (2010)
Medetomidine	0.3 mg/kg	IM	Clemens and Seeman (2011)
Meloxicam	0.1–0.3 mg/kg q24h	PO, SC	Harkness et al. (2010)
Meperidine	10–20 mg/kg q2–3h	SC, IM	Flecknell (2009)
Syrup	0.2 mg/mL drinking water		Huerkamp (1995)
Morphine	2–5 mg/kg q4h	SC, IM	Flecknell (2009)
Nalbuphine	1–2 mg/kg q3h	IM	Heard (1993)
Oxymorphone	0.2–0.5 mg/kg q6–12h	SC	Harkness et al. (2010)
Pentazocine	10 mg/kg q2–4h	SC	Heard (1993)
Piroxicam	6 mg/kg q24h	PO	Dobromylskyj et al. (2000)
Tramadol	5–10 mg/kg q12–24h	PO	Morrisey and Carpenter (2011)

IM = intramuscular; PO = per os; SC = subcutaneous.

use through extended and uninterrupted periods of analgesia without the need for frequent drug administration.

Imaging Techniques

Great advances have been made in rodent and small animal imaging over the last decade. Many imaging modalities once used only in humans [e.g., digital x-ray imaging, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), dual-energy x-ray absorptiometry (DEXA) scan, and ultrasound] have been adapted for use with these species, producing images of exquisitely fine detail. In addition, novel imaging techniques have been developed for use in animal research, including *in vivo* optical imaging. Some x-ray units (e.g., Faxitron animal imaging and irradiation systems) have been designed for use specifically with small rodents. These units produce a highly magnified and clear resolution image of the field of interest.

For conventional x-ray imaging, an x-ray machine with high milliamperage, such as a 200- or 300-mA unit with the capability for low settings of peak voltage delivered (kVp) and small incremental changes, is ideal. High-detail film-screen combinations work well. Short exposure times of 1/40 second or less should be used to decrease the chance of motion

artifact. Suggested exposure factors are 42–46 kVp, 300 mA, 1/40 second, 40 SID, 7.5 mA·s. Alternatively, nonscreen film can be utilized with standard x-ray or dental units. Nonscreen film produces high-detail radiographs, but longer exposure times are needed. Digital imaging techniques offer substantial flexibility in image enhancement and delivery application. The best method of restraint is to sedate the animal and use gauze or adhesive tape to position the animal on the cassette. A radiolucent tube or stockinette with the patient positioned inside can be utilized for restraint of conscious animals.

Euthanasia

Suitable methods for euthanasia include inhalant anesthetic overdose, intravenous or intraperitional administration of pentobarbital at three to four times the anesthetic dose or commercial euthanasia solution, and exposure to a controlled release of carbon dioxide. Refer to Chapter 4 for more information on appropriate euthanasia methods.

THERAPEUTIC AGENTS

Suggested antimicrobial and antifungal drug dosages for the guinea pig are listed in Table 8.6. Antiparasitic agents are listed in Table 8.7, and miscellaneous agents are listed in Table 8.8.

INTRODUCTION TO DISEASES OF GUINEA PIGS

Most laboratory guinea pig colonies today are relatively free of the variety of agents that cause clinical disease. Sporadic outbreaks, however, may still occur, and it is important for individuals to have a basic knowledge of the most common infections, their potential impact on research, and their management or eradication. Presented here is a general overview of common bacterial, viral, fungal, and parasitic agents of guinea pigs and some standard diagnostic and treatment modalities.

Disease conditions are often difficult to reverse. Ill guinea pigs do not tolerate clinical procedures well and have a tendency to progressively decline rather than recover. As such, the early identification of health abnormalities is of great importance. Nonspecific signs of illness in the guinea pig include a hunched posture; a scruffy, unkempt coat; reduced appetite; and weight loss. Lethargy, disinterest in surroundings, discharge from the nose or eyes, and/or diarrhea may be present. Anorexia is a common, nonspecific sign of illness that necessitates aggressive support because it is a life-threatening condition. Nutritional support may be provided through the administration of food items (e.g., moistened food pellets and critical care formulas). Animals may be encouraged to eat by the provision of especially appealing food items or soft or gel-based diets; however, guinea pigs may refuse these items if they are unfamiliar. Sterile fluids may be administered to support hydration and to provide energy (e.g., dextrose solutions). Fluids can be administered SC, IP, IV, or intraosseously via the

Table 8.6. Antimicrobial and antifungal agents used in guinea pigs

Drug	Dosage	Route	Reference
Amikacin	2–5 mg/kg q8–12h	SC, IM	Harkness et al. (2010)
Amoxicillin	Not recommended—toxic		Flecknell (1987)
Ampicillin	Not recommended		Laber-Laird et al. (1996)
Cefaxolin	15 mg/kg q12h	IM	Laber-Laird et al. (1996)
Ceftiofur sodium	1 mg/kg q24h	IM	Harkness (1993)
Cephalexin	50 mg/kg q24h	IM	Richardson (1992)
Cephaloridine	10–25 mg/kg q8–24h	IM	Anderson (1994)
Chloramphenicol	50 mg/kg q12h	PO, SC, IM	Harkness et al. (2010)
Ciprofloxacin	7–20 mg/kg q12h	PO	Harkness et al. (2010)
Doxycycline	2.5 mg/kg q12h	PO	Allen et al. (1993)
Enrofloxacin	0.05–0.2 mg/mL drinking water for 14 d		Harkness and Wagner (1995)
	5–10 mg/kg q12h	PO, SC, IM	Harkness et al. (2010)
Gentamicin	2–4 mg/kg q8–24h	SC, IM	Harkness et al. (2010)
Griseofulvin	25–50 mg/kg q12h for 14–60 d	PO	Harkness et al. (2010)
	1.5% in DMSO for 5–7 d	Topical	Harkness et al. (2010)
Itraconazole	2.5–10 mg/kg q24h	PO	Morrisey and Carpenter (2011)
Ketoconazole	10–40 mg/kg q24h for 14 days	PO	Adamcak and Otten (2000)
Lime sulfur dip	Dilute 1:40 with water; dip q7d for 4–6 wk	Topical	Harkness et al. (2010)
Metronidazole	20 mg/kg q12h	PO	Morrisey and Carpenter (2004)
Neomycin	12–16 mg/kg q12h	PO	Anderson (1994)
Penicillin	Not recommended		Laber-Laird et al. (1996)
Sulfadimethoxine	10–15 mg/kg q12h	PO	Harkness et al. (2010)
Sulfamerazine	1 mg/mL drinking water		Anderson (1994)
Sulfamethazine	1 mg/mL drinking water		Anderson (1994)
Sulfaquinoxaline	1 mg/mL drinking water		Collins (1995)
Tetracycline	10–20 mg/kg q8–12h; use with caution, toxicities reported	PO	Burgmann and Percy (1993)
Trimethoprim–sulfa	15–30 mg/kg q12h	PO, SC	Harkness et al. (2010)
Tylosin	10 mg/kg q24h; use with caution	PO, SC	Harkness et al. (2010)

DMSO = dimethyl sulfoxide; IM = intramuscular, PO = per os; SC = subcutaneous.

femur. A dehydrated animal should be given 10 mL of maintenance fluids per 100 g of body weight daily via a parenteral or oral route until it is drinking normally. Oral rehydration solutions are available commercially. The parenteral administration of vitamin C is recommended if sufficient dietary consumption cannot be assured. Guinea pigs are extremely sensitive to antibiotics with a Gram-positive spectrum. Disturbance of their predominantly anaerobic flora leads to overgrowth of clostridial organisms and other bacteria. Antibiotics should be used only when indicated and with extreme care.

Table 8.7. Antiparasitic agents used in guinea pigs

Drug	Dosage	Route	Reference
Amitraz	0.3% solution q7d	Topical	Morrisey and Carpenter (2004)
Carbaryl powder (5%)	Dust q7d for 3 wk	Topical	Anderson (1994)
Fenbendazole	20 mg/kg q24h for 5 d	PO	Allen et al. (1993)
Ivermectin	0.2–0.5 mg/kg q7d for 3 wk	PO, SC	Harkness et al. (2010)
Lime sulfur dip	Dilute 1:40 with water, dip q7d for 6 wk	Topical	Anderson (1994)
Metronidazole	25 mg/kg q24h	PO	Morrisey and Carpenter (2004)
Piperazine citrate	2–5 mg/mL drinking water for 7 d, off 7 d, repeat		Anderson (1994)
Praziquantel	6–10 mg/kg	PO	Harkness et al. (2010)
Pyrethrin powder or shampoo	Dust/shampoo q7d for 3 wk	Topical	Harkness et al. (2010)
Selamectin	15 mg/kg	Topical	Eshar and Bdolah-Abram (2012)
Sulfadimethoxine	10–15 mg/kg q12h	PO	Harkness et al. (2010)
Sulfamerazine	1 mg/mL drinking water		Anderson (1994)
Sulfamethazine	1 mg/mL drinking water		Anderson (1994)
Sulfaquinoxaline	1 mg/mL drinking water		Collins (1995)
Thiabendazole	100 mg/kg q24h for 5 d	PO	Allen et al. (1993)

PO = per os; SC = subcutaneous.

Bacterial Diseases

Antibiotic Toxicity

Guinea pigs are highly sensitive to antibiotics, particularly those specific for Gram-positive organisms (Box 8.4). The normal intestinal flora of guinea pigs is predominantly composed of Gram-positive organisms such as streptococci and lactobacilli. Administration of antibiotics specific for Gram-positive bacteria destroys the normal flora and permits an overgrowth of Gram-negative organisms as well as clostridial organisms. Penicillins, including ampicillin and amoxicillin, lincomycin, clindamycin, erythromycin, bacitracin, streptomycin, and the cephalosporins, can induce toxicity and should be avoided. The offending antibiotics can cause toxicity when administered orally, parenterally, and even topically. *Clostridium difficile* appears to play the primary role in the enterotoxemia that follows antibiotic treatment. *Escherichia coli* has also been observed to produce a bacteremia in treated animals. Treatment is generally unrewarding; the disease is almost always fatal. At necropsy, the cecal mucosa is edematous and frequently hemorrhagic. Fluoroquinolones, trimethoprim–sulfonamide combinations, tetracycline, and chloramphenicol are among the less hazardous antimicrobials. All antibiotics should be administered with caution, using the lowest effective dosage. It has been recommended that lactobacilli be provided in the form of active-culture yogurt or dietary supplements concurrently and for 5 days after antibiotic administration to help restore the natural, intestinal bacterial flora. The efficacy of this, however, has not been proven. Alternatively, a slurry of fecal pellets freshly collected from healthy animals can be gavaged in an attempt to reestablish or maintain normal intestinal flora.

Table 8.8. Miscellaneous agents used in guinea pigs

Drug	Dosage	Route	Reference
Aminophylline	50 mg/kg	PO, SC	Morrisey and Carpenter (2011)
Atipamezole	1 mg/kg	SC, IP, IV	Flecknell (1997)
Atropine	0.05–0.1 mg/kg	SC	Harkness and Wagner (1995)
	10 mg/kg q20min (for organophosphate toxicity)	SC	Harkness and Wagner (1995)
Betamethasone	0.1–0.2 mL	IM, SC	Richardson (1992)
Calcium EDTA	30 mg/kg q12h	SC	Morrisey and Carpenter (2004)
Calcium gluconate	100 mg/kg	IM	Hoefer (1999)
Chlorpheniramine	5 mg/kg	SC	Borchard et al. (1990)
Cimetidine	5–10 mg/kg q6–12h	PO	Allen et al. (1993)
Cisapride	0.5 mg/kg q8–12h	PO	Morrisey and Carpenter (2004)
Cyclophosphamide	300 mg/kg q24h	IP	Laber-Laird et al. (1996)
Dexamethasone	0.5–2 mg/kg, then decreasing dose q12h for 3–14d	PO, SC	Harkness and Wagner (1995)
Diphenhydramine	12.5 mg/kg	SC	Laber-Laird et al. (1996)
Dopamine	0.08 mg/kg	IV	Laber-Laird et al. (1996)
Doxapram	2–5 mg/kg	IP, IV	Harkness (1993)
Ephedrine	1 mg/kg	IV	Laber-Laird et al. (1996)
Epinephrine	0.003 mg/kg prn	IV	Laber-Laird et al. (1996)
Furosemide	1–4 mg/kg q4–6h	IM, SC	Harrenstien (1994)
Glycopyrrolate	0.01–0.02 mg/kg	SC	Huerkamp (1995)
Heparin	5 mg/kg prn	IV	Laber-Laird et al. (1996)
Human chorionic gonadotropin	1000 IU per animal, repeat in 7–10d	IM	Morrisey and Carpenter (2011)
Kaopectate liquid	0.2 mL q6–8h per adult	PO	Johnson-Delaney (1996)
Lactobacilli	Administer during antibiotic treatment period, then 5–7d beyond cessation	PO	Collins (1995)
Lactulose syrup	0.5 mL/kg q12h	PO	Morrisey and Carpenter (2011)
Leuprolide acetate depot	0.2–0.3 mg/kg q28d	IM	Carpenter (2005)
Loperamide hydrochloride (Imodium A–D)	0.1 mg/kg q8h for 3d, then q24h for 2d	PO	Harkness and Wagner (1995)
Metoclopramide	0.5 mg/kg q8h	SC	Johnson-Delaney (1996)
Naloxone	0.01–0.1 mg/kg	SC, IP	Huerkamp (1995)
Oxytocin	0.2–3 IU/kg	SC, IM, IV	Anderson (1994)
Phenobarbital	10–20 mg/kg	IV, IP	Laber-Laird et al. (1996)
Potassium citrate	10–30 mg/kg q12h	PO	Morrisey and Carpenter (2004)
Prednisone	0.5–2.2 mg/kg	SC, IM	Anderson (1994)
Sulfacrate	25–50 mg/kg q12h	PO	Morrisey and Carpenter (2004)
Vitamin A	50–500 IU/kg	IM	Laber-Laird et al. (1996)
Vitamin B complex	0.02–0.2 mL/kg	SC, IM	Anderson (1994)
Vitamin C	50–100 mg q24h for deficiency	PO, SC	Morrisey and Carpenter (2011)
	10–30 mg/kg	SC, IM, PO	Morrisey and Carpenter (2011)
Vitamin E–selenium (Bo-Se)	0.1 mL/100–250g	SC	Anderson (1994)
Vitamin K	1–10 mg/kg q24h for 4–6d	IM	Harkness and Wagner (1995)
Yohimbine	0.5–1 mg/kg	IV	Harkness and Wagner (1995)

IM = intramuscular; IP = intraperitoneal; IV = intravenous; PO = per os; SC = subcutaneous.

Box 8.4

Guinea pigs are highly sensitive to antibiotics, particularly those specific for Gram-positive organisms.

Pneumonia and Respiratory Diseases

Bordetella bronchiseptica and *Streptococcus pneumoniae* are the primary bacteria that cause respiratory disease in guinea pigs. *Klebsiella pneumoniae*, *Pasteurella multocida*, *Streptococcus zooepidemicus*, *Citrobacter freundii*, and *Pseudomonas aeruginosa* have also been implicated. *Bordetella* and *Streptococcus* are common commensal organisms that may proliferate and induce respiratory infections when an animal is compromised or stressed. Younger animals and pregnant sows are particularly at risk for disease. Affected animals may have no clinical signs or may exhibit dyspnea, nasal discharge, sneezing, and other nonspecific signs such as anorexia, ruffled fur, and weight loss. Alternatively, sudden death may be observed. Pregnant sows may abort or produce stillborn offspring.

Bordetella bronchiseptica is moderately prevalent in modern facilities and transmitted primarily by aerosol. Clinical symptoms are rare, however, even in cases in which the organism has been isolated. It is thought that prior outbreaks attributed to *B. bronchiseptica* may have in fact been combined infections with other agents. The organism may be transmitted to the guinea pig from other infected species such as rabbits. *B. bronchiseptica* typically produces gross lesions of rhinitis, tracheitis, and pulmonary consolidation. Purulent bronchitis and bronchopneumonia with large accumulations of neutrophils and intraluminal debris are typically seen on histopathology. Otitis media, accompanied by head tilt, may also occur with *Bordetella* infections. Diagnosis can be achieved through bacterial culture, enzyme-linked immunosorbent assay (ELISA), or polymerase chain reaction (PCR). Fluoroquinolones, trimethoprim–sulfa, and chloramphenicol are antibiotics of choice; however, treatment is often unrewarding.

Streptococcus pneumoniae is the causative agent of diplococcal pneumonia. No outbreaks of this disease in guinea pigs have been reported in 20 years; it is thought that prior outbreaks may have resulted from combined infections with other agents. Clinical signs can be nonspecific, such as depression, anorexia, ruffled hair coat, and sudden death, or they may include nasal and ocular discharge, dyspnea, torticollis, and abortion or stillbirth. Fibrinous pleuritis, pericarditis, peritonitis, and marked consolidation of affected lung lobes are typical gross lesions. Otitis media, metritis, and other suppurative processes may also occur. Acute bronchopneumonia with fibrinous exudation and polymorphonuclear cell infiltration are typical microscopic changes. Diagnosis is by culture or PCR. Treatment may be approached as with *Bordetella* infections; however, a carrier state may be induced. Infected humans may serve as a source of infection. Face masks and other standard personal protective equipment should be employed, and workers with possible streptococcal infections should avoid any contact until a course of antibiotics has been completed. Zoonotic infection is possible.

Cervical Lymphadenitis

Cervical lymphadenitis, characterized by abscessation of the cervical lymph nodes, was once commonly seen in guinea pigs. It is now rare in a research setting. *Streptococcus equi* subspecies *zooepidemicus* of Lancefield's group C is the most common etiologic agent. *Streptobacil-*

lus moniliformis (transmitted from wild rodents) and other *Streptococcus* spp., however, are sometimes implicated. The condition is commonly called lumps. Initially, the swollen lymph nodes (commonly the cervical lymph nodes) are firm in consistency. As the disease progresses, the lymphatic tissue is replaced by thick, creamy, purulent exudate, resulting in prominent soft swellings in the ventral cervical region. The infection is spread by various routes, including aerosols, bite wounds, contaminated cage equipment (e.g., food hoppers that require animals to repeatedly stretch over their edge), and abrasion of the oral mucosa by rough food. The animals generally do not appear to be clinically affected by the abscessed lymph nodes. In some instances, only an inapparent upper respiratory tract infection exists. Alternatively, an acute, fatal, septicemic disease or pneumonia may occur. Occasionally, otitis interna, torticollis, nasal or ocular discharges, or abortions or stillbirths are noted. Treatment may include surgical incision; drainage and lavage of lesions with chlorhexidine or povidone iodine; and systemic antibiotic therapy with enrofloxacin, trimethoprim–sulfa, gentamicin, or chloramphenicol. Control is best accomplished by culling affected animals prior to rupture of lesions and appropriate environmental sanitization.

Salmonellosis

Salmonellosis is a highly lethal disease in guinea pigs although it is very rarely detected in modern research colonies. *Salmonella typhimurium* and *S. enteritidis* are the most common isolates. The most likely source of infection in a colony is the ingestion of contaminated water or food, especially fresh vegetables and fruit that have been inadequately washed before feeding.

Salmonellosis usually occurs as an acute to subacute fatal septicemia. Clinical signs may include anorexia, weight loss, light-colored soft feces, conjunctivitis, dyspnea, and abortion. Diarrhea is uncommon. Inapparent carriers may exist within a colony. Gross lesions may be absent at necropsy in peracute and acute cases. Massive splenomegaly frequently occurs along with multiple yellow–white nodules in the spleen, liver, and other organs. Multifocal granulomatous hepatitis, splenitis, and lymphadenitis, with infiltration by histiocytic cells and polymorphs, are frequently seen. A definitive diagnosis depends on the presence of typical lesions on histopathology and on recovery of organisms from the spleen, blood, or feces.

Treatment is usually not recommended in an outbreak because of the danger of species and interspecies spread and zoonotic potential. The most prudent approach is to euthanize all affected and contact animals and disinfect the environment thoroughly.

Tyzzer's Disease

Spontaneous cases of Tyzzer's disease, caused by *Clostridium piliforme*, have been reported in guinea pigs, though clinical disease is rare in modern laboratory facilities. Immunocompromised, stressed, and young animals are at highest risk. Affected animals may have a rough hair coat, appear lethargic or emaciated, develop diarrhea, and die suddenly. Typical gross necropsy findings include necrosis and inflammation of the ileum, cecum, and colon and multiple necrotic foci in the liver. Treatment with drugs in the tetracycline family or chloramphenicol may be attempted but is usually unrewarding and is not recommended.

Ulcerative Pododermatitis

Ulcerative pododermatitis, or bumblefoot, is frequently associated with *Staphylococcus* spp. infection. Predisposing factors include traumatic lesions of the feet such as may occur with

rough, poorly sanitized flooring. Obese animals as well as animals housed on wire flooring are especially prone to the disease. Affected animals have swollen, painful granulomatous lesions on the ventral surface of their forefeet. Amyloid deposits may develop in multiple organs (e.g., liver, spleen, adrenal glands) as a sequela of the disease. Affected animals should be moved to cages with solid floors and provided with soft, deep bedding. In mild cases, the feet can be treated with warm-water chlorhexidine soaks and the application of dimethyl sulfoxide (DMSO) containing a topical antibiotic and/or povidone iodine. Systemic antibiotics such as trimethoprim–sulfa, enrofloxacin, gentamicin, or chloramphenicol as well as dexamethasone should be given in severe cases. The feet should be treated with topical antibacterial ointment and bandaged. Analgesics should be provided to control pain from the foot lesions.

Mastitis

Bacterial mastitis is common in lactating sows. *Pasteurella* spp., *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., and others have been implicated. The mammary glands become warm, enlarged, and hyperemic and may produce blood-tinged milk. The young should be weaned immediately and appropriate antibiotic therapy instituted in the sow. Hot packs applied to the mammary glands are also helpful. As this is generally a painful condition, analgesics should be provided.

Conjunctivitis

Chlamydophila caviae is the causative agent of guinea pig inclusion conjunctivitis. Cyclic outbreaks of severe disease are common in enzootically infected colonies. Adult animals are frequently asymptomatic. Overt signs of disease are primarily seen in 1- to 3-week-old guinea pigs. Transmission may be by direct contact or aerosol. Clinical signs include a reddened conjunctiva, serous to purulent exudate, and photophobia. Definitive diagnosis is made from microscopic exam of conjunctival scrapings and identification of intracytoplasmic inclusion bodies in epithelial cells. The disease tends to be self-limiting, with lesions healing in 3 to 4 weeks. Sulfonamide ophthalmic ointments may be used to make the animal more comfortable. *C. caviae* should be considered a potential human pathogen.

Other agents have been implicated in conjunctivitis in the guinea pig, including *Streptococcus zooepidemicus*, coliforms, *Staphylococcus aureus*, and *Pasteurella multocida*. Concurrent infection with *C. caviae* is a possibility.

Cystitis and Urolithiasis

Urinary tract infections are most common in older sows. Disease is often subclinical until urinary blockage or severe infection occurs. Blood may be noted on the vulva or in the cage. Trimethoprim–sulfa or enrofloxacin are safe antibiotics for initial disease treatment, but final antibiotic selection should be based on culture and sensitivity testing. Urinary calculi are common. Foods high in calcium (e.g., alfalfa, clover, select fresh fruits and vegetables) should be avoided or limited because their ingestion may induce hypercalcuria with resultant urolithiasis. Occasionally, cystotomy is indicated for removal of a large urolith. Cystitis is uncommon in males; however, aged boars are prone to urethral blockage by proteinaceous plugs.

Mycotic Diseases

Dermatophytosis

Trichophyton mentagrophytes is the most common etiologic agent of ringworm in the guinea pig. Clinical infections are uncommon; however, guinea pigs frequently harbor the organism asymptomatically. Stressed animals are susceptible. Irregular areas of alopecia usually arise on the face and spread to the trunk and limbs. The lesions may appear crusty or scaly. Microscopic examination of a skin scraping can be used in diagnosis. *T. mentagrophytes* does not fluoresce under ultraviolet light. Topical antifungal creams, griseofulvin in dimethyl sulfoxide applied topically, or griseofulvin administered orally can be used for treatment. *T. mentagrophytes* is infective to humans and other animals.

Viral Diseases

Several viral agents such as Sendai, pneumonia virus of mice, and reovirus 3 have the ability to replicate briefly in the guinea pig. These agents do not cause apparent disease but the guinea pig can serve as a reservoir of virus for other rodents. Guinea pigs may become infected with other (non-Sendai) paramyxoviruses (e.g., human influenza, simian virus 5). Infected animals can be detected through serologic testing, although they exhibit no clinical signs or apparent ill effects. Viral transmission is believed to be through human contact or exposure to contaminated biologic materials.

Cytomegalovirus

Cytomegalovirus (CMV) is a common pathogen of guinea pigs. It belongs to the herpes virus family and is species specific. Infections rarely cause detectable clinical disease unless the animal is immunosuppressed or stressed. Infected animals may exhibit swelling and tenderness of the salivary gland area. Large, intranuclear and intracytoplasmic inclusion bodies, characteristic of cytomegaloviruses, may be detected in the salivary glands. Transmission occurs through shedding of the virus in the saliva or urine or transplacentally. The virus may persist in the host for years as an inapparent or latent infection.

Cavian Leukemia

Cavian leukemia induces a B-cell neoplasia and affects the hemolymphopoietic system. On blood examination, anemia and leukocytosis of 25,000–250,000 per cubic millimeter are often found, with lymphoblastic cells predominating. Clinical signs include enlarged peripheral lymph nodes, rough hair coat, lethargy, icterus, and dull eyes. Hepatomegaly and splenomegaly may result from cellular infiltration of the organs. Cavian leukemia has been associated with a C-type retrovirus, although the virus' role in disease development is still unknown.

Adenovirus

Guinea pig adenovirus most frequently causes no clinical signs of illness but has been associated with outbreaks of fatal pneumonia. Young as well as stressed animals are most susceptible to disease; transmission occurs through direct contact. Pathologic lesions include lung consolidation with nonsuppurative necrotizing bronchitis and bronchiolitis. Prior exposure may be detected through immunofluoresence assay (IFA) or ELISA serology; active infections are diagnosed through PCR of lung tissue or feces. The virus can be eradicated from

infected colonies through embryo rederivation and possibly through strategically testing and culling affected animals.

Lymphocytic Choriomeningitis Virus

Guinea pigs can become naturally infected with lymphocytic choriomeningitis virus (LCMV), an RNA arenavirus, through contact with saliva, nasal secretions, and urine. LCMV is also a frequent contaminant of transplantable tumor lines. Unlike in the mouse and hamster, the virus is not thought to propagate within the guinea pig. Naturally infected guinea pigs most frequently exhibit no clinical signs although experimentally infected animals may develop meningitis and paralysis. Lymphocytic choriomeningitis virus is zoonotic and can cause severe illness in humans, particularly those who are immunosuppressed.

Parasitic Diseases

Acariasis

Two species of mites, *Trixacarus caviae* and *Chirodiscoides caviae*, are the most common cause of acariasis in guinea pigs. These mites are commonly found on pet guinea pigs, but rarely in research facilities. *T. caviae* is a burrowing, sarcoptic mange mite that can produce alopecia, crusting, and severe pruritis that may lead to self-mutilation and debilitation. Severe infections can be fatal. Lesions are usually distributed on the neck, shoulders, lower abdomen, and inner thighs. The mite is often not readily detected by skin scrapings. *C. caviae*, a fur mite that does not burrow into the skin but attaches to the hair shaft, causes few clinical signs. Alopecia and pruritis, when present, are concentrated in the lumbar region and lateral aspect of the hindquarters. In heavy infestations, mites can be seen moving on the hair shafts. Mites are spread by direct contact with the host, animal bedding, or hair and debris. Treatments include permethrin or carbamate compounds (*Chirodiscoides*) or ivermectin or selamectin (*Trixacarus*).

Pediculosis

Gliricola porcelli and *Gyropus ovalis* are biting lice that cause occasional alopecia, rough hair coat, and mild pruritis. Lice are spread by direct contact with the host or by contaminated bedding. Treatments include pyrethroid flea powder dusts, lime sulfur dips, and ivermectin.

Protozoans

Eimeria caviae, the intestinal coccidia of guinea pigs, is usually nonpathogenic but occasionally causes colitis, diarrhea, and death. Recently weaned animals are primarily affected. The sporulation time is 3–10 days; thus good sanitation will disrupt the life cycle of the parasite. Sulfonamides can be used to treat infected guinea pigs.

Cryptosporidium wrairi is infrequently found in research facilities; however, up to 50% morbidity and mortality has been seen in infected colonies. Juvenile animals are most frequently affected. Clinical signs include lethargy, rough hair coat, diarrhea, weight loss, a potbellied appearance, and emaciation. Affected animals often have a greasy-appearing hair coat. Transmission occurs through ingestion of oocysts in feces or indirectly through contact with contaminated water, food, or fomites. At necropsy, the small and large intestines usually contain watery ingesta. Hyperemia of the small intestine and serosal edema of the cecum

may be seen. Smears of fresh feces or mucosal scrapings examined with phase contrast microscopy are generally the best method of diagnosis. There is no effective treatment; however, addition of sulfamethazine to the water supply has been reported to suppress outbreaks. The environment can be cleaned with 5% ammonia to destroy the oocysts. Although some *Cryptosporidium* spp. (e.g., *C. parvum*, *C. felis*) are believed to be zoonotic, *C. wrairi* has been shown to only infect guinea pigs and is not currently considered a zoonotic threat.

The cysts of *Balantidium caviae* are commonly found in the feces of guinea pigs, but the organism is relatively nonpathogenic. *Klossiella cobaye* is considered a nonpathogenic renal coccidian.

Nematode Infection

Paraspidodera uncinata are small worms that reside in the cecal and colonic mucosa of guinea pigs. Infections are usually considered insignificant; however, heavy infections can cause diarrhea and unthriftiness. Transmission is by ingestion of infective eggs. Sanitation is important in controlling infection. Ivermectin, piperazine, or thiabendazole are effective treatments.

Neoplasia

The prevalence of tumors in guinea pigs is low, even in aged animals, with respiratory and integumentary tumors predominating. Genetic factors appear to play an important role in the incidence of neoplasia. Bronchogenic neoplasms are the most frequently reported primary pulmonary neoplasia, with up to a 35% incidence in animals over 3 years of age. The most common bronchiogenic neoplasm is the benign papillary adenomas that may initially induce clinical signs suggestive of an infectious pneumonia. Trichofolliculomas are the predominant neoplasms of the integumentary system. Trichofolliculomas are benign basal cell epitheliomas that present as solid masses most commonly over the lumbosacral area. The skin growth can be easily removed surgically. Other commonly reported integumentary tumors include fibrosarcomas, lipomas, sebaceous gland adenomas, and hemangiomas. Mammary gland tumors, predominantly benign fibroadenomas, have been reported. Tumors of the reproductive system are not uncommon. Leiomyomas and leiomyosarcomas are the most frequently reported tumors of the uterus; teratomas are the most commonly reported ovarian neoplasia. Inbred guinea pig strains exhibit a significantly increased incidence of cavian leukemia compared with outbred strains. The disease develops most frequently in animals under 3 years of age, with resultant high mortality. Tumors of the cardiovascular, urinary, and alimentary systems are rare. It has been proposed that the low incidence of spontaneous tumors in guinea pigs may be partially due to tumor protective effects of both Kurloff cells and the serum enzyme asparaginase that has been credited for antitumor activity when administered to select other species.

Miscellaneous Conditions

Alopecia

Hair pulling and barbering are common behaviors in guinea pigs. Animals may chew their own hair or that of a cagemate. The location of hair loss can usually provide a clue about whether it is self-inflicted or a result of barbering by a dominant cagemate.

Self-barbering occurs just caudal to the shoulders. Dominancy or agonistic barbering occurs on the rump, back, ears, and around the eyes. There is no specific treatment for barbering other than removing the offending animal from the group. Providing good quality hay or enrichment items may be of some benefit. Measures to reduce stress levels should also be considered.

Intensively bred sows frequently exhibit a bilateral alopecia over the back and rump during advanced pregnancy and lactation. Nutrition, genetic, and endocrine factors may be involved. Around the time of weaning, a thinning of hair occurs during the transition from juvenile fur to more mature hair.

Trauma

The most commonly encountered traumatic injury in guinea pigs is fracture of the tibia. Animals, especially young, can suffer a leg fracture if their foot becomes trapped in wire flooring. Fractures, diaphragmatic hernias, and liver contusions with hemorrhage into the peritoneal cavity may occur in animals that are improperly handled or dropped. Depending on the fracture location, a simple, lightweight splint may be used to support the fracture during bone healing.

Malocclusion

Tooth overgrowth in guinea pigs usually involves premolars and anterior molars (Box 8.5). Maxillary premolars and molars overgrow laterally, abrading the cheek, whereas the mandibular premolars and molars overgrow medially, causing abrasion of and/or entrapping the tongue. The condition is easily overlooked because the cheek teeth are difficult to see on clinical exam. Affected animals exhibit excessive salivation, halitosis, chronic weight loss, and tongue trauma. Malocclusion tends to be a lifelong problem, requiring continual observation and repeated treatment. The offending teeth can be trimmed with pediatric rongeurs or a dental bur in a sedated or lightly anesthetized animal. Affected animals should not be bred because there is a genetic predisposition to malocclusion.

Box 8.5

Tooth overgrowth in guinea pigs usually involves premolars and anterior molars. The condition is easily overlooked because the cheek teeth are difficult to see on clinical exam.

Heat Stress

Guinea pigs are highly susceptible to heat stress. Heat stress may occur even in moderate temperatures in areas of high humidity and/or poor ventilation. Clinical signs include excessive salivation, rapid shallow breathing, hyperemia of extremities, and elevated body temperature. Rapid cooling with cool water or ice water, steroids, and supportive care may be attempted; however, treatment is often unrewarding.

Water Deprivation

Animals can die of water deprivation even when ample water appears available to them. This may occur when water is provided by a device with which the animal is unfamiliar; when water devices are placed too high or are inaccessible, particularly to weanlings; and when water is unpalatable. Other causes of dehydration include malfunctioning automatic watering devices or sipper tubes (e.g., tubes that are plugged with foreign objects or air locks) as well as the actions of dominant individuals that prevent subordinate animals from drinking.

Scurvy

The guinea pig requires an exogenous source of vitamin C (6 mg daily for maintenance), due to a genetic deficiency of the enzyme L-gulonolactone oxidase, which is involved in the conversion of L-gulonolactone to L-ascorbic acid. Vitamin C deficiency frequently occurs when guinea pigs are fed rabbit chow or outdated and/or improperly stored guinea pig chow. To ensure that appropriate nutrients, including vitamin C, are provided, guinea pig chow should be purchased only from reputable commercial vendors; stored in a cool, dry place; and used within the manufacturer's recommended time following milling. For many years, the shelf life of guinea pig chows was only 3 months after milling due to the rapid degradation of vitamin C. Chows containing microencapsulated vitamin C (L-ascorbyl-2 polyphosphate) have been developed that have a more prolonged (6-month) shelf life. Signs of scurvy usually appear within 2 weeks after guinea pigs have been deprived of vitamin C. Clinical signs include reluctance to move, unkempt appearance, swelling around the joints, and infrequently diarrhea. The most prominent gross lesions seen at necropsy are hemorrhages in the muscle and periosteum, particularly around the stifle joint (Figure 8.4) and rib cage, and epiphyseal enlargement at the costochondral junction. Affected guinea pigs should receive 30 mg/kg per day of parenteral (preferable) or oral ascorbic acid until recovery is evident. Animals may experience ill-effects with only marginal hypovitaminosis C such as may occur during periods of partial or full anorexia or feeding of improperly stored diets.

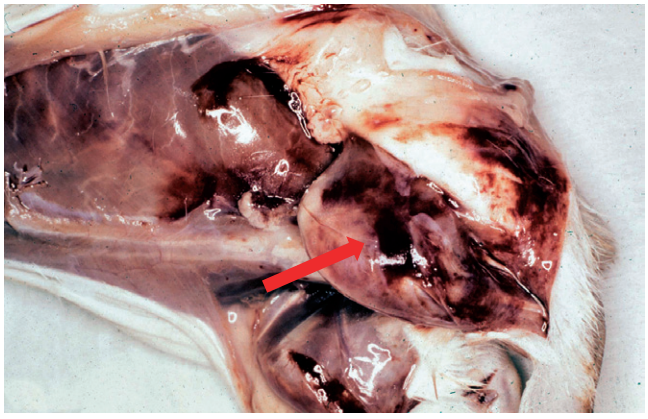

Fig 8.4. Typical scurvy lesion with hemorrhage in stifle area. (Photo courtesy of ACLAM.)

These animals are more prone to upper respiratory disease, bacterial infections, and conjunctivitis.

Circumanal Sebaceous Accumulations

Excessive accumulations of sebaceous secretions occur in the folds of the circumanal and genital region in adult male guinea pigs. The sebaceous secretions can also form a plug that accumulates in the folds between the two halves of the scrotum.

Preputial Infection and Vaginitis

Male guinea pigs occasionally develop preputial infections caused by lodging of foreign material in the preputial folds. Breeding males housed on bedding may be affected when pieces of bedding adhere to the moist prepuce following copulation and are drawn into the preputial fornix. Treatment involves removing the bedding particles and cleansing the area.

Vaginitis in female guinea pigs is usually caused by entrapment of wood chips or other bedding material in the vagina, causing a foreign body reaction. Treatment includes washing the area carefully and swabbing away the bedding material. It may be desirable to place the animal on a different type of bedding until the area has healed.

Dystocia

The most common cause of dystocia is incomplete relaxation of the pubic symphysis due to fusion and ossification of the pubic symphysis. Normally, 2–24 hours before parturition, the pubic symphysis of a pregnant sow becomes separated, allowing the birth canal to increase in diameter. If sows are not initially bred prior to 7 months of age, the pubic symphysis will often fuse and ossify. Dystocia can also result from an excessively large or malformed fetus, large litter, obesity, pregnancy toxemia, and uterine inertia. A sow in dystocia often presents with a bloody or greenish-brown discharge. Uterine inertia may respond to oxytocin; however, if parturition has not begun within 15 minutes after administration, a cesarean section should be performed.

Pregnancy Toxemia (Ketosis)

Two different patterns of disease are associated with pregnancy toxemia in the guinea pig: the fasting or metabolic form and the circulatory or toxic form. The clinical signs are similar, and both forms usually occur in late pregnancy. Animals exhibit depression, acidosis, ketosis, proteinuria, and ketonuria. They produce clear urine with pH lowered from around 9 to 5–6.

The fasting or metabolic form, called ketosis, occurs in obese sows during the last 2–3 weeks of pregnancy. Sows during their first or second pregnancy are more frequently affected. Stress factors such as changes in feeding routines or environment may precipitate the disease. Affected animals usually become comatose and die within 5–6 days after onset of symptoms. A similar condition may be observed in obese, aged males. A reduced carbohydrate intake with subsequent mobilization of fat as a source of energy apparently is involved. Lesions seen at necropsy include fatty liver, fatty kidneys, ample fat stores, and an empty stomach. Treatments that may be tried include lactated Ringer's solution, calcium gluconate, 5% glucose, and corticosteroids; however, prognosis is poor. The incidence can be reduced in breeding sows by controlling food intake to prevent obesity and by breeding at a body weight of 450–500 g. Breeding sows should not be disturbed by changing diets, moving to new quarters, or other factors that might induce fasting.

In the circulatory or toxic form, a large gravid uterus compresses the aorta caudal to the renal vessels, which causes uteroplacental ischemia. Blood pressure is reduced in the uterine vessels, causing placental necrosis and hemorrhage, thrombocytopenia, ketosis, and death. The guinea pig has been identified as a possible animal model for preeclampsia in pregnant women.

Gastric Dilation, Cecal Torsion, Typhlitis

Gastric dilation, cecal torsion, and acute typhlitis occur sporadically in guinea pigs of all ages. Animals are usually found dead with no previous indication of disease.

Diabetes Mellitus

A spontaneous diabetes mellitus that occurs in animals 3–6 months of age has been reported. Affected animals are not clinically ill but have hyperglycemia, glycosuria, and rarely ketonuria. Exogenous insulin is not required for survival. An infectious agent is thought to be involved.

Soft Tissue Calcification

Soft tissue or metastatic calcification occurs most often in guinea pigs over 1 year of age. Affected animals may be unthrifty and exhibit muscle stiffness or lameness. Mineral deposition may be confined to the soft tissue around the elbows and ribs or may be more widespread to the lung, trachea, heart, aorta, liver, kidney, stomach, uterus, and sclera. This syndrome is thought to be attributable to a dietary imbalance of two or more minerals—for example, low magnesium and high phosphorus—and is one reason why guinea pigs should not be fed diets formulated for other species. Multiple linear chalky deposits on affected organs can be seen grossly at necropsy.

Nutritional Muscular Dystrophy

A myopathy has been associated with vitamin E/selenium deficiency. Clinical signs may include depression, hindlimb weakness, conjunctivitis, and reduced reproductive performance in sows. Affected animals usually have elevated serum creatine phosphokinase (CPK) levels. Treatment with vitamin E/selenium injections may be of benefit.

Osteoarthritis

Spontaneous osteoarthritis of the stifle and other joints has been described. Obesity, hypovitaminosis C, and joint injury may contribute to joint degeneration.

Aging

Aged guinea pigs develop fecal impactions composed of soft, cecal feces within the anus. Loss of muscle tone and/or an inability to eat feces directly from the anus may play a role. Multiple ovarian cysts, fatty infiltration of the pancreas, and segmental nephrosclerosis are frequently observed at necropsy of aged animals.

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CHAPTER 8 REVIEW

Fill In the Blank

1. The guinea pig is also commonly known as a _____, a derivation of its scientific name.
2. The _____ breed of guinea pigs have short, coarse hair arranged in whorls or rosettes.
3. The _____ breed of guinea pigs have long, fine, silky hair.
4. The soft palate is continuous with the base of the tongue and possesses an opening called the _____ _____.
5. Guinea pigs lack the enzyme L-gulonolactone oxidase and thus require vitamin _____ in their diet.
6. Guinea pigs have a unique leukocyte, the _____ cell, which is a mononuclear lymphocyte with a large intracytoplasmic inclusion.
7. The term for parturition in the guinea pig is _____.
8. The thymus of the guinea pig is not located within the thoracic cavity as in other rodents, but instead surrounds the _____.
9. The female guinea pig is called a _____ and the male is called a _____.
10. Guinea pigs have _____ digits on their forefeet and _____ digits on their hindfeet.

Match Up

Match the following with their respective descriptions:

A. Scurvy
B. Altricial
C. Precocious
D. 7 months
E. 12 months
F. 45 days
G. 68 days
H. Lumps
I. Sore hocks
J. Bumblefoot
K. Preyer
L. *Bordetella bronchiseptica*
M. *Gliricola porcelli*
N. *Streptococcus zooepidemicus*
O. *Chlamydophila caviae*
P. *Chirodiscoides caviae*

(*Continued*)

11. _____ Reflex that involves cocking ears in response to sound
12. _____ Causative agent of inclusion conjunctivitis
13. _____ Biting louse
14. _____ Gestation length
15. _____ Age that sows should be bred prior to
16. _____ What ulcerative pododermatitis is commonly called
17. _____ Deficiency of vitamin C causes this
18. _____ Term that describes newborn guinea pigs
19. _____ Causative agent of cervical lymphadenitis
20. _____ Causative agent of acariasis

Critical Thinking

21. A group of guinea pigs have recently undergone a tympanic bullae procedure as part of a research study. Several of them appear hunched in their cage and reluctant to move. Others are salivating excessively. You check the surgical chart and see they were initially given an injection of ketamine IM and then administered isoflurane via face mask for the procedure. What is the most likely problem?

9 Chinchillas

The chinchilla is a caviomorph (New World hystricognath) rodent closely related to guinea pigs, degus, and porcupines. It belongs to the family Chinchillidae. The two most common species are *Chinchilla chinchilla* (formerly *C. brevicaudata*), the short-tailed chinchilla, and *C. laniger*, the long-tailed chinchilla. Specific information in this chapter refers to *C. laniger*, the species most commonly encountered in research. The word chinchilla is derived from the name Chincha, a native people of the Andes. Chinchillas are native to South America, once inhabiting the rocky slopes of the Andes Mountains in Peru, Bolivia, Chile, and Argentina. Extensive hunting for their pelts has rendered them nearly extinct in the wild; fortunately, these animals breed successfully in captivity. Average adult weights range from 400 to 600 g; females are slightly larger than males. They range from 25 to 35 cm (10–14 in.) in length. The standard color is a smoky blue–gray that ranges from very light to very dark; selective breeding has produced a number of color mutations, including beige, white, black, brown, silver, and violet. They are virtually odorless and easy to maintain.

USES

The chinchilla is a common research model in otolaryngological investigations, specifically auditory studies, including otitis media, tinnitus, noise-induced hearing loss, and cochlear implants. Their cochlear size and affiliated neural connections closely approximate human anatomy and physiology, as do their auditory range and sensitivity. They have large tympanic bullae, capsules that contain an extension of the thin, boney cavities housing the middle ear, allowing easy surgical access to the middle ear, cochlea, and surrounding structures.

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Additionally, there is a lack of susceptibility to naturally occurring middle ear infections. Chinchillas show a less pronounced presbycusis (age-related hearing loss) as they age; study of this phenomenon is facilitated by their relatively longer life span compared with other rodent species. This approximates the age-related hearing loss in the human male, thus providing a naturally occurring model for related otology studies. Chinchillas are also used in research investigations involving Chagas disease, listeriosis, and *Pseudomonas* infections.

BEHAVIOR

In their native environment, chinchillas live in groups of several hundred in rock crevices or burrows at elevations above 800 m (2600 ft). They are social, inquisitive animals, with family groups of two to five sharing the same burrow. They rarely fight, although females can be quite aggressive toward strange males during breeding. Chinchillas have several predator-avoidance mechanisms. One is referred to as fur slip, which allows them to escape perceived dangerous conditions. When fighting or handled roughly, they release a large patch of fur to "slip" away. Chinchillas will also spray urine at perceived intruders of their territory. Although normally nocturnal or crepuscular, chinchillas may engage in periods of activity including jumping and climbing during daylight hours. They are dexterous, using their front paws to grasp food in the manner of most other rodents. Chinchillas are not a particularly vocal species, but will elicit a number of communicative sounds. They produce a series of low-frequency chirps during exploratory behavior, a rapid series of grunts as a contact call, bark and growl when threatened, and squeal or shriek when injured. They do not hibernate. Chinchillas, like guinea pigs, are creatures of habit and do not adapt well to change.

Like most rodents, these animals possess an innate preference for contact with the vertical perimeter of a bounded space (i.e., wall following, corner burrowing, and group aggregation), avoiding the perceived threats of open areas (this orientation response is called thigmotaxis). Knowledge of this behavioral preference can be employed to more quickly acclimate animals to handling by holding them securely with as much surface contact as possible.

ANATOMIC AND PHYSIOLOGIC FEATURES

Chinchilla biologic and reproductive data are listed in Table 9.1. Their compact bodies are covered with exceptionally dense velvetlike fur with up to 60 hairs from each follicle; their long, bushy tails aid in balance. Four toes are present on all feet, each with a small claw. The plantar surfaces are devoid of fur. Their coat grows in waves, starting at the head and progressing toward the tail. The position of a pale bar line present in each hair indicates the stage of growth of the coat. In the furrier industry, the pelt is most valuable when the bar line is at the end of the hair all over the body. Relative to the size of their bodies, they have large heads with long vibrissae, prominent eyes, and batlike ears. The thymus gland is entirely intrathoracic. The dental formula is 2(I 1/1, C 0/0, P 1/1, M 3/3). The chinchilla's teeth are open rooted, growing throughout their life span, and incisors are yellowish in

Table 9.1. Chinchilla biologic and reproductive data

Adult body weight: Male	400–500 g
Adult body weight: Female	400–600 g
Life span	9–18 y
Body temperature	37°–38°C (98.6°–100.4°F)
Heart rate	100–150 beats per minute
Respiratory rate	40–80 breaths per minute
Food consumption	30–40 g/d; 5.5 g/100 g per day
Water consumption	10–20 mL/d; 8–9 mL/100 g per day
Breeding onset: Male	7–9 mo
Breeding onset: Female	4–5 mo
Estrous cycle length	30–50 d
Gestation period	105–120 d
Postpartum estrus	Fertile
Litter size	1–6
Weaning age	6–8 wk
Breeding duration	3 y
Chromosome number (diploid)	64

Source: Adapted from Harkness et al. (2010) and Quesenberry et al. (2004).

contrast to the molars, which are cream colored. The jaws have an abnormal relative size with the mandible being wider than the maxilla. The oral cavity is narrow, primarily filled by the tongue. The chinchilla has a palate like that of the guinea pig. The soft palate is continuous with the base of the tongue and possesses an opening called the palatal ostium. The gastrointestinal tract is remarkably long as they are hindgut fermenters. The jejunum nearly fills the entire abdomen, the cecum is large, and the proximal colon is highly sacculated, in contrast to the terminal colon, which is smooth. The cecum holds less intestinal contents than that of the rabbit or guinea pig. Typical of rodents, chinchillas cannot vomit and are coprophagic.

Sexing chinchillas can be difficult because the external genitalia of males and females are similar. The female's prominent, cone-shaped clitoris may be mistaken for a penis at a quick glance. Males have a greater anogenital distance and larger genital papilla. In the male, there is no true scrotum; rather, the testicles are located in the inguinal canals or subcutaneously in the anal region. The female chinchilla is unique in that the vagina has a separate external opening between the rectum and urethral openings that remains closed by the vaginal membrane except during estrus and parturition. The female has two uterine horns, two cervices, and three pairs of mammary glands, two lateral and one inguinal.

Hematologic values for chinchillas reported in the literature are extremely variable. There are, however, several distinctive characteristics of their hematologic and urinary profiles. A seasonal effect on hematologic values of laboratory-adapted chinchillas has been reported. The red blood cell count and hemoglobin levels are highest in the winter season, while the

white blood cell count is highest in the winter and early spring when reproductive processes increase. The lymphocyte is the predominant white blood cell. Chinchillas have a higher oxygen–hemoglobin affinity than other rodents and rabbits, probably due to their natural mountainous habitat. Urine is normally alkaline, with a pH of 8.5, and contains varying amounts of calcium carbonate crystals. The chinchilla is able to withstand long periods without water (8 days to 2 weeks) and thus can excrete concentrated urine. The specific gravity often exceeds 1.045. White and red blood cells are normally not present in the urine, and casts are rare. The presence of a few squamous epithelial cells is normal, especially in females. Selected hematologic and biochemical values are listed in Appendix 1, "Normal Values."

BREEDING AND REPRODUCTION

Puberty occurs at 4–12 months of age, depending upon the sex and the time of year the chinchilla is born. Females reach puberty at 4–5 months of age and males at 7–9 months. If young are born in the spring, puberty occurs in the fall; if born in the fall, puberty occurs approximately 1 year later. The chinchilla is seasonally polyestrous, with the main breeding season occurring between November and May. A harem system is generally used whereby one male has access to as many as 12 females. Females (does) are quite selective in their choice of a male (buck) for breeding and can be very aggressive toward the male. For pair breeding, the female is dominant and should be taken to the male's cage. For harem systems, a collar can be placed around the female's neck and a small exit hole provided for the male to escape the aggressive female if necessary. The estrous cycle lasts 30–50 days with an average of 38 days. Breeding occurs at night and is characterized by multiple intromissions; ovulation is usually spontaneous. Detection of the large vaginal plug either in the female or on the cage floor is a reliable indicator that mating occurred the previous day. Gestation is lengthy, with an average of 111 days. A postpartum estrus occurs 2–48 hours after parturition, and a postlactational estrus 35–84 days after parturition.

Near parturition, the chinchilla may become inactive and anorectic; the vagina begins to open and takes on a bluish color. The female chinchilla does not make a nest. Parturition normally occurs in the early morning with all kits born within several hours. Dystocia is uncommon, but if labor takes longer than 4 hours, a cesarean section is advisable. Chinchillas are prone to retained placentas or feti and should be evaluated by abdominal palpation after parturition. The normal litter size is two, but it may be as high as six. A disproportionate number of males are born: the ratio is 119 males to 110 females. Newborns are precocious, fully furred, with teeth, and with their eyes and ears open and frequently vocal. Milk letdown is sometimes sluggish and may be treated with an injection of oxytocin. Lactating females can develop gastrointestinal stasis and tympanites 2–3 weeks postpartum that results in gaseous distension of the stomach and intestines and is frequently accompanied by hind limb paralysis. An intravenous or intraperitoneal small-animal dose of calcium gluconate has been reported to diminish symptoms. Young begin to eat solid food within 1 week of birth. The use of a dust bath is contraindicated in the presence of newborns to prevent dust from entering their eyes and oral cavities. Generally, chinchillas are weaned at 6–8 weeks of age, but can be weaned as early as 3 weeks.

HUSBANDRY

Housing and Environment

Chinchillas are active animals, a fact which should be considered during caging selection (Box 9.1). Multilevel cages work well to accommodate their activity level. As with other rodent species, solid-bottom cages with hardwood chips or recycled paper products are preferred. If wire-mesh flooring is used, the mesh should be of small enough gauge, for example 15 mm × 15 mm, to prevent limb injuries. Floor space and cage height recommendations have not been published for laboratory chinchillas; breeders and fanciers have found 1–2 ft^2 per animal with a minimum height of 12 inches to be suitable. Rabbit caging can be used, but must be modified to prevent accidental escape of chinchillas through catch (drop) pan openings. When using rabbit caging, the spacing of metal floor bars or perforated flooring should be carefully evaluated. The temperature and humidity parameters are different than for most rodents: housing conditions include a temperature of 16°–21°C (61°–70°F), humidity between 30% and 60%, 10–15 air changes per hour, and 14 hours of light per day. It is important to avoid an environment with poor ventilation and high temperature and humidity as these animals are heat sensitive and prone to matted fur when housed in these conditions.

Box 9.1

Chinchillas are active animals that are best housed in a multilevel cage.

Sanitation

The frequency of primary enclosure sanitation depends much on the type of housing used. Solid-bottom cages and feeding and watering receptacles should be cleaned weekly and disinfected with chemicals, hot water 61.6°–82.2°C (143°–180°F), or a combination of both. Detergents and chemical disinfectants enhance the effectiveness of hot water, but they must be thoroughly rinsed from all caging surfaces with fresh water. Suspended cages usually require less frequent sanitation. Catch pans from suspended cages should be changed three times a week. Refer to Chapter 4, "Mice," for a more detailed description of disinfection methods.

Environmental Enrichment and Social Housing

An animal's social needs should be carefully considered when housing animals in the laboratory environment, with the aim to increase normal behavior and decrease abnormal or stereotypic behaviors. Chinchillas are frequently housed in pairs or small groups to address their social needs. Multilevel caging and feeding twice daily can be used to enrich the chinchilla's environment. A pumice gnawing stone to wear the teeth is available commercially and should be placed in the cage to promote normal gnawing behavior. Alternatively, fresh branches from willow, beech, hazelnut, or unsprayed fruit trees can also be used for gnawing

enrichment. Items such as plastic dumbbells or balls placed in the cage or stainless steel chains hung on the outside of the cage are well utilized by chinchillas. Huts with a flat top can be used to allow animals to jump onto another surface. PVC piping cut in small sections or aligned in an L-shaped design allow for seclusion. Dust baths, which aid in the absorption of skin oils, are an important part of the chinchilla's normal grooming behavior and are necessary to maintain a healthy coat (Box 9.2). Enclosed or open pans with a dust depth of 2–3 centimeters should be offered several times weekly. Sanitized chinchilla dust is available commercially; alternatively, silver sand and fuller's earth at a 9:1 ratio can be used. Playground sand and talc are not recommended. Formal approval should be secured from the researcher, the IACUC, and the facility veterinarian prior to the introduction of any enrichment items.

Box 9.2

Chinchillas should be provided with a dust bath several times weekly.

Feeding and Watering

Chinchillas should be fed a commercial pelleted laboratory chinchilla chow that contains 16%–20% protein, 2%–5% fat, and approximately 18% fiber. The pellets should contain a minimum of 2700 cal per kilogram of diet. Guinea pig or rabbit chow is adequate; however, the smaller pellets in these diets are more difficult for the animal to grasp. Adults eat an average of 30–40 g of food per day, which should be offered from a J-feeder or other elevated self-feeder. Good quality grass hay, such as timothy or alfalfa, that is free of mold and insecticides, should be fed *ad libitum* from a hay rack; cubed hay can be placed directly on the floor of the cage. The diet can be supplemented with small amounts of fresh fruits, vegetables, oatmeal, raisins, or sunflower seeds, but excessive supplementation can result in obesity and digestive tract upsets and should be avoided. Dietary changes should be made slowly to avoid enteric problems. Chinchillas should be provided with fresh, potable water *ad libitum* by bottle or automatic watering system.

TECHNIQUES

Handling and Restraint

Chinchillas are easy to handle and respond best to a light touch and gentle approach. They are not aggressive biters but will bite if agitated. To carry a chinchilla a short distance, lift it by the base of the tail and place the animal on the opposite forearm, up against the body, with its head toward the elbow. To restrain a chinchilla, grasp the base of the tail with one hand and place the other hand over the shoulders and thorax as depicted in Figure 9.1. Figure 9.2 shows an alternative method of restraining a docile chinchilla. Care must be taken to avoid grasping the fur or rough handling as this can result in the loss of a patch of fur, called

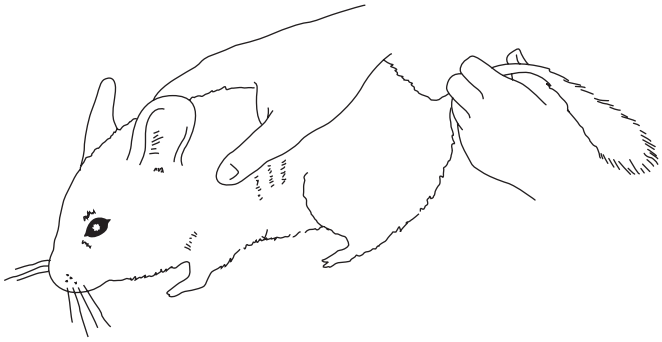

Fig 9.1. Restraint of the chinchilla.

Fig 9.2. Restraint of a docile chinchilla.

Box 9.3

Grasping the fur or roughly handling the chinchilla can result in fur slip and must be avoided.

fur slip (Box 9.3). Additionally, never try to grasp an escaping chinchilla by the end of the tail as this can result in a degloving injury.

Identification

Cage cards are most often used as a form of general identification. There are several permanent methods to identify chinchillas individually, including ear tagging or tattooing and

Table 9.2. Adult chinchilla blood volumes and sample size

	Volume (mL)
Total blood	27–48
Single sample	3–5
Exsanguination	10–18

Source: Adapted from Harkness et al. (2010).
Note: Values are approximate.

subcutaneous (SC) placement of a microchip for quick electronic identification. Dyes, markers, or clipped fur can be used as temporary identification methods.

Blood Collection

Assuming the animal is mature, healthy, and on an adequate plane of nutrition, the blood volume of most species averages 7% of the body weight in grams. Table 9.2 lists approximate adult chinchilla blood volumes. A typical rule of thumb for blood withdrawal suggests that up to 10% of the circulating blood volume can be withdrawn from normal, healthy animals every 2–3 weeks with minimal adverse effect. Small amounts of blood, 0.3–1 mL, can be collected from peripheral veins such as the lateral saphenous, cephalic, and femoral. The femoral vein is located at the medial aspect of the hind limb; blood can be collected using the technique employed to sample a cat's medial saphenous vein. The ventral tail artery is an alternate site. Larger quantities of blood can be collected from the jugular vein. For jugular venipuncture, anesthesia may be necessary; the chinchilla is positioned with the head and neck extended upward and the forelimbs extended downward over the edge of a table with a second person drawing the blood sample. Anesthesia is required for collection from the cranial vena cava transverse sinus (venous system encircling the auditory bullae), and heart. These sites involve greater risk due to the potential for inapparent vessel laceration and hemorrhage. Cardiac sampling is not recommended except as a terminal procedure.

Urine Collection

Urine can be collected using a metabolic cage, gentle digital pressure on the bladder, clean-catch method, or cystocentesis. Chinchillas that are annoyed will usually urinate, projecting their urine with accuracy for several feet.

Drug Administration

Palatable drugs can be incorporated into the food or water. Liquids can be administered orally using a small syringe or directly into the stomach via a stomach tube. Subcutaneous injections with a maximum volume of 8 mL per site can be given between the shoulders or in the flank area. Intramuscular injections can be given in the lumbar muscle and in the rear leg using the quadriceps or semitendinosus muscle. Smaller-gauge needles, such as 23 to 25 gauge, should be used for intramuscular injections, and no more than 0.3 mL of a solution should be administered in one site. The intraperitoneal route can also be used for volumes

Table 9.3. Maximum adult chinchilla injection volumes

Route	Volume (mL)
IP	10
SC	8–25
IM	0.3
IV	1
PO	5

Source: Adapted from Quesenberry et al. (2004), Strake et al. (1996), and Lukas (1999).

Table 9.4. Anesthetic agents and tranquilizers used in chinchillas

Drug	Dosage	Route	Reference
Inhalants			
Isoflurane	To effect	Inhalation	Hoefer (1994)
Sevoflurane	To effect	Inhalation	Morrisey and Carpenter (2004)
Injectables			
Acepromazine	0.5 mg/kg	IM	Johnson-Delaney (1996)
Alphadolone–alphaxalone	20–30 mg/kg	IM	Green (1982)
Diazepam	2.5 mg/kg	IP	Green (1982)
Fentanyl–droperidol	0.20 mL/kg	IM	Green (1982)
Ketamine	40 mg/kg	IM	Anderson (1994)
Ketamine	40 mg/kg	IM	Hoefer (1994)
+ acetylpromazine	0.5 mg/kg	IM	
Ketamine	20 mg/kg	IP, IM	Flecknell (1987)
+ diazepam	5 mg/kg	IP, IM	
Ketamine	5–10 mg/kg	IM	Morrisey and Carpenter (2004)
+ midazolam	0.5–1 mg/kg	IM	
Ketamine	35–40 mg/kg	IM	Anderson (1994)
+ xylazine	4–8 mg/kg		
Pentobarbital	35–40 mg/kg	IP	Anderson (1994)
Tiletamine–zolazepam	20–40 mg/kg	IM	Hoefer (1994)

IM = intramuscular; IP = intraperitoneal; IV = intravenous.

up to 10 mL. Intravenous injections are difficult but possible using the small peripheral vessels in the ears or legs. Suggested maximum dosing volumes are summarized in Table 9.3.

Anesthesia, Surgery, and Postoperative Care

A variety of agents that can be used to tranquilize and anesthetize chinchillas are listed in Table 9.4. Food should be withheld from chinchillas for 6 hours prior to anesthesia.

Atropine can be administered to decrease salivary secretions 15 minutes before the induction of anesthesia. Ketamine can be administered alone for chemical restraint or noninvasive procedures and mixed with acepromazine or xylazine for longer and/or more painful procedures. A ketamine and pentobarbital combination can also be used. Pentobarbital is reported to be short acting in the chinchilla, and is associated with cardiovascular depression and hypothermia. Ophthalmic ointment should be used in the eyes of chinchillas anesthetized with ketamine or ketamine combinations as this drug suppresses the blink reflex.

Inhalation anesthetic agents can be given via a chamber or face mask. Isoflurane is recommended, but sevoflurane can also be used. A nose cone or face mask is generally used to maintain anesthesia as chinchillas are difficult to intubate. Care should be used during induction because some animals hold their breath and then take deep rapid breaths of the anesthetic agent, which can result in death if the concentration of anesthetic gases is high. The use of ophthalmic ointment is recommended to prevent drying of the ocular membranes by the anesthetic gases. Care should be taken to scavenge waste anesthetic gases for the safety of personnel in the area. A combination of reflexes, such as the toe pinch and palpebral, should be used to monitor the depth of anesthesia.

Two of the most common surgical interventions performed in chinchillas include a simple castration and opening and drainage of an abscess. Males have open inguinal canals that require closure during castration. A number of surgical procedures involving the auditory system are commonly performed for research.

Surgery should be done in an aseptic manner and blood loss should be minimized. Use of a circulating water blanket to support euthermia is necessary. Animals should be recovered on a clean, dry paper or cloth towel, separated from any direct bedding to prevent aspiration or ingestion of fine bedding particles. Anesthetized animals should never be placed in an enclosure with conscious cagemates. Postoperative care should include hydration maintenance and a method to prevent hypothermia; the administration of warmed lactated Ringer's or normal saline will help facilitate both thermoregulation and hydration during recovery. A nonambulatory animal should be turned every 30 minutes to prevent hypostatic pulmonary congestion. It is important to maintain hydration and nutrition levels in the postoperative period to support normal metabolic processes and gut physiology. Select, commercially available supportive and critical care products for laboratory animals are provided in Appendix 3. Unless approved by the IACUC, analgesics should be provided as a postprocedural standard of care. It must be assumed that procedures which cause pain or distress in humans will cause pain or distress in other animals unless documented to the contrary. Analgesics that may be utilized in chinchillas are listed in Table 9.5.

Imaging Techniques

Great advances have been made in rodent and small animal imaging over the last decade. Many imaging modalities once used only in humans [e.g., digital x-ray imaging, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), dual-energy x-ray absorptiometry (DEXA) scan, and ultrasound] have been adapted for use with these species, producing images of exquisitely fine detail. In addition, novel imaging techniques have been developed for use in animal research, including *in vivo* optical imaging. Some x-ray units (e.g., Faxitron animal imaging and irradiation systems) have been

Table 9.5. Analgesia agents used in chinchillas

Drug	Dosage	Route	Reference
Acetylsalicylic acid	100–200 mg/kg q6–8h	PO	Johnson-Delaney (1996)
Buprenorphine	0.05 mg/kg q8–12h	SC	Morrisey and Carpenter (2004)
Butorphanol	0.2–2 mg/kg q4h	IM, SC	Morrisey and Carpenter (2004)
Carprofen	4 mg/kg q24h	SC	Richardson (1997)
	4 mg/kg q24h	PO	Harkness et al. (2010)
Flunixin meglumine	1–3 mg/kg q12h	SC	Hoefer (1999)
Ketoprofen	1 mg/kg q12–24h	SC	Harkness et al. (2010)
Meloxicam	0.1–0.3 mg/kg q24h	PO, SC	Harkness et al. (2010)
Meperidine	10–20 mg/kg q6h	IM, SC	Smith and Burgmann (1997)
Morphine	2–5 mg/kg q2–4h	SC	Harkness et al. (2010)
Tramadol	5–10 mg/kg q12–24h	PO	Morrisey and Carpenter (2011)

IM = intramuscular; PO = per os; SC = ubcutaneous.

designed for use specifically with small rodents. These units produce a highly magnified and clear resolution image of the field of interest.

For conventional x-ray imaging, an x-ray machine with high milliamperage, such as a 200- or 300-mA unit with the capability for low settings of peak voltage delivered (kVp) and small incremental changes, is ideal. High-detail film–screen combinations work well. Short exposure times of 1/40 second or less should be used to decrease the chance of motion artifact. Suggested exposure factors are 42–46 kVp, 300 mA, 1/40 second, 40 SID, 7.5 mA·s. Alternatively, nonscreen film can be used with standard x-ray or dental units. Nonscreen film produces high-detail radiographs, but longer exposure times are needed. Digital imaging techniques offer substantial flexibility in image enhancement and delivery application. The best method of restraint is to sedate the animal and use adhesive tape to secure it to the cassette in the appropriate position. A radiolucent tube or stockinette with the patient positioned inside can be used for restraint of conscious animals.

Euthanasia

Chinchillas may be euthanized with inhalation anesthetic agent overdose, by injection of pentobarbital at three to four times the anesthetic dose, or with a commercial euthanasia solution given by the intravenous or intraperitoneal route. Although a controlled administration of carbon dioxide is an approved method of euthanasia, pre-sedation or the use of injectable agents provide a smoother and more esthetically acceptable euthanasia. Refer to Chapter 4 for a more detailed description of euthanasia methods.

THERAPEUTIC AGENTS

Suggested chinchilla antimicrobial and antifungal drug dosages are listed in Table 9.6. Antiparasitic agents are listed in Table 9.7, and miscellaneous agents are listed in Table 9.8.

Table 9.6. Antimicrobial and antifungal agents used in chinchillas

Drug	Dosage	Route	Reference
Amikacin	2–5 mg/kg divided q8h–12h	SC, IM, IV	Harkness et al. (2010)
Amoxicillin	Do not use		Morrisey and Carpenter (2004)
Ampicillin	Do not use		Morrisey and Carpenter (2004)
Captan powder	1 tsp/2 cups dust, add to dust box	Topical	Hoefer (1994)
Cephalosporin	25–100 mg/kg q6h	PO	Johnson-Delaney (1996)
Chloramphenicol	50 mg/kg q12h	PO, SC, IM	Harkness et al. (2010)
Chlortetracycline	50 mg/kg q12h	PO	Allen et al. (1993)
Ciprofloxacin	5–15 mg/kg q12h	PO	Smith and Burgmann (1997)
Doxycycline	2.5 mg/kg q12h	PO	Johnson-Delaney (1996)
Enrofloxacin	10 mg/kg q12h	SC, IM, PO	Hoefer (1994)
Gentamicin	2 mg/kg q12h	SC, IM, IV	Harkness et al. (2010)
Griseofulvin	25 mg/kg q24h for 30–60 d	PO	Jenkins (1992)
Itraconazole	5 mg/kg q24h	PO	Mitchell and Tully (2009)
Lime sulfur dip	Dilute 1:40 with water, dip q7d for 6 wk	Topical	Anderson (1994)
Metronidazole	10–20 mg/kg q12h; use with caution	PO	Harkness et al. (2010)
Neomycin	15 mg/kg q24h	PO	Burgmann and Percy (1993)
Oxytetracycline	50 mg/kg q12h	PO	Collins (1995)
Penicillin	Do not use		Morrisey and Carpenter (2004)
Sulfadimethoxine	25–50 mg/kg q24h for 10–14 d	PO	Smith and Burgmann (1997)
Sulfamerazine	1 mg/mL drinking water		Anderson (1994)
Sulfamethazine	1 mg/mL drinking water		Anderson (1994)
Tetracycline	0.3–2 mg/mL drinking water		Burgmann and Percy (1993)
	10–20 mg/kg q8–12h	PO	Harkness et al. (2010)
Trimethoprim–sulfa	30 mg/kg q12h	PO, SC, IM	Harkness et al. (2010)
Tylosin	10 mg/kg q24h	PO, SC, IM	Collins (1995)

IM = intramuscular; IV = intravenous; PO = per os; SC = subcutaneous.

Table 9.7. Antiparasitic agents used in chinchillas

Drug	Dosage	Route	Reference
Albendazole	25 mg/kg q12h for 2 d	PO	Donnelly (2004)
Carbaryl powder (5%)	Dust q7d for 3 wk	Topical	Anderson (1994)
Fenbendazole	20 mg/kg q24h for 3–5 d	PO	Harkness et al. (2010)
Ivermectin	0.2 mg/kg q7d for 3 wk	PO, SC	Anderson (1994)
Lime sulfur dip	Dilute 1:40 with water, dip q7d for 4–6 wk		Harkness et al. (2010)
Metronidazole	25 mg/kg q12h for 5 d; use with caution	PO	Morrisey and Carpenter (2004)
Piperazine citrate	100 mg/kg q24h for 2 d	PO	Johnson-Delaney (1996)
Praziquantel	5–10 mg/kg, repeat in 10 d	PO, SC, IM	Smith and Burgmann (1997)
Pyrethrin powder or shampoo	weekly for 3 wk	Topical	Harkness et al. (2010)
Sulfadimethoxine	25–50 mg/kg q24h for 10 d	PO	Harkness et al. (2010)
Sulfamerazine	1 mg/mL drinking water		Anderson (1994)
Sulfamethazine	1 mg/mL drinking water		Anderson (1994)
Thiabendazole	50–100 mg/kg q24h for 5 d	PO	Allen et al. (1993)

IM = intramuscular; PO = per os; SC = subcutaneous.

Table 9.8. Miscellaneous agents used in chinchillas

Drug	Dosage	Route	Reference
Atropine	0.05–0.1 mg/kg	SC	Harkness et al. (2010)
Calcium-EDTA	30 mg/kg q12h	SC	Hoefer (1994)
Calcium gluconate	100 mg/kg	IP	Richardson (1997)
Cimetidine	5–10 mg/kg q6–12h	IM, PO, SC	Smith and Burgmann (1997)
Cisapride	0.5 mg/kg q8–12h	PO	Morrisey and Carpenter (2004)
Dexamethasone	0.6 mg/kg	IM	Anderson (1994)
Diphenhydramine	1–2 mg/kg q12h	SC, PO	Morrisey and Carpenter (2004)
Doxapram	5–10 mg/kg	IP, IV	Harkness (1993)
Fluoxetine	5–10 mg/kg q24h	PO	Donnelly (2004)
Furosemide	2–5 mg/kg q12h	PO, SC	Morrisey and Carpenter (2004)
Glycopyrrolate	0.01–0.02 mg/kg	SC	Huerkamp (1995)
Lactobacilli	Administer during antibiotic treatment period, then 5–7 days beyond cessation	PO	Collins (1995)
Lactulose syrup	0.5 mL/kg q12h	PO	Morrisey and Carpenter (2011)
Metoclopramide	0.5 mg/kg q8h	SC	Johnson-Delaney (1996)
Oxytocin	0.2–3 IU/kg	SC, IM, IV	Anderson (1994)
Prednisone	0.5–2.2 mg/kg	SC, IM	Anderson (1994)
Pseudoephedrine	1.2 mg/animal q12h	PO	Richardson (1997)
Sucralfate	25–50 mg/kg	PO	Morrisey and Carpenter (2004)
Vitamin B complex	0.02–0.2 mL/kg	SC, IM	Anderson (1994)
Vitamin K	1–10 mg/kg	IM	Johnson-Delaney (1996)
Yohimbine	0.5–1 mg/kg	IV	Harkness et al. (2010)

EDTA = ethylenediaminetetraacetic acid; IM = intramuscular; IP = intraperitoneal; IV = intravenous; SC = subcutaneous.

INTRODUCTION TO DISEASES OF CHINCHILLAS

The many improvements in environmental controls, preventative medicine, and husbandry for chinchillas have led to healthier laboratory chinchillas. Most are relatively free of the variety of agents that cause clinical disease; however, a decreased incidence does not mean that infections will not occur. Presented here is a general overview of common bacterial, viral, fungal, and parasitic agents of chinchillas and some standard diagnostic and treatment modalities.

The most common causes of disease in chinchillas relate to husbandry and feeding inadequacies. Poor housing conditions such as high humidity, poor ventilation, and overcrowding can contribute to respiratory disease, and a number of skin conditions are traced to diet. Like most rodents, chinchillas hide signs of disease as a survival mechanism. Healthy chinchillas are active and inquisitive and carry their tails high over their back, while ill animals have a dull coat and are indifferent to their surroundings.

Bacterial Diseases

Respiratory Disease

Pasteurella spp., *Bordetella* spp., *Streptococcus* spp., and *Pseudomonas aeruginosa* alone or in combination can cause respiratory disease in chinchillas. Symptoms may include anorexia, depression, nasal discharge, and dyspnea. Young are particularly prone to disease of the lower respiratory tract or pneumonia. Predisposing factors include overcrowding, high humidity, drafty housing, poor ventilation, and stress. Tetracycline or drugs in the tetracycline family are antibiotics of choice except for *P. aeruginosa* infections, which are more sensitive to gentamicin. Prognosis is poor if chronic respiratory disease is present.

Bite Wounds and Abscess Formation

Bite wounds with secondary abscess formation can be seen in chinchillas housed in group arrangements. *Streptococcus* spp. and *Staphylococcus* spp. are most commonly involved. The wounds and/or abscesses should be drained and surgically debrided. Enrofloxacin or chloramphenicol is a good antibiotic to initiate therapy pending results of culture and sensitivity testing.

Enteritis

Enteritis is a common finding in chinchillas and can usually be traced to poor management. A number of conditions may be involved, including colic, intussusception, diarrhea, mucoid enteritis, fecal impaction, and rectal prolapse. *Pseudomonas* spp., *Pasteurella* spp., *Proteus* spp., *Salmonella* spp., and *Escherichia coli* are frequently involved and can also cause a septicemia with or without enteritis. Affected animals can be listless, be dehydrated, produce soft or liquid feces, or have an absence of feces. Onset is often acute, which makes it difficult to treat. Predisposing factors include a sudden diet change, inappropriate or prolonged antibiotic use, overcrowding, stress, and diets too low in fiber and too high in fat and protein. Antibiotic therapy and fluid replacement are recommended for treatment. Keeping a chinchilla clean and comfortable is an important part of the nursing care that should be provided for animals with enteritis. Gentamicin, sulfonamides, and neomycin are antibiotics of choice for enteritis.

Enterotoxemia

Clostridium perfringens type D has been reported to cause disease in chinchillas. The highest incidence occurs in young animals, 2–4 months of age. Animals may be found dead without signs of illness or may have diarrhea and appear to be experiencing abdominal pain. Enterotoxemia has been prevented by immunization with toxoid. See the next section, "Antibiotic Toxicity" for additional information.

Antibiotic Toxicity

Care should be taken when selecting antibiotics for use in chinchillas because many agents can suppress normal gut flora. Antimicrobials that have a selective Gram-positive spectrum, such as clindamycin, lincomycin, erythromycin, and ampicillin, should be avoided. Changes in enteric pH or normal gut flora can result in bacterial overgrowth and enterotoxemia such as that caused by *Clostridium* spp., resulting in severe diarrhea and acute death. Broad-

spectrum antibiotics used for short periods of time are less likely to upset the normal gut flora.

Supplementation with a lactobacillus product has been reported to help replenish favorable gut flora. Providing an oral electrolyte solution such as those used for human infants and/or fluids given subcutaneously or intravenously may also be beneficial.

Pseudomonas Infections

Chinchillas are very susceptible to *Pseudomonas aeruginosa* and are most often infected by contaminated drinking water. Clinical symptoms may include conjunctivitis, otitis, pneumonia, dermal pustules, enteritis, mesenteric lymphadenopathy, metritis, and septicemia with acute death. Foci of necrosis are most commonly seen in the liver, kidney, and spleen at necropsy along with other general signs of septicemia. Gentamicin may be used to treat individual animals. Prevention includes chlorination of the drinking water to 10 ppm or acidifying the drinking water to a pH of 2.5–3. A polyvalent bacterin vaccine is available for chinchillas that provides effective protection from *P. aeruginosa* infection.

Listeriosis

Chinchillas are highly susceptible to infection with *Listeria monocytogenes*, which is still common in fur-ranched but not in laboratory chinchillas. The disease can be sporadic in a colony or produce high mortality. All ages are affected, and encephalitic and enteric forms of the disease are seen. Clinical signs may include anorexia, diarrhea, depression, ataxia, circling, convulsions, and paralysis. Common disease syndromes include encephalitis, septicemia, and abortion. The disease is usually peracute in chinchillas, with death occurring within 48–72 hours after onset of signs. Treatment with chloramphenicol or tetracycline can be attempted; however, it is generally unrewarding because affected animals die so rapidly. There has been some success with autogenous vaccines.

Pseudotuberculosis

Yersinia pseudotuberculosis can cause an acute or chronic contagious disease in chinchillas. The acute form is manifested as a septicemia. Anorexia, depression, progressive weight loss, intermittent diarrhea, or sudden death may be seen in the chronic form. Palpably enlarged mesenteric lymph nodes are a hallmark of this disease. Yellow–white foci of necrosis and caseous nodules are seen in the liver, spleen, mesenteric lymph nodes, and intestines. Tetracycline can be used, but treatment is usually ineffective.

Mastitis

Mastitis is fairly common and should be suspected if previously healthy neonates become restless, then lethargic from hunger as mammary glands become painful and edematous. Treat with antibiotics based on culture and sensitivity; sulfonamides may be effective. Local application of hot packs is usually beneficial. Neonates should be fostered to another female or hand-raised.

Metritis

Proteus vulgaris, *E. coli*, *Pseudomonas* spp., *Staphylococcus* spp., and *Streptococcus* spp. are the most common organisms that cause metritis, which is often precipitated by a retained

placenta or fetus. Clinical signs include swelling and discoloration of the vulva, white to brown putrid exudate, and fever. The use of oxytocin to expel the placenta or fetus, flushing the vagina and uterus with saline, and parenteral antibiotic administration per culture and sensitivity are indicated. Offspring should be hand-reared to prevent cross-contamination.

Mycotic Diseases

Dermatophytosis

The most common cause of ringworm in the chinchilla is *Trichophyton mentagrophytes*. Lesions can occur anywhere on the body but typically appear as small, scaly areas of alopecia on the nose, ears, and feet. Ultraviolet light is not useful for diagnosis since *Trichophyton* does not fluoresce. Animals with lesions should be treated promptly or culled due to the zoonotic potential of this fungal agent. Treatment with oral itraconazole for a duration of 5–6 weeks is effective. Dust baths should not be shared between cages as this is a potential mode of fungal infection transmission.

Viral Diseases

There are few reports of viral agents causing disease. Lymphocytic choriomeningitis virus has been reported to affect chinchillas. Chinchillas infected with human influenza A virus are a model for childhood otitis media. Chinchillas are susceptible to human herpes virus 1.

Parasitic Diseases

Intestinal parasitism is generally uncommon. Infestation with coccidia, tapeworms, and nematodes are rare in well-managed facilities. Cystic subcutaneous masses caused by the intermediate stage of *Multiceps serialis* are occasionally seen; these masses may be surgically removed. The dog is the definitive host of this parasite, and the chinchilla functions as the intermediate host; transmission is by ingestion of contaminated feed. Chinchillas normally harbor high numbers of *Giardia* in their intestines. Stress and poor husbandry can cause numbers to increase and predispose animals to opportunistic infections. Affected animals usually have intermittent illness with sticky, black feces. Albendazole or fenbendazole are recommended treatments; metronidazole is contraindicated as it has been associated with liver failure in chinchillas.

Neoplasia

Neoplasia is uncommon in the chinchilla; there are few reports of its occurrence.

Miscellaneous Conditions

Malocclusion

Malocclusion, or slobbers as it is commonly called, can be seen with the incisors or molars and results in overgrowth and abnormal wear of the teeth. Chinchillas may have a selective appetite in the early stages that progresses to anorexia and excessive drooling. This causes the fur around the chin, chest, and forepaws to become wet and hence the term slobbers. Malocclusion of the incisors is easy to visualize; however, sedation and careful examination may be necessary to examine the molars. Radiography is frequently required to evaluate the condition. Treatment involves trimming the overgrown teeth with a dental bur or rongeur, under sedation. Routine trimming of the teeth is usually necessary, and affected animals

should not be bred because there may be a hereditary predisposition to the condition. Placement of a gnawing pumice stone in the cage is recommended.

Choke

Esophageal choke occurs when the entrance of the trachea is occluded by a large piece of food or other foreign body. It is more common in animals that are fed raisins, fruits, and nuts or that consume bedding material. The chinchilla may cough, retch, and struggle in an effort to dislodge the offending material. The animal's condition will deteriorate rapidly if it is unable to free the obstruction.

Constipation

Constipation is a more frequent problem than diarrhea in chinchillas; it is evidenced by straining to defecate but producing only a few thin, short, hard pellets. Feeding a diet that is too low in fiber is the usual cause. Provision of a commercial, pelleted chinchilla diet or the provision of small amounts of fresh food such as apples, raisins, carrots, or lettuce and alfalfa cubes may resolve the problem.

Conjunctivitis

Conjunctivitis without clinical signs of respiratory infection is usually caused by irritation from the dust bath or dirty bedding. Treating with ophthalmic ointment, improving husbandry, and discontinuing dust baths for a few weeks are recommended.

Fur Chewing

Fur chewing is a serious problem in the chinchilla industry. The exact cause remains unknown; however, it appears to be a vice and seems to occur more in certain lines. Loud noises, improper diet, hormonal imbalance, poor housing conditions, stress, and boredom have all been incriminated. Affected chinchillas are motley and have a lion's-mane appearance because they chew all of the fur within reach on their lower body to a short length. Hairballs are commonly found in the stomach at necropsy. These animals should not be bred.

Fur Slip

When chinchillas are handled roughly, become agitated during handling, or fight, the fur may be released in patches. The underlying skin appears clean and smooth. This release of fur occurs in response to the effect of adrenaline on the erector pili muscles. The fur may take up to 5 months to regrow.

Penile Hair Rings

Male chinchillas can accumulate a ring of twisted hair around the penis and under the prepuce. The condition can affect their breeding ability because the ring of hair can cause irritation, infection, and damage to the penis. Treat by applying a sterile lubricant to the penis and prepuce and gently rolling off the accumulated ring of hair.

Heat Stroke

Chinchillas are very prone to heatstroke, particularly if the humidity is high. Affected animals are usually found in a prostrate condition, panting, and have an elevated body

temperature. Treatment involves cooling the animal's body with cold water until the temperature returns to normal. In addition, administering intravenous fluids can help to stabilize the cardiovascular system. Care should be taken not to place cages near windows with direct sunlight.

Fractures

Fractures of the tibia are commonly seen when a chinchilla is grabbed by its hind limb or catches its leg in the cage. This bone is very fragile as it is longer than the femur, and the fibula is nearly nonexistent, giving the tibia little support. Surgical repair can be a challenge because the bone cortices are thin and the lumen narrow; thus, wire or external fixation stabilized by bandages is more effective than an intramedullary pin. The chinchilla should be placed in a small cage to limit mobility until the fracture heals.

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CHAPTER 9 REVIEW

Multiple Choice

1. The chinchilla is a popular animal for _____ studies.
   A. auditory
   B. liver
   C. kidney
   D. cardiovascular

2. All are true statements regarding the chinchilla's anatomic and physiologic features *except*
   A. Palms and soles of feet are devoid of fur.
   B. Body is covered with very soft, thick fur.
   C. Teeth are open rooted and grow throughout life.
   D. Terminal colon is highly sacculated.
3. Gestation in the chinchilla is _____ days.
   A. 68
   B. 30
   C. 111
   D. 175
4. This happens when chinchillas are handled roughly or become agitated during handling.
   A. fur slip
   B. choke
   C. slobbers
   D. degloving
5. The predominant leukocyte is
   A. neutrophil
   B. lymphocyte
   C. monocyte
   D. eosinophil
6. Chinchillas are very susceptible to this organism and are most often infected by contaminated drinking water.
   A. *Proteus vulgaris*
   B. *Listeria monocytogenes*
   C. *Clostridium perfringens*
   D. *Pseudomonas aeruginosa*
7. Chinchillas normally harbor high numbers of _____ in their intestines.
   A. *Multiceps*
   B. *Giardia*
   C. *Syphacia*
   D. *Aspiculuris*
8. Fractures of this bone are common when a chinchilla is grabbed by its hind limb.
   A. femur
   B. patella
   C. tibia
   D. fibula

(*Continued*)

9. Bacteria commonly isolated from chinchilla bite wounds
   A. *Staphylococcus*
   B. *Listeria*
   C. *Salmonella*
   D. *Pasteurella*
10. Puberty in the male chinchilla occurs at ____ months.
   A. 2
   B. 4
   C. 6
   D. 8

Critical Thinking

11. The chinchillas in your facility are individually housed in large shoebox-type cages with hardwood chips as bedding. They currently have no environmental enrichment. Make several suggestions to address the social needs of the animals.

10 Rabbits

Rabbits, cottontails, and hares are of the order Lagomorpha and are collectively referred to as lagomorphs. Although often confused as a rabbit, hares belong to the genus *Lepus* and are in many ways quite dissimilar to rabbits. Cottontails are descendants of the North and South American rabbit and are classified in the genus *Sylvilagus*. Rabbits native to Europe have given rise to only one species of rabbit, *Oryctolagus cuniculus*, which translates to "hare dug out of the earth." The laboratory rabbit is derived from this European rabbit. The three genera, *Lepus*, *Sylvilagus*, and *Oryctolagus*, cannot interbreed to produce viable offspring. Rabbit breeds can vary markedly in size, with a range of 1–9 kg (2.2–19.8 lb). There are numerous coat color variations, including albino, black, silver, and belted.

BREEDS

Though more than 50 breeds of rabbits are recognized in the United States, only 3 are commonly used in research: the New Zealand White (NZW), the Dutch Belted, and occasionally the Flemish Giant. The NZW is a medium-sized albino rabbit weighing between 3 and 5 kg (6.6–11.0 lb). It is the most commonly used breed in biomedical research; it is also used for meat production. The Dutch Belted is a small breed, possessing a black or chocolate coat with a white belt encircling the thorax and a white facial blaze and feet. It weighs between 0.9 and 1.8 kg (2.0–4.0 lb) and is second in importance as a laboratory breed (Figure 10.1). The Flemish Giant is most often fawn or gray coated, weighs between 6.5 and 9 kg (14.3–19.8 lb), and is occasionally used for research.

Clinical Laboratory Animal Medicine: An Introduction, Fourth Edition.
Karen Hrapkiewicz, Lesley Colby, and Patricia Denison.
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Fig 10.1. Dutch Belted rabbit in enrichment hut. (Photo courtesy of Bio-Serv.)

Both spontaneously derived mutant strains of rabbits (e.g., Watanabe heritable hyperlipidemic rabbit, St. Thomas Hospital strain rabbit) and purposefully produced transgenic rabbits (e.g., β-MyHC-Q^{403} transgenic rabbit) are now commonly used and have expanded the use of rabbits as models of human disease and conditions.

USES

The rabbit has been, and continues to be, used extensively in biomedical research. Their popularity is partially due to their body size (between that of rodents and the larger mammals), their ease of handling, and their unique anatomical and physiological characteristics. In addition, rabbits are frequently selected due to their position on the evolutionary scale, closer to humans than rodents, but less evolved and with lower perceived sentience than nonhuman primates and other large mammals.

The rabbit has an extensive history of use and range of applications in biomedical research. Considerable research has been conducted to elucidate the mechanisms of lipid metabolism and hypercholesterolemia such as through study of the Watanabe heritable hyperlipidemic rabbit strain with a spontaneous genetic mutation of its low-density lipoprotein receptor as is similar in human familial hypercholesterolemia. Rabbits have been used widely in the production of antibodies to characterize immune function as well as vaccine development. An interesting bit of history and an example of the use of one species (rabbit) to benefit another (dog) is the development of the rabies vaccine by Louis Pasteur in the late 1800s. Juvenile animals are susceptible to naturally occurring and induced mucoid enteropathy, a condition that facilitates investigation into human inflammatory bowel disease, cystic fibrosis, and cholera. Rabbits have also been used in the development of a wide range of surgical

models (including the development of surgical laser use) and as models for acute respiratory distress syndrome, asthma, cerebral embolism, cardiovascular disease, endometriosis, and glaucoma.

BEHAVIOR

In general, rabbits are gentle and docile animals and are not normally aggressive toward human handlers. They tend to be curious but easily startled. When frightened or painful, they may emit a high-pitched scream or display in the form of foot stomping, growling, or snorting. As with other prey species, rabbits become still when frightened or stressed and may rarely show obvious signs of pain. Rabbits very rarely bite but may do so if painful. Other signs of pain include inactivity, dullness, a hunched stance, bruxism (tooth grinding), hiding, excessive or lack of grooming, aggression, and anorexia. Sexually mature rabbits are territorial and will usually fight if housed together. In contrast, if immature females are housed together at 3 months of age or younger, they often establish long-term stable groups. Trauma, pseudopregnancies, and infertility can, however, occur in group-housed rabbits. Rabbits can be effectively litter-box trained: they typically choose one area of their cage for defecation and urination.

Depending on environmental conditions, wild rabbits are either nocturnal or diurnal. They are crepuscular foragers, feeding predominantly at dusk and dawn, and hide in burrows or in thickets of brush during daylight hours. Rabbits housed indoors in controlled environments tend to have alternating periods of wakefulness and rest throughout the day and night. Nests for offspring are constructed from fur plucked from the abdomen and dewlap (ventral neck) area.

Rabbits prefer to occupy areas near the perimeter or walls of their enclosure, avoiding the perceived threats of open areas (this orientation response is called thigmotaxis). Knowledge of this behavioral preference can be employed to more quickly acclimate animals to handling by holding them securely with as much surface contact as possible.

ANATOMIC AND PHYSIOLOGIC FEATURES

Rabbit biologic and reproductive data are listed in Table 10.1. The rabbit has a compact body and heavily muscled back and rear legs. Their lightweight skeleton comprises only 7% of their total body weight. This muscle–to–skeletal bone ratio makes them particularly susceptible to fracture of their lumbar vertebrae (most often occurring at the L7–S1 junction). They have long ears with prominent vessels, have a short tail, and are covered with very fine, soft fur. Rabbits have five digits on the forelimbs, four digits on the hind limbs, and toenails capable of inflicting scratches to handlers. They have no footpads; fur covers the plantar and palmar surfaces of the tarsus and carpus. In addition to their use of feces and urine, rabbits have chin, anal, and inguinal glands that are used for territorial scent marking.

Each eye of the rabbit has an approximately 190° field of vision, but, due to the wide placement of eyes on their head, they possess only a very limited field of binocular vision. Harderian lacrimal glands are located behind the well-developed nictitating membranes and

Table 10.1. Biologic and reproductive data for rabbits

Adult body weight: Male	2–5 kg
Adult body weight: Female	2–6 kg
Life span	5–6 y or more
Body temperature	38.5°–40°C (101.3°–104°F)
Heart rate	130–325 beats per minute
Respiratory rate	30–60 breaths per minute
Tidal volume	4–6 mL/kg
Food consumption	5 g/100 g per day
Water consumption	5–12 mL/100 g per day
Breeding onset: Male	6–10 mo
Breeding onset: Female	4–9 mo
Estrous cycle length	Induced ovulator
Gestation period	29–35 d
Postpartum estrus	48 hours
Litter size	4–10
Weaning age	4–6 wk
Breeding duration	1–3 y
Chromosome number (diploid)	44

Source: Adapted from Harkness et al. (2010).

aid in lubrication. The blood flow through the large ears of the rabbit is a primary means of body temperature regulation.

Domestic rabbits resemble rodents in many respects. The principal anatomic feature differentiating them is an additional pair of incisors, known as peg teeth, which are smaller and positioned behind (posterior to) the upper incisors on the maxilla. The purpose of these short, cylindrical teeth is not clear. When the jaw is at rest, the mandibular incisors should be positioned between the two sets of maxillary incisors. The rabbit dental formula is 2(I 2/1, C 0/0, P 3/2, M 3/3); a diastema, or open space, is present between the incisors and premolars. All of the teeth are open rooted, growing continuously throughout life. The incisors grow up to 10 cm (4 in.) in 1 year. The oral cavity is small and the tongue is relatively large, contributing to the difficulty of oral intubation. The esophagus of the rabbit is unique in that it has three striated muscle layers throughout the entire length, and mucous glands are absent. Rabbits, like rats, cannot vomit due to anatomic positioning of their cardia and stomach. Unlike rodents, their stomachs are not divided and are thin walled, which may lead to postmortem stomach rupture secondary to significant gas accumulation following death or handling of abdominal contents at necropsy. The small intestine is relatively short, making up approximately 12% of the total length of the gastrointestinal tract. The large intestine consists of the cecum, the proximal colon, and the distal colon. Combined, the intestinal contents may account for up 20% of the body weight, which is significant when calculating drug dosages based on body weight. For hindgut fermentation of their herbivorous diet, rabbits have a large, well-developed cecum that is approximately 10 times the size of the stomach and contains approximately 40% of the intestinal contents. There are two types of

Box 10.1

There are two types of feces produced by rabbits: firm, dry daytime fecal pellets and soft, moist nutrient-rich nighttime feces called cecotrophs that the rabbit normally ingests.

feces produced by rabbits: firm, dry daytime fecal pellets and soft, moist nutrient-rich nighttime feces called cecotrophs (Box 10.1). The cecotrophs are covered with mucus to protect them from the acid pH of the stomach and are high in water, nitrogen, electrolytes, and B vitamins. These feces contain twice the protein and half the fiber of the firm, dry fecal pellets. Rabbits may ingest these from the cage floor or more frequently will ingest them directly from the anus. Healthy rabbits should never have empty stomachs due to their coprophagic behavior. Similar to guinea pigs, rabbits have bands of smooth muscle (taeniae) that course longitudinally along portions of the proximal colon resulting in the formation of sacculations (haustra) in this intestinal segment. The taeniae and haustra do not extend the entire length of the colon. The fusus coli separates the proximal and distal colon. It functions as a differential pacemaker for peristaltic waves of the proximal and distal colon and in the physical separation of colonic fiber contents. The distal colon, devoid of both taeniae and haustra, is smooth and loosely coiled in appearance.

Two lymphoid organs are associated with the gastrointestinal system of the rabbit: the sacculus rotundus at the ileocecal–colic junction, and the vermiform appendix at the tip of the cecum. The sacculus rotundus is considered to be analogous to the bursa of Fabricius in birds. These two lymphoid organs contain more than 50% of the total lymphoid tissue of the rabbit, accounting for the relatively small size of the spleen. The liver has four lobes with the gallbladder positioned between liver lobes to the right of the midline. Compared to other laboratory animals, rabbits produce relatively large quantities of bile that empty into the duodenum via the common bile duct.

Rabbits are obligate nose breathers. Their lungs have six lobes with each side having a cranial, middle, and caudal lobe. The right caudal lobe is further divided into a lateral and medial lobe. The heart is small relative to their body size and their right atrioventricular valve is bicuspid instead of tricuspid, as occurs in other mammals. Their thymus persists into adulthood, not regressing as commonly occurs in many other adult mammals.

In contrast to most other mammals, the rabbit kidney is unipapillate rather than multipapillate, thus cannulating the kidneys of rabbits is more easily accomplished. Rabbits are the only known mammals in which the renal tubules can be dissected free from the kidney with an intact basement membrane, facilitating its use in renal physiology studies.

The genital tract of the female, like that of most domestic animals, is bicornate but differs in that the two horns of the uterus open into the vagina through separate cervices. The uterine horns are curled and lie in the caudal abdomen dorsal to the urinary bladder. The placenta is hemochorial, similar to the human. The female has four to five pairs of mammary glands and nipples. The males have only rudimentary nipples visible under their fur. In the mature male, the scrotal pouches are located anterior and lateral to the penis—unlike in most

other mammals, where the scrotal sacs are caudal to the penis. Inguinal canals remain open for life. The testes generally descend around 12 weeks of age. Lateral to the external genitalia of both sexes are the paired, hairless inguinal pouches, which usually contain white-to-brown odiferous secretions from the inguinal glands. One unfamiliar with rabbit anatomy could conceivably mistake an inguinal pouch for the anus.

Mature female rabbits often have a prominent dewlap beneath their chin. Mature male rabbits normally have larger heads than females. Differentiating males from females can be accomplished readily in adults by gently pressing the skin back from the genital opening. The female has a short slitlike opening; pressing the skin back exposes the mucosal surface of the vulva. In the male, this procedure will cause the penis to evert and be readily visible. In the male, testicles may or may not be palpable due to the open inguinal canals. In the newborn, when pressure is applied against the genital orifice, the penis will evert, appearing as a cone-shaped protuberance, whereas the mucosa of the vulva protrudes only laterally and anteriorly, still maintaining the appearance of a slit-shaped opening. The posterior end of the vulva does not evert due to its close attachment near the anus.

There are several distinct characteristics of the hematologic, clinical chemistry, and urinary profiles of rabbits. Rabbit neutrophils, called pseudoeosinophils or heterophils, have intracytoplasmic eosinophilic granules that cause them to resemble eosinophils. Pelger–Huet anomaly may be observed, which is a hyposegmentation of the heterophil nuclei due to incomplete differentiation. True eosinophils have larger, darker granules than heterophils. Lymphocytes are the predominant leukocyte. Basophils are more common than in other mammals, making up 2%–7% of the leukocyte population. Serum calcium is not maintained within a tightly defined range, but rather fluctuates and reflects dietary intake and may be as high as 15 mg/dL. Aortic and kidney calcification may develop with prolonged, high dietary calcium carbonate levels. Rabbits produce approximately 50–75 mL/kg of urine daily that has an alkaline pH of approximately 8.2 and a specific gravity ranging between 1.003 and 1.036. It may appear cloudy owing to the high quantities of calcium carbonate and ammonium magnesium phosphate crystals and, to the inexperienced observer, may be mistaken for a purulent discharge. Dietary porphyrins will occasionally cause the urine color to vary from dark red to orange. Porphyrin-stained urine can be differentiated from hematuria through microscopic identification of red blood cells in urine sediment or through observation of porphyrin fluorescence. Rabbits differ from most other mammals in that calcium and magnesium ions are excreted primarily in the urine rather than in the bile. Hematologic and biochemical parameters for the rabbit are listed in Appendix 1, "Normal Values."

BREEDING AND REPRODUCTION

Adult females are known as does; adult males are bucks. Young rabbits are kits or kittens although they are frequently and incorrectly referred to as bunnies. The onset of puberty varies with breed. Smaller breeds generally mature earlier, and larger breeds mature later. Does tend to reach sexual maturity prior to bucks. NZW does are initially bred between 4 and 6 months at a weight of 3–4 kg. NZW bucks are first bred at approximately 6–7 months. Rabbits do not have a definite estrous cycle. Does have 7–10 days of receptivity followed

by a short period, 1–2 days, during which they are not receptive to the male. They are induced ovulators like the cat and the ferret. The average reproductive life of a doe is approximately 3 years but may extend to 5–6 years. The average reproductive life of a buck is 5–6 years.

During breeding, agonistic behavior prevails, with chasing, squealing, tail flagging, enurination or urine spraying, and combat. In the wild, courtship chasing between adults is an early form of sexual activity. When rabbits are bred, the female should be taken to the male's cage as she may attack him in her own cage, and he may become distracted by scents in the female's environment. If the female is receptive, she will display lordosis (a flattening of the back and raising of the pelvis to position the perineum toward the male) and copulation will take place soon after introduction of the female. The buck elevates his hindquarters, walks stiff-legged, and lays his tail flat across his back. This maneuver supplies a visual stimulus to the doe. Additionally, inguinal gland secretions serve as an olfactory stimulus. The buck may turn his hindquarters and eject a small amount of urine toward a doe or at another male, followed by some form of circling about the cage. On occasion, a doe will refuse to mate with one buck but will readily accept another. She also may accept a buck once but refuse him a second time. Females may attempt to castrate males, so rabbits should be paired for only short periods and observed closely. It is normal behavior for the male to emit a cry at the time of copulation. After ejaculation, the male falls off the female to either side or backward as both feet are off the ground simultaneously at the time of ejaculation.

Ovulation occurs about 10 hours after mating. Approximately 25% of matings are anovulatory. To facilitate artificial insemination, ovulation can also be induced by administration of human chorionic gonadotropins or other luteinizing hormones. Pseudopregnancies are a common occurrence in rabbits and are caused by sterile matings, mounting by other does, or stimulation by a nearby male. Pseudopregnancy may extend over 16–22 days. Nest building may be observed during this time.

The gestation period is heavily influenced by breed, but it is also influenced by parity, nutritional status, and environmental factors and ranges from 29 to 35 days, with an average of 31 days. Most pregnancies maintained over 34 days result in stillbirths. Pregnancy can be detected by ultrasonography at day 7 postmating, by radiography at day 11, and by palpation at day 14. A nest box with appropriate contact bedding should be provided during the last few days of gestation and through the first month. Females begin building a nest 3–4 days prior to parturition by plucking fur from their dewlap and sides. Parturition, or kindling, most frequently occurs in the early morning and is usually rapid (approximately 10–15 minutes) but can last 1–2 days. Both anterior and breech presentations are normal. Dystocia, or difficult delivery, is uncommon. Does may breed immediately after parturition.

Litter size is influenced by breed and parity, with primiparous does producing smaller litters. NZW litters usually consist of seven to nine kits. Regardless of breed, kits nurse only once a day, either early in the morning or late in the evening, and usually for only 3–5 minutes. The young are altricial (blind, deaf, and hairless); their ears open at 8 days, eyes open at 10 days, and they begin to eat solid food at about 16–17 days of age. They are unable to self-regulate their body temperature until after 7 days of age. Does will not retrieve kits from outside of the nest box and will leave them to die. Kits may be weaned at 4–6 weeks of age. Orphaned rabbits should be fostered if at all possible. Colostrum is not a necessity as most passive immunity is acquired through the yolk sac prior to birth. Females may act

savagely toward kits that are not their own. Therefore great care must be taken when introducing kits to unfamiliar females. Commercial milk replacer for lagomorphs is available; orphans may be fed via stomach tube or syringe.

HUSBANDRY

Housing and Environment

Rabbits are frequently housed in front-opening, stainless steel or plastic cages. Although metal grid or slatted flooring is still used, solid-bottom plastic flooring with perforated holes for excreta passage is more common. These cages are often designed as drawerlike units that can be slid out from the cage rack, facilitating animal examination. Excreta trays with or without paper liners are placed beneath the rabbit cages to facilitate cleaning. The provision of solid resting boards within a section of the cage may be beneficial for animal comfort and thermoregulation. Adult rabbits greater than 5.4 kg must be provided with at least 0.46 m^2 (5.0 ft^2) of floor space per rabbit and 40.5 cm (16 in.) of cage height to enable normal postural movements. See Table 10.2 for additional space requirement information. Additional cage space is required for does with litters. Newer caging designs allow for social housing by opening a panel between two adjacent cages. Group housing may be accomplished with select, compatible, same-sex animals. Females are more amenable to group housing if their social group is established prior to or soon after weaning. It is not advised to attempt to group house sexually intact adult males due to interanimal aggression. Successful group housing of males, castrated prior to weaning, has been reported but should be approached with great caution. Group housing may be accomplished by using the animal housing room, or a component thereof, as the primary enclosure and providing the appropriate floor space per animal. Hardwood bedding, straw, paper products, or other suitable material may be placed on the floor to absorb urine and feces. Rabbits housed in groups will tend to use a consistent location for urination and defecation that should be at least spot-cleaned daily. Feed and watering devices should be elevated from the floor and sanitized weekly; the primary enclosure (room) should be sanitized at least every 2 weeks. Enrichment items such as open, small-animal carriers or plastic barrels can be added as "burrows," as can other enrichment devices specifically designed for laboratory animal use (see "Environmental

Table 10.2. Recommended cage space for rabbits

Weight (kg)	Floor Area per Animal (ft^2)	Height (inches)[a]
<2	1.5	16
Up to 4	3.0	16
Up to 5.4	4.0	16
>5.4	≥5.0	16

Source: Guide for the Care and Use of Laboratory Animals (ILAR, 2011).

[a]From cage floor to cage top.

Fig 10.2. Group housing of rabbits. (Photo courtesy of Wayne State University.)

Enrichment and Social Housing"). In all group housing arrangements, the provision of hiding places allows submissive animals to retreat if necessary (Figure 10.2).

As stated in the *Guide for the Care and Use of Laboratory Animals* (the *Guide*; ILAR, 2011), the room temperature should be maintained within a range of 16°–22°C (61°–72°F), a temperature lower than recommended for other species, and humidity between 30% and 70%. Rabbits are fairly resistant to cooler temperature, but they can quickly develop hyperthermia at increased temperatures. Ten to 15 air changes per hour are suggested to maintain adequate ventilation. This, however, is only a guideline, and determination of optimal ventilation should include careful consideration of the amount of heat, humidity, and volatile compounds expected to be produced within the housing room. Room light timers should be programed to deliver a consistent duration of light each day (12–14 hours for maintenance; 14–16 hours for optimal breeding).

Sudden, loud noises may startle rabbits. Low-level background noise can be used to reduce their reaction to noises created by general husbandry practices. Elevated noise levels may produce physiologic changes, so rabbits should not be housed close to vocal species such as dogs, pigs, or nonhuman primates or near noisy equipment such as cage washers.

Environmental Enrichment and Social Housing

The *Guide* states that an animal's social needs should be carefully considered when housing animals in the laboratory environment. Ideally, enrichment should increase the opportunity for normal behavior for the species and decrease the incidence of abnormal or stereotypic behaviors. Rabbits tend to be social and should, if possible, be group or pair housed as described previously. Contact with personnel can be a form of enrichment for rabbits if they are gently and consistently handled when young. Novel objects or food items may also be used to enrich a rabbit's environment. Objects such as balls, plastic or metal chains, wooden blocks, Nylabones, PVC tubing, huts, and other commercial items designed for use with laboratory animals may be provided. Even a simple metal bowl becomes an enrichment item as it is pushed around the cage. Studies have shown that interaction with items that the rabbit directly manipulates (manipulanda) decreases with time, and they should be frequently

rotated to maintain novelty. These objects should be sturdy, sanitizable, and nontoxic. Rabbits tend to chew on enrichment items; these should be made of durable materials that are resistant to destruction and carefully observed for sharp edges or broken pieces. Food items such as carrots, lettuce, hay cubes, loose hay, or commercially produced consumables (e.g., Bunny Blocks or Rabbit Snax) can be provided as treats and to eliminate boredom. They must be provided in limited quantities. As in all species, enrichment items must be evaluated to determine their likelihood to harm or injure the animal and to induce uncontrolled experimental variation. Formal approval should be secured from the researcher, the IACUC, and the facility veterinarian prior to the introduction of any enrichment items.

Feeding and Watering

Rabbits are strict herbivores and readily accept firm, dry pelleted commercial feed. Nutritional requirements vary with age, activity, and reproductive state. Maintenance diets for adult rabbits should contain at least 12% protein and 10% crude fiber. Higher fiber diets that contain approximately 22.5% fiber are used to reduce obesity and prevent hairball, or trichobezoar, formation. All feed should be purchased from a reputable, commercial vendor, have a known mill date, and be appropriately stored to maintain nutritional content. Food consumption varies with age, ambient temperature, and water availability. Animals should be limit fed as *ad libitum* feeding will often lead to obesity, especially in NZW rabbits. Once-to-twice daily feeding of a total of approximately 5 g of commercial laboratory rabbit feed per 100 g of body weight per day will maintain an adult, medium-sized rabbit. During growth, gestation, or lactation, rabbits require an approximate 20% increase in caloric intake. Food pellets should be provided in elevated self-feeders (e.g., J-type hoppers attached to a cage side or door) rather than in an open bowl that becomes easily contaminated. Feeders should facilitate the addition of food without disturbing cage occupants. Offering a limited amount of high-fiber, low-calcium grass hay can help to maintain the health of the rabbit's gastrointestinal system. Alfalfa hay should be provided in only limited quantities due to its high calcium levels.

The average water consumption of a rabbit is 50–150 mL/kg of body weight per day, higher than many other species. Water consumption increases with ambient temperature, while food intake decreases. Rabbits readily accept water bottles as well as automatic watering systems, which are much more efficient for production colonies and the research environment. Rabbits will not eat when water is unavailable.

Sanitation

Excreta trays should be cleaned as often as needed to control odor and ammonia levels. Daily cleaning is optimal. The carbonate and phosphate crystals from urine precipitate form a scale residue on cages and animal room floors. Soaking or rinsing with an acid solution (e.g., phosphoric acid, hydroxyacetic acid) is often required to remove urine scale. Primary enclosures, water bottles, automatic watering systems, and feed hoppers should be disinfected with chemicals, hot water 61.6°–82.2°C (143°–180°F), or a combination of both. Detergents and chemical disinfectants enhance the effectiveness of hot water, but they must be thoroughly rinsed from all surfaces with fresh water. Refer to Chapter 4, "Mice," for a more detailed description of disinfection methods.

Fig 10.3. Restraint of a New Zealand White rabbit.

TECHNIQUES

Handling and Restraint

Rabbits should be handled gently but firmly. They are prone to kick their hind limbs, which can cause deep scratches in their handlers. A rabbit should be lifted by obtaining a firm grip on the loose skin over the scruff of the neck with one hand; the other hand should be used to support the animal's hindquarters and limit back leg movement as shown in Figure 10.3. If the hind limbs are not supported, the rabbit may kick out with such force as to fracture its lumbar spine, most commonly at the L7–S1 junction.

To carry a rabbit, a "football hold" provides a secure restraint technique and accommodates their need for physical contact. To accomplish this, one arm is used to cradle the rabbit and hold it firmly against the handler's body while concealing the rabbit's head in the bend of the elbow. The handler's other arm is placed over the rabbit's back and grasps the scruff of the neck to provide slight pressure so the rabbit is less likely to struggle (Figure 10.4). Rabbits may slip and slide on stainless steel examining tables and may try to jump. Using a towel for traction or as a wrap, coupled with a hand behind the rump and a firm grasp of the scruff ensures that the animal does not escape and sustain injury (Figure 10.5). Commercially available restraint devices—including metal or plastic restrainers as well as flexible, but snug-fitting, cloth bags (e.g., cat bags)—are available and primarily serve to control the body while the head or ears are manipulated. Caution must be exercised when using a restrainer as an improperly restrained rabbit may struggle and cause serious injury. A

Fig 10.4. Football hold for carrying a rabbit.

Fig 10.5. Restraint of rabbit for examination on a table.

restrained animal should never be left unattended. Hypnosis may be used to examine the ventral aspect of the animal, to trim teeth or toenails, or to obtain a radiograph. Cover the rabbit's eyes and gently roll the animal onto its back; use care to center it and stroke the abdomen cranial to caudal. This will often have a calming or hypnotic effect, the degree of which will vary between individuals. Hypnosis should not be used for invasive or painful procedures.

Identification

Cage cards are commonly used as a form of general identification. Individual identification methods include ear tags, ear tattoos, or subcutaneous (SC) placement of a microchip. Fur

Table 10.3. Adult rabbit blood volumes and single sample size

	Volume (mL)
Total blood	160–480
Single sample	20–40
Exsanguination	60–160

Source: Adapted from Harkness et al. (2010).
Note: Values are approximate.

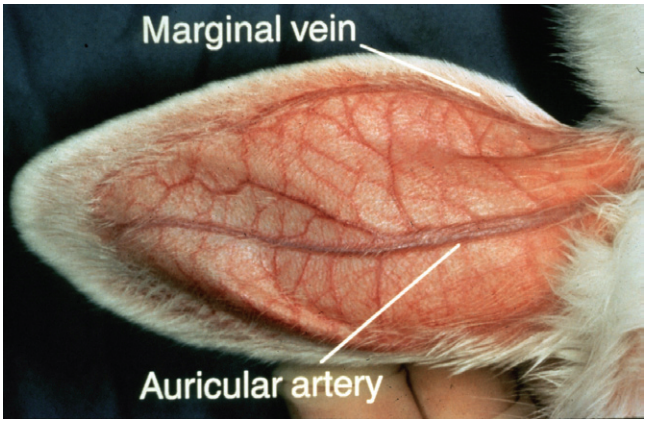

Fig 10.6. Blood vessels of the rabbit ear. (Photo courtesy of ACLAM.)

dyes or clipped areas of fur as well as ear markings with a permanent pen can serve as temporary methods of identification.

Blood Collection

Approximate blood volumes for adult rabbits are listed in Table 10.3. Assuming the animal is mature, healthy, and on an adequate plane of nutrition, the blood volume of most species averages 7% of the body weight in grams. A typical rule of thumb for blood withdrawal suggests that up to 10% of the circulating blood volume (or 1% of the body weight) can be withdrawn every 2–3 weeks from normal, healthy animals with minimal adverse effect. A larger volume or more frequent blood collection may be possible if fluid replacement is provided and the hematocrit is monitored.

The marginal ear, cephalic, and lateral saphenous veins can be used to obtain small amounts of blood. These veins easily collapse with too much negative pressure. The auricular artery, also known as the central ear artery, is easily visible on the dorsal surface of the pinnae, where it extends from the ear base to the ear tip. It is one of the best sites for collection of larger blood samples. Individuals must be familiar with the anatomy of the auricular vessels (Figure 10.6). Warming the ear with a heat lamp or warm water and gently plucking the hair over the vessel stimulates blood flow. Topical application of an irritant such as 40%

d-limonene or citrus oil can induce vasodilation but must be removed with 70% ethanol immediately postcollection to avoid undue skin irritation. Acepromazine, administered intramuscularly at a dosage of 1–5 mg/kg body weight, provides light sedation and also causes vasodilation. Infusing a small amount of local anesthetic such as 2% xylocaine, subcutaneously, or applying a topical anesthetic such as lidocaine–prilocaine cream (EMLA cream) 15 minutes to 1 hour prior to sampling can reduce discomfort at the collection site. Tubes that apply a slight vacuum allow a more rapid collection of up to 30–50 mL of blood. Hemostasis of the veins is easily achieved with slight pressure, but the artery must be held off for several minutes to prevent continued hemorrhage and hematoma formation. The application of a blood-clotting powder, such as HemaBlock, to the puncture site can speed hemostasis.

The jugular vein is another recommended site for blood collection that is often overlooked. The rabbit's head and front legs must be restrained securely to extend the neck, just as a cat would be restrained, to expose the jugular veins. Blood collection can also be performed with the rabbit in dorsal recumbency, but this method may be best facilitated with light sedation.

Cardiac puncture can be used to collect larger quantities of blood from an anesthetized rabbit. This technique should be performed only as a terminal procedure due to the high risk of lung laceration, pericardial bleeding, cardiac tamponade, and possible mortality. The rabbit is placed in dorsal recumbency and a 1.5-inch, 18-gauge needle can be inserted just to the left of the xiphoid process in a craniodorsal approach at a 30° angle from the surface of the thorax. Alternatively, the rabbit may be placed in right lateral recumbency and the chest palpated to locate the strongest heartbeat; the needle is introduced slowly between the ribs toward the opposite side of the chest.

Urine Collection

Urine can be collected as a clean catch sample or by gentle expression of the bladder. Cystocentesis provides another means of obtaining a clean urine sample. To perform this technique, the rabbit should be anesthetized or sedated. Using a V-trough or other restraint device, the rabbit is placed on its back and the lower abdomen is gently palpated to locate the bladder. Once the bladder is immobilized between the thumb and forefinger, a small-gauge needle (22 or 23 gauge) attached to a syringe is slowly advanced from the midline into the bladder and urine is aspirated, and then the needle is withdrawn. Cystocentesis cannot be used if the bladder is not large enough to be manually isolated unless it is performed under ultrasound guidance. Care must be taken to ensure the needle is accurately placed; if blood or some other substance is aspirated, immediately withdraw the needle and recognize that a bowel or blood vessel may have been inadvertently entered.

Metabolic cages provide an efficient means of collecting both urine and feces as they are designed to separate the two forms of excreta. Finally, urethral catheterization may be used to collect urine from anesthetized rabbits. Catheters must always be handled aseptically to avoid the possible introduction of bacteria into the bladder, producing an infection.

Drug Administration

Maximum dosing volumes for the various routes are listed in Table 10.4. Palatable drugs may be administered to rabbits by incorporating them in the food or water. Medicated

Table 10.4. Approximate maximum dosing volumes in rabbits

Route	Amount
IP	20.0 mL/kg
SC	10.0–20.0 mL/kg
IM	0.5 mL per site
IV	2.0 mL/kg bolus
	10.0 mL/kg infusion
ID	0.05–0.1 mL per site
PO	15.0 mL/kg

Source: Diehl et al. (2001) and Suckow and Douglas (1997).

custom diets are available through commercial laboratory animal feed vendors. Liquid medications can be administered by placing the tip of a syringe in the corner of the mouth and slowly injecting small amounts repeatedly. Oral administration of unpalatable liquids may be accomplished using an orogastric tube. A bite block must be used to protect the orogastric tube. A simple bite block can be fashioned from a syringe casing modified with a hole for passage of the tube. Plastic blocks are available commercially and are smooth to ensure no injury to the tissues of the mouth. The placement of the tube into the stomach must always be verified prior to dosing, to ensure drugs are not accidentally introduced into the trachea and lungs. Placement verification can usually be accomplished by using an empty syringe to gently aspirate; if the tube is in the trachea, the syringe will fill with air. A small volume of sterile water may also be introduced; a cough is usually elicited if the tube is in the trachea. An alternative method to check placement is to use a stethoscope pressed against the abdomen to listen for bubbles in the stomach as air is injected through the tube. Stainless steel feeding needles (13 gauge, 16 in.) can also be used to administer drugs orally. A nasogastric tube can be placed if continuous fluid administration into the stomach is required.

Subcutaneous injections should be administered under the loose skin between the shoulder blades or over the back, with a total maximum volume of 20 mL/kg delivered over multiple sites. Intramuscular injections with a maximum volume of 0.5–1.0 mL per site can be given in the large lumbar (epaxial) muscles along the spinal column and in the large muscles (quadriceps, thigh) of the hind limb. When injecting the thigh muscles, care must be taken to avoid the sciatic nerve that courses through the sciatic notch and along the posterior aspect of the hind limb (Figure 10.7). The lumbar muscle site is preferred for intramuscular injection.

Intravenous (IV) injections are most frequently given in the marginal ear vein. Application of a local anesthetic (e.g., lidocaine and prilocaine cream) at the puncture site can decrease animal discomfort. A maximum volume for bolus injection is approximately 2 mL/kg; up to 10 mL/kg may be given via a slow infusion. Continuous infusion of fluids or compounds can be performed using a venous catheter and a small automatic syringe pump that is contained in a jacket worn by the rabbit or by extending an IV line from a pump to the animal with use of a swivel-tether that permits normal movement. As an alternative to externalized

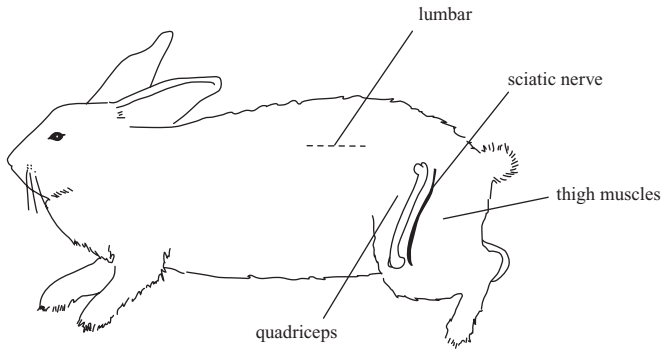

Fig 10.7. Intramuscular injection sites in the rabbit. (Adapted from Timm and Jahn, 1980, Practical methodology of the rabbit. In *Practical Methodology* [slide series], © Regents University of California.)

catheters, a vascular access port (VAP) may be surgically implanted and used for both dosing and blood withdrawal. Aseptic technique and a defined operating procedure must be followed when accessing the VAP to minimize the chances for infection and to maintain catheter patency. Regardless of the route of administration, large volumes should be warmed to body temperature prior to administration.

Intradermal (ID) injections are frequently used for the production of polyclonal antibodies. For ID skin injection, the skin should be clipped, cleaned with an antiseptic, held tautly, and the needle slowly advanced with the bevel facing up. The point of the needle (23 or 25 gauge) should enter just under the epidermal layer and into the dermis to produce a small, raised bleb. To stimulate a rabbit to produce a high titer of antibodies that can later be collected from its blood, purified antigens are suspended in a liquid medium and mixed with an antigenic stimulant (a component of the adjuvant) that induces a robust immune response. Historically, Freund's complete adjuvant (FCA) and Freund's incomplete adjuvant (FIA) were most frequently used. FCA is an oil-in-water suspension containing highly antigenic, killed *Mycobacterium* organisms. When used, FCA is mixed with the antigen of interest and injected in multiple ID locations along the animal's back and side, but avoiding the scruff region as this is where rabbits are frequently restrained. FCA is such a strong immunostimulant that it must only be administered one time per animal; injection site reactions often occur days to weeks later due to the body's reaction to the *Mycobacterium* component of the adjuvant. Skin lesions may include small nodules, diffuse swelling, and open abscesses or scabbing. Lesions are less likely to be significant if smaller injection volumes (0.05–0.1 mL) are used. If the animal's immune system must be further stimulated, FIA should be used in place of FCA. FIA is identical to FCA but lacks the *Mycobacterium* component. Given the complications associated with the use of Freund's adjuvants, alternative adjuvants including aluminum compounds, liposomes, and monophosphoryl lipid A have been developed and are now frequently used. Postimmunization monitoring for pain and distress is a necessary component of any antibody production protocol.

Anesthesia, Surgery, and Postoperative Care

Agents commonly used for anesthesia and tranquilization in rabbits are listed in Table 10.5. Although rabbits cannot vomit, withholding food for 2–6 hours before surgery allows for

Table 10.5. Anesthetic agents and tranquilizers used in rabbits

Drug	Dosage	Route	Reference
Inhalants			
Isoflurane	1.5%–5% to effect	Inhalation	Gillett (1994)
Sevoflurane	To effect	Inhalation	Morrisey and Carpenter (2004)
Injectables			
Acepromazine	1–5 mg/kg	IM, SC	Gillett (1994)
Acepromazine (for vasodilation with minimal sedation)	0.5–1 mg/kg	IM	Morrisey and Carpenter (2004)
Acepromazine	1 mg/kg	IM	Flecknell (2009)
+ butorphanol	1 mg/kg		
Bupivicaine	<1.5 mg/kg	Infiltrated SC	Harkness et al. (2010)
Diazepam	5–10 mg/kg	IM	Gillett (1994)
	1–5 mg/kg	IV	Gillett (1994)
Fentanyl–droperidol	0.15–0.44 mg/kg	IM	Wixson (1994)
Fentanyl/fluanisone	0.2–0.5 mL/kg	IM	Flecknell (2009)
Fentanyl/fluanisone	0.3 mL/kg	IM	Flecknell (1996)
+ diazepam	1–2 mg/kg	IM, IV, IP	
Fentanyl/fluanisone	0.3 mL/kg	IM	Flecknell (2009)
+ midazolam	1–2 mg/kg	IV, IP	
Ketamine	40 mg/kg	IM	Jenkins (1993)
+ acepromazine	0.5–1 mg/kg		
Ketamine	25 mg/kg	IM	Flecknell (1987)
+ diazepam	5 mg/kg	IM	
Ketamine	10 mg/kg	IV	Gil et al. (2003)
+ diazepam	2 mg/kg	IV	
Ketamine	25 mg/kg	IM	Flecknell (1997)
+ medetomidine	0.5 mg/kg	IM	
Ketamine	35 mg/kg	IM	Difilippo et al. (2004)
+ medetomidine	0.5 mg/kg	IM	
+ buprenorphine	0.03 mg/kg	IM	
Ketamine	25 mg/kg	IM	Stieve et al (2008)
+ midazolam	5 mg/kg	IM	
Ketamine	30 mg/kg	IM	Vachon et al. (1999)
+ midazolam	0.2 mg/kg	IM	
+ xylazine	3 mg/kg	IM	
Ketamine	10 mg/kg	IV	Flecknell (1987)
+ xylazine	3 mg/kg	IV	
Ketamine	30–40 mg/kg	IM	Gillett (1994)
+ xylazine	3–5 mg/kg	IM	
Ketamine	10 mg/kg	IN	Robertson and Eberhart (1994)
+ xylazine	3 mg/kg	IN	
Ketamine	35 mg/kg	IM	Lipman et al. (1990)
+ xylazine	5 mg/kg	IM	
+ acepromazine	0.75 mg/kg	IM	

(*Continued*)

Table 10.5. (*Continued*)

Drug	Dosage	Route	Reference
Ketamine	35 mg/kg	IM	Marini et al. (1992)
+ xylazine	5 mg/kg	IM	
+ butorphanol	0.1 mg/kg	IM	
Lidocaine	<2 mg/kg	Infiltrated SC	Harkness et al. (2010)
Medetomidine	0.1–0.5 mg/kg	SC, IM	Flecknell (2009)
Medetomidine	0.25 mg/kg	IM	Ko et al. (1992)
+ midazolam	0.5 mg/kg	IM	
+ propofol	2 mg/kg	IV	
Medetomidine	0.25 mg/kg	IM	Ko et al. (1992)
+ propofol	4 mg/kg	IV	
Midazolam	0.5–2 mg/kg	IV, IM, IP	Flecknell (1987)
	2 mg/kg	IN	Robertson and Eberhart (1994)
	4 mg/kg	IM, IP	Flecknell (1987)
Pentobarbital	20–45 mg/kg	IP, IV	Harkness and Wagner (1995)
Propofol	7.5–15 mg/kg	IV	Adam et al. (1990)
Thiopental (1%)	15–30 mg/kg	IV	Wixson (1994)
Tiletamine–zolazepam	Generally not recommended (except IN) due to nephrotoxicity		Doerning et al. (1992)
Tiletamine–zolazepam	10 mg/kg—only if no renal compromise	IN	Robertson and Eberhart (1994)
Urethane	1000 mg/kg	IP, IV	Flecknell (1987)
Xylazine	2–5 mg/kg	IM	Flecknell (2009)

IM = intramuscular, IN = intranasal, IP = intraperitoneal, IV = intravenous, SC = subcutaneous.

a more accurate body weight and can help decrease the pressure applied to the diaphragm by intestinal contents. Atropine esterase, which hydrolyzes atropine, is present in the serum of about one-third of the domestic rabbit population, significantly decreasing atropine efficacy. Glycopyrrolate, another anticholinergic, can be used instead of atropine to prevent bradycardia and decrease salivary and tracheobronchial secretions. Rabbits can be challenging to anesthetize because they may have variable responses to anesthetics, they are easily stressed and difficult to intubate, and many of their reflexes are unreliable indicators of the depth of anesthesia. With the correct technique and experience, most of these obstacles are easily overcome.

There are a number of injectable agents that can provide adequate anesthesia for short procedures. Ketamine, a dissociative anesthetic agent, can be mixed with a number of agents

to prolong anesthesia and provide better analgesia. The ketamine/xylazine (K/X) combination remains a popular anesthetic choice. Medetomidine combined with ketamine produces a slightly longer anesthetic period than is provided by K/X. Butorphanol or buprenorphine can be added to K/X or ketamine/medetomidine (K/M) for better analgesia. The K/X combination has been implicated in several cases of myocardial fibrosis when used repeatedly in both Dutch and NZW rabbits. K/X has also been associated with sciatic nerve damage and self-mutilation, necessitating caution in hindlimb intramuscular injections. Xylazine and medetomidine are alpha-2-adrenergic agonists that provide sedation and analgesia. Atipamezole can be given to reverse the effects of alpha-2 agonists and speed recovery. Ketamine/diazepam IV offers another option. A fentanyl/droperidol combination or acepromazine can be administered subcutaneously or intramuscularly for light sedation.

Propofol (5–15 mg/kg) IV has been used in rabbits and appears to be safe for very short-term (3–5 min) anesthetic maintenance. Long-term infusion of propofol has been reported to cause significant hypotension, hypoxemia, lipemia, and death in rabbits. Tiletamine hydrochloride has been associated with renal nephrotoxicity and should be avoided in rabbits. Caution should be exercised when using sodium pentobarbital or other barbiturates due to the narrow margin of safety between surgical anesthesia and death. In addition, barbiturates provide variable analgesia and cause respiratory and cardiovascular depression and hypothermia. Studies have shown that plasma levels of certain biochemical parameters may be significantly affected by anesthesia and should be interpreted with caution.

The intranasal administration of certain anesthetic and tranquilizing agents has been described. Drug absorption occurs through the vessels of the cribriform plate, leading directly to the central nervous system. This route should be used with caution as some agents have been shown to elicit variable responses in rabbits and may even cause acute respiratory failure and death.

Inhalant agents, such as isoflurane and sevoflurane, are safe and commonly used in rabbits. Although rabbits can be induced via face mask, great care must be taken in restraining them to avoid injury. Administration of isoflurane to rabbits via a face mask or anesthetic induction chamber is associated with apnea and a marked bradycardia. Premedication with acepromazine, diazepam, or another suitable sedative will allow for an easier and safer masking induction. A laryngeal mask airway (LMA) device can be used alternatively to deliver inhalant agents. The LMA is a device that is introduced through the oral cavity and pharynx and covers the laryngeal opening without entering the larynx or trachea.

Rabbit intubation can be difficult due to the small size of the oral cavity and difficulty in visualizing the larynx. To intubate a rabbit, a long (40 mm), thin (size 0 or 1) laryngoscope blade or an otoscope can be used to allow visualization of the larynx. A flexible stylet inside the appropriately sized endotracheal tube (2–3 mm outside diameter in 1–3 kg rabbits; 3–6 mm outside diameter in larger rabbits) can be helpful in guiding the tube into the trachea. Rotating the tube as it passes over the epiglottis facilitates passage into the trachea. A topical spray of lidocaine can be used to anesthetize the epiglottis. Rabbits must be adequately anesthetized to avoid laryngospasm, which makes intubation even more difficult.

Alternatively, a blind intubation method can be used that, when properly performed, is both faster and less traumatic than the laryngoscope method. The rabbit's head is held with the palm of the hand on the back of the skull and the thumb and index finger placed along the length of each mandible. The most important step is to extend the neck adequately, with

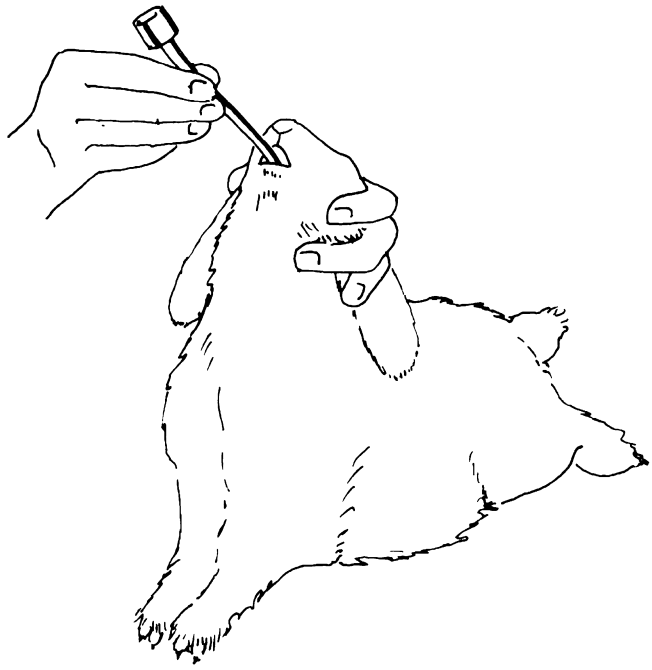

Fig 10.8. "Blind" endotracheal intubation of the rabbit. (Adapted from Harkness and Wagner, 1995, *The Biology and Medicine of Rabbits and Rodents*, 4th ed., Philadelphia: Williams & Wilkins.)

the nose pointing straight up or even slightly hyperextended so the path to the trachea is straight and minimally obstructed (Figure 10.8). The endotracheal tube is passed over the tongue until it is at the entrance to the larynx as evidenced by condensation in the tube when the rabbit exhales. During exhalation, the tube should be quickly and firmly passed forward while rotating it so that it enters the trachea. This method can be mastered with patience and practice.

The depth of anesthesia is best gauged by the rate and depth of respiration and the degree of jaw tension. Other indicators of the depth of anesthesia, in decreasing order of reliability, are the ear pinch, pedal reflex, and palpebral reflex. The ear pinch is more sensitive and persists longer than the others. If necessary, a drop of doxapram can be placed under the tongue to stimulate respiration. Rabbits are sometimes used in experimental studies that require surgical manipulations, including placement of indwelling vascular catheters, reproductive tract surgeries [ovariohysterectomies (OHEs) and castrations], orthopedic surgeries, and gastrointestinal surgeries.

For surgical prepping, clipping rabbit hair is difficult at best. Using a sharp 40 blade held flat against the skin, stretching the skin taut, and clipping slowly and carefully are important steps to avoid traumatizing the skin. The skin should be lightly scrubbed in preparation for surgery.

The incision for an OHE in a rabbit should be made lower on the abdomen, similar to an OHE approach in a cat. The bladder should be gently emptied prior to prepping the

surgical site. The bladder is thin walled and it may rupture if pressure is applied too vigorously. Rabbits have a very narrow linea alba and veins that tear easily. Additionally, care must be taken to ligate both cervices of the rabbit that enter into the vagina separately, especially in larger does. In the male, both inguinal canals remain open throughout life and must be sutured closed during a castration to avoid herniation of abdominal contents.

Rabbits are adept at removing sutures; it is best to use metal staples, clips, or a subcuticular pattern when closing the incision. After clipping, rabbit fur grows back in an uneven pattern. For optimal postoperative recoveries, surgeries should be performed with attention to aseptic technique, maintaining body temperature, and blood pressure.

Analgesics must be administered for pain control in rabbits that undergo surgical procedures. Unless documented to the contrary and approved by the IACUC, it must be assumed that procedures that cause pain or distress in humans will cause pain and distress in other animals. Rabbits experiencing pain may show clinical signs, including depression, grinding of teeth, anorexia, and reluctance to move. They are notoriously stoic in response to pain; those unfamiliar with the species will often overlook subtle indications of a pain response. Analgesic agents for rabbits are listed in Table 10.6. Opiates such as butorphanol and buprenorphine are frequently used. Butorphanol is indicated for mild postoperative pain; buprenorphine is indicated for acute and chronic visceral pain. Buprenorphine can cause marked sedation if given too early in the anesthetic recovery period. Stanozol can be used to stimulate the appetite of rabbits following surgery. Ibuprofen can be used to decrease adhesion formation. Newer nonsteroidal anti-inflammatory agents such as carprofen provide good, long-lasting (up to 24 hours) analgesia for mild to moderate pain.

Postoperative care should include hydration support of the anesthetized animal (e.g., subcutaneous, intraperitoneal, or intravenous administration of a warmed solution such as saline, saline–dextrose, lactated Ringer's) and support of normal body temperature through the use of a circulating water heating blanket. A nonambulatory animal should be turned at least every 30 minutes to prevent hypostatic congestion. It is important to maintain hydration and nutrition levels in the postoperative period to support normal metabolic processes and gut physiology. Select, commercially available supportive and critical care products for laboratory animals are provided in Appendix 3.

Imaging Techniques

Great advances have been made in small-animal imaging over the last decade. Many imaging modalities once used only in humans [e.g., digital x-ray imaging, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), dual-energy x-ray absorptiometry (DEXA) scan, and ultrasound] have been adapted for use with these species, producing images of exquisitely fine detail. In addition, novel imaging techniques have been developed for use in animal research, including *in vivo* optical imaging.

For conventional x-ray imaging, an x-ray machine with high milliamperage, such as a 200- or 300-mA unit with the capability for low settings of peak voltage delivered (kVp) and small incremental changes, is ideal. High-detail film–screen combinations work well. Short exposure times of 1/40 second or less should be used to decrease the chance of motion artifact. Suggested exposure factors are 42–46 kVp, 300 mA, 1/40 second, 40 SID, 7.5 mA·s. Alternatively, nonscreen film can be used with standard x-ray or dental units. Nonscreen film produces high-detail radiographs, but longer exposure times are needed. Digital imaging

Table 10.6. Analgesic agents used in rabbits

Drug	Dosage	Route	Reference
Acetaminophen	200–500 mg/kg	PO	Gillett (1994)
Acetylsalicylic acid	100 mg/kg q4h	PO	Flecknell (2009)
Buprenorphine	0.01–0.05 mg/kg q8–12h	SC, IV	Flecknell (2009)
	0.5 mg/kg q12h	Per rectum	Huerkamp (1995)
Butorphanol	0.1–0.5 mg/kg q4h	SC, IV	Dobromylskyj et al. (2000)
Butorphanol	5 mg/kg	IM	Wixson (1994)
+ xylazine	4 mg/kg		
Carprofen	1.5 mg/kg q24h	PO	Flecknell (2009)
	4 mg/kg q24h	SC	Flecknell (2009)
Fentanyl	25 μg/h patch for up to 72h		Foley et al. (2001)
Flunixin meglumine	1–2 mg/kg q12–24h	SC	Heard (1993)
Ibuprofen	2–7.5 mg/kg q12–24h	PO	Harkness et al. (2010)
	10 mg/kg ~q4h	IV	Flecknell (2009)
	2–7.5 mg/kg q4h	PO	Morrisey and Carpenter (2004)
	7.5 mg/kg q6–8h	PO	Smith and Burgmann (1997)
Ketoprofen	3 mg/kg q24h	SC, IM	Flecknell (2009), Heard (2009)
Meloxicam	0.6–1 mg/kg	SC, PO	Flecknell (2009)
	0.1–0.5 mg/kg q12–24h	PO, SC	Harkness et al. (2010)
Meperidine	5–10 mg/kg q2–3h	SC, IM	Harkness et al. (2010)
Syrup	0.2 mg/mL drinking water		Huerkamp (1995)
Morphine	2–5 mg/kg q2–4h	SC, IM	Flecknell (2009)
Nalbuphine	1–2 mg/kg q4–5h	IM, IV	Heard (1993)
Oxymorphone	0.05–0.2 mg/kg q6–12h	SC, IM	Harkness et al. (2010)
Pentazocine	5–10 mg/kg q4h	IM, IV	Flecknell (2009)
Piroxicam	0.2 mg/kg q8h	PO	Dobromylskyj et al. (2000)
Tramadol	10 mg/kg q24h	PO	Harkness et al. (2010)

IM = intramuscular; IN = intranasal; IP = intraperitoneal; IV = intravenous; PO = per os; SC = subcutaneous.

techniques offer substantial flexibility in image enhancement and delivery application. The best method of restraint is to sedate the animal and use gauze, adhesive tape, or positioning devices to restrain the animal in the appropriate radiographic position.

Euthanasia

Rabbits are most frequently euthanized by an overdose of barbiturate or barbiturate-based euthanasia solution given intravenously. This method is painless, fast, easily accomplished, and relatively inexpensive. Inhalant anesthetic overdose can be used but is less favorable as the animal may experience an excitation phase during anesthetic induction. Rabbits that are used for polyclonal antibody production are usually exsanguinated under anesthesia for the terminal collection of blood and harvesting of the antibody-rich serum. Of note, many commercial supplies of sodium pentobarbital cause hemolysis because of high levels of

alcohol, propylene glycol, or both. An alternate formulation should be obtained to avoid hemolysis.

THERAPEUTIC AGENTS

Table 10.7 lists a variety of antimicrobial and antifungal agents that can be used in rabbits. Refer to Table 10.8 for a list of antiparasitics for use in rabbits and to Table 10.9 for miscellaneous drugs used in rabbits.

INTRODUCTION TO DISEASES OF RABBITS

Improvements in environmental controls and husbandry have led to the identification and eradication of a majority of infectious agents in laboratory rabbits. Most laboratory rabbit colonies today are relatively free of the viruses, bacteria, parasites, and fungi that cause clinical disease. Basic knowledge about the variety of infectious agents that may infect a rabbit population is imperative to being able to quickly and appropriately respond to a disease outbreak if it does occur. Rabbits tend to be quite stoic, so detecting clinical signs can be a challenge. Some common signs include anorexia and refusal of treats, depression, hunched posture, and lethargy. Anorexic rabbits must be treated promptly to avoid the life-threatening condition of gastrointestinal stasis. Treatment includes fluid therapy (approximately 90–100 mL/kg per day) and nutritional support to maintain intestinal function and prevent dehydration and intestinal atony. Oral rehydration solutions and critical care products are available commercially. Presented here is a general overview of common bacterial, viral, fungal, and parasitic agents of rabbits and some standard diagnostic and treatment modalities.

Bacterial Diseases

Bacterial Pneumonia and Respiratory Diseases

Pasteurellosis is the single most common and troublesome disease of domestic rabbits. It is caused by *Pasteurella multocida*, a Gram-negative coccobacillus. Rabbits may harbor the organism in the upper respiratory tract without showing clinical signs. From the respiratory tract, the organism may spread along the nasolacrimal ducts, eustachian tubes, down the trachea, hematogenously, and venereally (Figure 10.9). Following stress, a variety of syndromes may develop, including rhinitis, conjunctivitis, bronchopneumonia, otitis media and interna, genital infections, abscesses, and septicemia. *P. multocida* is readily spread from one rabbit to another by direct contact, fomites contaminated with nasal secretions, and even aerosolization within a room. A variety of *P. multocida* isolates appear to differ in virulence. Some forms are associated with a rapidly fatal septicemia, whereas other strains induce a slowly progressive rhinitis.

Sneezing and the presence of a serous to mucopurulent nasal discharge characterize the upper respiratory form of pasteurellosis, also known as snuffles. This is the most common clinical presentation of infection. Exudate is often seen on the inner aspect of the forelegs.

Table 10.7. Antimicrobial and antifungal agents used in rabbits

Drug	Dosage	Route	Reference
Amikacin	2–5 mg/kg q8–12h	SC, IM	Harkness et al. (2010)
Amoxicillin	Do not use		Morrisey and Carpenter (2004)
Ampicillin	Do not use		Morrisey and Carpenter (2004)
Cefaxolin	60 mg/kg q12h	PO	Laber-Laird et al. (1996)
Cephalexin	11–22 mg/kg q8–12h	PO, SC	Harkness et al. (2010)
Cephaloridine	11–15 mg/kg q12h	IM	Gillett (1994)
Cephalothin	12.5 mg/kg q6h for 6d	IM	Russell et al. (1981)
Chloramphenicol	30 mg/kg q8–12h	PO, SC, IM, IV	Harkness et al. (2010)
Chlortetracycline	50 mg/kg q12h	PO	Morrisey and Carpenter (2004)
Ciprofloxacin	10–20 mg/kg q12–24h	PO	Morrisey and Carpenter (2004)
Clindamycin	Do not use		Morrisey and Carpenter (2004)
Doxycycline	2.5 mg/kg q12h	PO	Carpenter et al. (1995)
Enrofloxacin	5–15 mg/kg q12h; limit injections as they may cause muscle necrosis and sterile abscesses	PO, IM, SC	Morrisey and Carpenter (2004); Hernandez-Divers (2005)
	200 mg/L drinking water for 14d		Broome and Brooks (1991)
Erythromycin	Do not use		Morrisey and Carpenter (2004)
Gentamicin	4 mg/kg q24h	SC, IM	Morrisey and Carpenter (2004)
Griseofulvin	12.5–25 mg/kg q24h for 30–45d	PO	Hernandez-Divers (2005)
Ketoconazole	10–40 mg/kg q24h	PO	Morrisey and Carpenter (2004)
Lime sulfur dip (2.5%)	Dip q7d for 4–6wk		Morrisey and Carpenter (2004)
Metronidazole	20 mg/kg q12h	PO	Harkness et al. (2010)
Miconazole	q24h for 14–28d	Topical	Harkness and Wagner (1995)
Minocycline	6 mg/kg q8h	IV	Nicolau et al. (1993)
Neomycin	30 mg/kg q12h	PO	Burgmann and Percy (1993)
Oxytetracycline	50 mg/kg q12h	PO	Burgmann and Percy (1993)
	1 mg/mL drinking water		Burgmann and Percy (1993)
Penicillin G, benzathine	42,000–60,000 IU/kg q48h	SC, IM	Russell et al. (1981)
Penicillin G, procaine	40,000 IU/kg q24h	IM	Hernandez-Divers (2005)
Silver sulfadiazine 0.01% cream	q24h	Topical	Donnelly (2005)
Sulfadimethoxine	10–15 mg/kg q12h for 10d	PO	Harkness and Wagner (1995)
Sulfamethazine	1 mg/mL drinking water		Burgmann and Percy (1993)
Sulfaquinoxaline	1 mg/mL drinking water		Burgmann and Percy (1993)
Tetracycline	50 mg/kg q8–12h	PO	Burgmann and Percy (1993)
	250–1000 mg/L drinking water		Gillett (1994)
Trimethoprim–sulfa	30 mg/kg q12–24h	PO, IM, SC	Harkness et al. (2010)
Tylosin	10 mg/kg q12h	PO, SC, IM	Hernandez-Divers (2005); Harkness et al. (2010)
Vancomycin	50 mg/kg q8h	IV, IM	Harkness et al. (2010)

IM = intramuscular; IV = intravenous; PO = per os; SC = subcutaneous.

Table 10.8. Antiparasitic agents used in rabbits

Drug	Dosage	Route	Reference
Albendazole	7.5–20 mg/kg q24h (use with caution)	PO	Morrisey and Carpenter (2011)
Amprolium (9.6%)	0.5 mL/500 mL drinking water for 10 d		Harkness and Wagner (1995)
	5 mL/gal drinking water for 5 d		Hernandez-Divers (2005)
Carbaryl powder (5%)	Twice weekly	Topical	Harkness and Wagner (1995)
Doramectin (1%)	0.2 mg/kg repeat in 10 d	SC	Voyvoda et al. (2005)
Fenbendazole	10 mg/kg, repeat in 2 wk prn	PO	Hillyer (1994)
	50 ppm in feed for 2–6 wk		Okerman (1994)
Fipronil	Do not use, toxic		Morrisey and Carpenter (2004)
Imidacloprid	1 cat dose q30d	Topical	Morrisey and Carpenter (2004)
Ivermectin	0.4 mg/kg q7–14d	PO, SC	Hillyer (1994)
Lasalocid	120 ppm in feed		Harkness and Wagner (1995)
Lime sulfur (2%–3%)	Dip q7d for 4–6 wk		Jenkins (1995)
Lufenuron	30 mg/kg q30d	PO	Morrisey and Carpenter (2004)
Mebendazole	50 mg/kg	PO	Harkness and Wagner (1995)
	1 g/kg of feed		Harkness and Wagner (1995)
Metronidazole	20 mg/kg q12h	PO	Harkness et al. (2010)
Monensin	0.002%–0.004% in feed		Harkness et al. (2010)
Piperazine	200 mg/kg, repeat in 2–3 wk	PO	Hillyer (1994)
	2–5 mg/mL drinking water for 7 d		Hillyer (1994)
Praziquantel	5–10 mg/kg, repeat in 10 d	PO, SC, IM	Allen et al. (1993)
Pyrantel pamoate	5–10 mg/kg, repeat in 2–3 wk	PO	Quesenberry (1994)
Pyrethrin	Shampoo/powder weekly for 4–6 wk		Harkness et al. (2010)
Selmectin	6 mg/kg	Topical	Morrisey and Carpenter (2004)
Sulfadimerazine	2 g/L water		Harkness et al. (2010)
Sulfadimethoxine	50 mg/kg once, then 25 mg/kg q24h for 10–20 d	PO	Hillyer (1994)
Sulfamethazine	100 mg/kg q24h	PO	Gillett (1994)
	0.77 g/L drinking water		Harkness et al. (2010)
	0.5%–1% in feed		Harkness et al. (2010)
Sulfaquinoxaline	0.04%–0.1% in drinking water		Harkness and Wagner (1995)
	125–250 ppm in feed		Harkness et al. (2010)
Thiabendazole	50–100 mg/kg q24h for 5 d	PO	Morrisey and Carpenter (2004)
	50 mg/kg q24h, repeat in 3 wk	PO	Hernandez-Divers (2005)
Tilmicosin	25 mg/kg once	SC	McKay et al. (1996)

IM = intramuscular; PO = per os; SC = subcutaneous.

Table 10.9. Miscellaneous agents used in rabbits

Drug	Dosage	Route	Reference
Atipamezole	1 mg/kg	SC, IP, IV	Flecknell (1997)
Atropine	0.1–3 mg/kg	SC	Harkness et al. (2010)
Barium	10–14 mL/kg	PO	Quesenberry (1994)
Bromelin enzyme	1–2 tablets q24h for 3–5 d	PO	Quesenberry (1994)
Calcium-EDTA	27 mg/kg q6h for 2–5 d (make at 10 mg/mL in 5% dextrose or saline)	SC	Johnson-Delaney (1996)
Calcium gluconate (10% solution)	5–10 mL 3–5 mL	PO IV	Raphael (1981)
Cholestyramine	2 g per animal q24h for 18–21 d, gavage with 20 mL water	PO	Harkness and Wagner (1995)
Cimetidine	5–10 mg/kg q8–12h	PO, SC, IM, IV	Morrisey and Carpenter (2004)
Cisapride	0.5 mg/kg q8–24h	PO	Smith and Burgmann (1997)
Dexamethasone	0.2–0.6 mg/kg	SC, IM, IV	Gillett (1994)
Dexamethasone	0.5–2 mg/kg	IM, IV	Paul-Murphy and Ramer (1998)
Digoxin	0.005 mg/kg q12–24h	PO	Pariaut (2009)
Diltiazem	0.5 mg/kg q8–24h	PO	Pariaut (2009)
Diphenhydramine	2 mg/kg q8–12h	PO, SC	Morrisey and Carpenter (2004)
Doxapram	2–5 mg/kg q15min	IV	Huerkamp (1995)
Enalapril	0.5 mg/kg q12–24h	PO	Pariaut (2009)
Flumaznil	0.1–10 mg/kg	IV	Flecknell (2009)
Furosemide	2–5 mg/kg q12h	PO, SC, IM, IV	Paul-Murphy and Ramer (1998)
Glycopyrrolate	0.01 mg/kg 0.1 mg/kg	IV IM, SC	Flecknell (2009) Flecknell (2009)
Hairball laxative, feline	1–2 mL per animal q24h for 3–5 d	PO	Quesenberry (1994)
Human chorionic gonadotropin	20–25 IU per animal	IV	Harkness and Wagner (1995)
Iron dextran	4–6 mg/kg once	IM	Morrisey and Carpenter (2004)
Lactobacillus	1 notch daily of paste; or 1/4–1/2 tsp powder, or per package label; mix into food	PO	Johnson-Delaney (1996)
Loperamide hydrochloride (Imodium A-D)	0.1 mg/kg q8h for 3 d, then q24h for 2 d	PO	Harkness and Wagner (1995)
Meclizine hydrochloride (Antivert)	2–12 mg/kg q24h	PO	Harkness and Wagner (1995)
Metoclopramide	0.2–1 mg/kg q6–8h	PO, SC	Harkness and Wagner (1995)
Naloxone	0.01–0.1 mg/kg	IV, IM	Gillett (1994)
Oxytocin	0.1–3 IU/kg	SC, IM	Harkness and Wagner (1995)

Table 10.9. (*Continued*)

Drug	Dosage	Route	Reference
Pancreatic enzyme concentrate (Viokase-V)	Mix 1 tsp with 3 Tbsp yogurt, let stand 15 min, then give 2–3 mL q12h	PO	Allen et al. (1993)
Papain enzyme	1–2 tablets per animal q24h for 3–5 d	PO	Quesenberry (1994)
Pineapple juice (fresh)	10 mL per medium- sized animal q24h for 3–5 d	PO	Quesenberry (1994)
Polysulfated glycosaminoglycan	2.2 mg/kg q3d for 21–28 d, then q14d	IM, SC	Jenkins (1995)
Prednisolone	0.25–0.5 mg/kg q12h for 3 d, then q24h for 3 d, then q48h	PO	Quesenberry (1994)
	0.5–2 mg/kg q12h	PO	Morrisey and Carpenter (2004)
Prochlorperazine	0.2–0.5 mg/kg q8h	PO	Harcourt-Brown (2002)
Ranitidine	2–5 mg/kg q12h	PO	Harcourt-Brown (2002)
Simethicone	65–130 mg/animal q1h for 2–3 treatments	PO	Krempels et al. (2000)
Sodium bicarbonate	2 mEq/kg	IV, IP	Harrenstien (1994)
Stanozolol	1–2 mg	PO	Harkness and Wagner (1995)
Sucralfate	25 mg/kg q8–12h	PO	Harkness and Wagner (1995)
Verapamil (Calan)	0.2 mg/kg q8h for 9 treatments	SC	Harkness and Wagner (1995)
Vitamin A	500–1000 IU/kg once	IM	Johnson-Delaney (1996)
Vitamin B complex	0.02–0.4 mL/kg q24h	IM	Johnson-Delaney (1996)
Vitamin E–selenium (Bo-Se)	0.25 mL per rabbit	IM	Johnson-Delaney (1996)
Vitamin K	1–10 mg/kg prn	IM	Johnson-Delaney (1996)
Yohimbine	0.2–1 mg/kg	IM, IV	Gillett (1994)

EDTA = ethylenediaminetetraacetic acid; IM = intramuscular; IP = intraperitoneal; IV = intravenous; PO = per os; SC = subcutaneous.

Turbinate atrophy may be a sequela with some strains of *P. multocida*. A subacute to chronic suppurative conjunctivitis with epiphora may also be a clinical manifestation. The infection results in loss of fur around the medial canthus of the eye and is usually seen in conjunction with rhinitis. Animals may live for many months with these symptoms or the disease may progress to one of the other clinical forms.

The pulmonary form of pasteurellosis may be acute or chronic. The acute disease appears as a bronchopneumonia with variable consolidation of the lungs; cyanosis is often a prominent feature. Chronic pneumonia is often asymptomatic. At times, laboratory rabbits die of pneumonia without observable clinical signs because their limited exercise allows them to

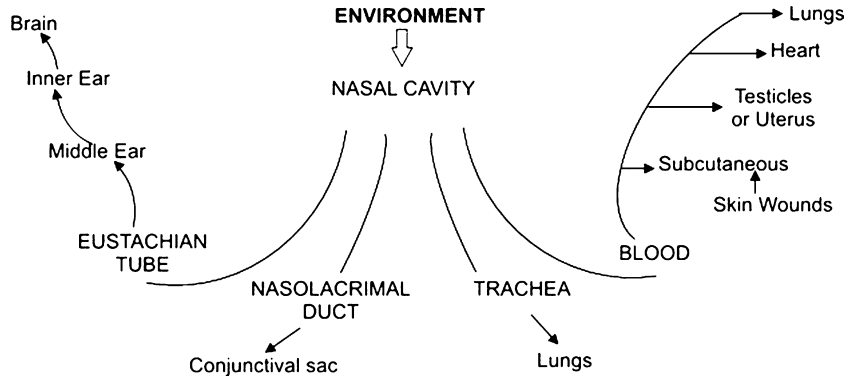

Fig 10.9. Pasteurellosis progression. (Adapted from ACLAM *Diseases of Rabbits* slide.)

mask the severity of disease. Alternatively, rabbits with severe pneumonia may exhibit signs such as anorexia, depression, dyspnea, moist lung sounds (rales), and death.

Suppurative otitis media and otitis interna are common manifestations of this organism. Otitis interna causes vestibular derangements and torticollis, commonly called head tilt or wryneck. The head tilt may be so severe that the head is rotated nearly 180°.

Reproductive tract infections in rabbits include pyometra in the doe and orchitis in the buck. Venereal transmission has been described. Large, subcutaneous abscesses may be seen with *P. multocida* infections. The pyometra or abscess contents tend to be yellow–white and have a very thick, creamy consistency that is difficult to aspirate. Incising the abscess and removing the thick purulent material may be necessary to initiate successful treatment of the infection although means to minimize environmental contamination should be considered.

Definitive diagnosis is most often made by culture of the organism. Screening colonies through use of enzyme-linked immunosorbent assays (ELISA) or polymerase chain reaction (PCR) tests can also be a helpful, but not fully reliable, means of identifying infected animals. To exclude the adverse effects of this organism on research results, only *Pasteurella*-free rabbits should be used; these animals are readily available. There is no commercially available vaccine. Eradication of the organism is extremely difficult with antibiotic treatment as the organism is harbored in the nasal passages, pharynx, and tympanic bullae. Antibiotics often used in treating pasteurellosis include penicillin G (40,000 U/kg), enrofloxacin (5 mg/kg, twice a day for 14 days), and tilmicosin (25 mg/kg). A degree of caution should be exercised in using antibiotics such as penicillin G for prolonged periods because rabbits are susceptible to developing a fatal diarrhea caused by clostridial enterotoxemia (Box 10.2).

Box 10.2

A degree of caution should be exercised in using antibiotics such as penicillin G for prolonged periods because rabbits are susceptible to developing a fatal diarrhea caused by clostridial enterotoxemia.

Bordetella bronchiseptica has been recovered from the respiratory tracts of both normal and diseased rabbits. Thus, its status as a pathogen is not well established. It may facilitate *P. multocida* infections of the lower respiratory tract and should be regarded as a potential pathogen in young rabbits 4–12 weeks of age. Rabbits are frequently asymptomatic carriers of *Bordetella* spp. and should not be housed near guinea pigs because they may inadvertently infect them.

Cilia-associated respiratory (CAR) bacillus isolates from rabbits differ significantly from isolates obtained from infected rodents, suggesting that isolates may be host specific. The organism appears to be an opportunistic invader of the respiratory tract and is found between the cilia of the respiratory epithelium. Naturally occurring infections are usually asymptomatic but may cause a slight nasal discharge. Histologically, there is slight hypertrophy and hyperplasia of the laryngeal, tracheal, and bronchial epithelium. Antimicrobial treatments for the CAR bacillus have not been evaluated for efficacy in rabbits.

Bacterial Enteritis and Miscellaneous Enteric Diseases

As a group, enteric diseases are second only to pasteurellosis as a health problem in domestic rabbits (Box 10.3). Of the common diseases encountered, the enteric diseases are probably the least understood. In some cases, diarrhea is associated with a specific, well-defined agent, but in many cases, the cause of enteric disease is obscure. Specific enteric diseases include coccidiosis, enterotoxemia, Tyzzer's disease, and salmonellosis. Those of uncertain etiologies include colibacillosis and mucoid enteropathy.

Box 10.3

As a group, enteric diseases are second only to pasteurellosis as a health problem in domestic rabbits.

Symptomatic treatment may be the best initial step in treating individual rabbits with acute diarrhea. Maintaining hydration and body temperature as well as changing the diet to one containing higher fiber and lower protein should be the focus of treatment. It is important to keep the rabbit's anus free from fecal impaction and the hindquarters clean and dry. Analgesics may be indicated in depressed, anorexic rabbits.

Colibacillosis

Colibacillosis might appropriately be classified as a specific enteric disease; however, the role of the *Escherichia coli* organism is not completely understood. The type of *E. coli* that most frequently causes disease in rabbits is known as enteropathogenic *E. coli* (EPEC). Enteropathogenic organisms do not produce enterotoxins, and they do not invade the intestinal mucosa. Rather, the EPECs adhere to receptors on the enterocytes and probably release a Shiga-like cytotoxin. It is known that *E. coli* is either absent or present in low numbers in the gut of normal rabbits. In some cases of severe diarrhea, the organisms are isolated in large numbers. Due to this finding, it was once thought that *E. coli* was not a primary pathogen of rabbits, but this has since been disproven. Outbreaks of colibacillosis may be

seen in sucklings (1–2 weeks old) or weanlings (4–6 weeks old) because of the prevalence of receptors, which allow EPEC attachment.

Clinical signs are nonspecific and may be attributed to any of the enteric pathogens. Gross findings include an edematous and hemorrhagic cecum. Histologically, there may be villous atrophy, edema, congestion, hemorrhage, and characteristic attaching and effacing intestinal lesions with large numbers of Gram-negative coliform bacilli attached to the epithelium. Tentative diagnosis can be made through observation of the characteristic histologic lesions and by identification of nonhemolytic, facultatively anaerobic *E. coli* on fecal culture. A definitive diagnosis can be made with microscopy, serotyping, or biotyping.

Treatment is largely symptomatic. Systemic antibiotics (gentamicin, chloramphenicol, and neomycin), intestinal protectants such as bismuth subsalicylate, fluids and electrolytes, and other supportive measures may be beneficial. Alteration of the diet to reduce the amount of concentrates fed to no more than 2–3 oz per day and providing added roughage, such as hay, is often more effective than are antibiotics in reducing mortality.

Enterotoxemia

Clostridium spiroforme is the most common pathogen associated with enteritis in recently weaned (4- to 8-week-old) rabbits. Sporadic infections are most common, but epizootics do occur. In addition, *C. perfringens* and *C. difficile* have also been implicated in this disease complex but are lesser pathogens. It is now known that clostridial species are either absent or found in low numbers in the large intestine of rabbits. A disruption of the normal microflora of the gut appears to play an important role in the disease process of adult rabbits. Clostridial organisms may be isolated as the sole cause of enteric disease, but concurrent infections with *E. coli*, *C. piliforme*, or rotavirus are not uncommon. Changes in feed, weaning, antibiotic therapy, and concurrent infections may all be stressors that allow colonization of *C. spiroforme* and enterotoxin production. Weaning causes a change in gut flora, which most likely allows the pathogen to proliferate. A depression in antibacterial resistance favors the increased growth of toxin-producing bacteria. The enterotoxins cause damage to the enterocytes, with functional impairment and subsequent diarrhea, dehydration, and death.

A hemorrhagic and edematous cecum is typically seen in these cases. Gram stains of a smear of cecal contents can be helpful in identifying curved or coiled Gram-positive bacilli. Anaerobic cultures are also indicated for trying to obtain a presumptive diagnosis of clostridia-mediated diarrhea. A definitive diagnosis requires the demonstration of the enterotoxin (iota toxin) or causative organism. Cytotoxicity assays, ELISAs, and PCR tests are *in vitro* tests that can identify *Clostridia* spp.–associated toxins in the fecal material of rabbits. Treatment consists mainly of supportive therapy since there is little evidence that enterotoxemia can be effectively treated with antibiotics. Adding copper sulfate to the diet may reduce toxin production by *Clostridia* species. The administration of oral probiotics may have beneficial effects in weanlings as this may help reestablish a normal gut flora.

Several antibiotics have been associated with outbreaks of enterotoxemia, including erythromycin, clindamycin, lincomycin, and streptomycin. Penicillin and its derivatives are routinely used in rabbits but have also been associated with enterotoxemia and must be used with caution. Parenteral administration is preferred over oral administration. Antibiotics that are generally considered to be safe in rabbits include chloramphenicol, enrofloxacin,

trimethoprim/sulfa, gentamicin, neomycin, vancomycin, and metronidazole. Preventive measures for animals receiving antibiotics include the oral administration of *Lactobacillus* spp. preparations to inhibit toxins or cholestyramine to absorb toxins.

Proliferative Enteropathy

Proliferative enteropathy (PE) is caused by infection with *Lawsonia intracellularis*, which is an obligate, intracellular, curved rod–shaped, argyrophilic bacterium located within the apical cytoplasm of infected crypt epithelial cells. PE has a worldwide distribution and affects a wide variety of species (e.g., hamsters, ferrets, and pigs). Since *L. intracellularis* isolates from different species have little genetic variation, intraspecies and/or interspecies transmission appears likely. Epizootics of the disease are usually confined to younger animals. Infection occurs primarily in weanlings, when passive maternal immunity declines. Transmission is fecal–oral, and then the organisms infect intestinal proliferating crypt epithelial cells and multiply. The intestine appears to be the only tissue infected. Most animals develop subclinical infection but shed the bacterium in feces, contributing to environmental contamination. Stressors, such as overcrowding, transport, change in diet, and experimental manipulations, may predispose to clinical infection.

Clinical signs include abdominal distention with sloshing sounds from the intestines, hunched posture, depression, polydipsia, anorexia, hypothermia 37°–38.5°C (99°–101°F), and constipation followed by profuse mucoid diarrhea. Affected animals may be emaciated and/or dehydrated. Grossly, rabbits have proliferative jejunitis and/or ileitis characterized by thickening and corrugation of the small intestinal mucosa and the presence of semifluid mucinous contents within lumens of colon and cecum. Microscopically, intestinal crypts are elongated and often branched and lined by multilayered immature enterocytes. Special stains (Warthin Starry silver stain, PAS stain) can be used to demonstrate the curved bacteria consistent with *L. intracellularis* in apical cytoplasm of enterocytes and within crypt lumens.

Diagnosis should be made based on the combination of clinical signs and results of necropsy, histopathology, bacteriology, and/or demonstration of intralesional organisms by histochemistry, electron microscopy, immunohistochemistry, ELISA, and/or PCR. Bacterial culture and isolation must be performed by use of cultured enterocytes because *L. intracellularis* does not proliferate in cell-free media.

Treatment is usually ineffective, although intense fluid therapy and broad-spectrum antibiotics may help rabbits in the early stages of this disease. Antibiotics must be administered with caution due to their potential impact on the intestinal flora. Provision of a heat source to prevent hypothermia is another important part of supportive therapy. Preventive measures include decreasing food intake when shipping and for 48 hours postshipment.

Tyzzer's Disease

Clostridium piliforme is a Gram-negative, spore-forming bacillus and is the etiologic agent of Tyzzer's disease. It is an obligate intracellular pathogen that can cause an acute hemorrhagic typhlocolitis, and is difficult to grow *in vitro*. Infections are most frequently observed in epizootic outbreaks of weanling rabbits between 6 and 12 weeks of age; morbidity and mortality can be very high. Though asymptomatic infections with fecal shedding are not uncommon, clinically affected animals present with acute and profuse diarrhea, anorexia, dehydration, lethargy, and fecal staining of the hindquarters. Death follows in 12–48 hours.

Rabbits with chronic infections may show weight loss and muscle wasting. Stresses such as overcrowding, shipping, poor ventilation, and improper nutrition are precipitating factors that elicit an acute diarrheal disease. At necropsy, focal necrosis of the liver and intestinal mucosa is seen.

A diagnosis is based upon identifying Periodic Acid–Schiff stained or silver-positive intracytoplasmic filamentous bacilli in histologic sections of the liver, heart, or cecum. Alternatively, a Giemsa-stained impression smear of the hepatic lesions may reveal the typical bacilli. ELISA, immunofluoresence assay (IFA), and PCR assays can also be used to identify the presence of antibodies to the bacterium.

Oxytetracycline is suggested for treatment; however, the prognosis for animals showing clinical signs of Tyzzer's disease is grave. Institution of sound husbandry practices and elimination of environmental stress factors are crucial in controlling an outbreak in a rabbit colony. Spores can persist in the environment for extended periods; thus 1% peracetic acid or 0.3% sodium hypochlorite or a vaporized sterilant should be used to clean laboratory surfaces.

Salmonellosis

Salmonella spp. infections are a relatively uncommon cause of enteric disease in laboratory rabbits, but are acutely fatal and characterized by septicemia. Diarrhea or abortions may be noted. Although various antibiotics may be effective in eliminating clinical signs of salmonellosis, treatment of affected animals is contraindicated due to the zoonotic potential of this organism and the possibility of inapparent carriers.

Miscellaneous Bacterial Infections

Staphylococcus aureus is considered a normal bacterial inhabitant of the rabbit nasopharynx, conjunctiva, and skin. It is one of the most common causes of conjunctivitis in rabbits. Pathogenic infections may occur and manifest in the forms of septicemia, dermatitis, and abscesses. Staphylococcal mastitis often causes septicemia in the doe and multiple abscesses in nursing kits maintained in unsanitary conditions. The organism can be identified through culture and its relative virulence assessed by PCR. Penicillin, enrofloxacin, cephalosporins, aminoglycosides, and chloramphenicol, in conjunction with topical treatment are sometimes effective. Antibiotic treatment of staphylococcal infections, however, is frequently discouraging.

Mastitis occurs sporadically in lactating does and occasionally during pseudopregnancy. Blue breast is a common term for mastitis as the skin over the mammary gland changes from pink to red then to bluish purple. Several microorganisms, including *Staphylococcus aureus*, *Pasteurella* spp., and *Streptococcus* spp., are causative agents. Causative organisms may be introduced by means of splinters from nest boxes, trauma from the teeth of nursing young, or unsanitary housing conditions. Treatment involves isolating the affected doe and treating her with appropriate antibiotics; lancing and flushing abscesses may be necessary. The young should be removed from the doe but not foster nursed on another doe because of the probability of spreading the disease to a healthy animal.

Necrobacillosis or Schmorl's disease, caused by *Fusobacterium necrophorum*, is characterized by ulceration of the skin and by subcutaneous swellings of the face, neck, and oral cavity. Septicemia with infection of the jugular vein can occur as a secondary infection. Although the organism is commonly present in the digestive tract of rabbits, the incidence

of disease is low. Infection is thought to develop through fecal contamination of skin wounds. The recommended treatment for affected rabbits includes debridement, drainage of abscesses, provision of systemic antibiotics such as penicillin, tetracycline, or metronidazole, and improved sanitation. Lesions frequently reoccur. *Fusobacterium necrophorum* is transmissible to humans.

Tularemia is caused by *Francisella tularensis*. It is a Gram-negative coccobacillus that infects vertebrates, especially rabbits and rodents, and usually causes sudden death of affected animals. Typical lesions at necropsy are small foci of necrosis in the spleen and liver. The organism can be transmitted by direct contact, via aerosols or ingestion, or by bloodsucking arthropods. The disease is seen in wild rabbits but is rare in domestic rabbits because of lack of exposure to the appropriate arthropod vectors. *Francisella tularensis* can cause a potentially fatal zoonotic disease in humans, seen primarily in hunters and wildlife personnel.

Pseudomonas aeruginosa is an uncommon pathogen in rabbits. Skin infections with blue-green discoloration may occur around the dewlap and in other areas in which the skin is in contact with moisture; infection may extend to other organs. Treatment involves removing the fur from the affected region, cleaning the lesion, and administering topical or systemic antibiotics. Affected animals' drinking water should be cultured and evaluated as the possible source of contamination.

Spirochete Infections: Venereal Spirochetosis

Venereal spirochetosis, also known as rabbit syphilis, vent disease, and treponematosis, is caused by *Treponema paraluiscuniculi*, a Gram-negative, spiral-shaped rod that can be propagated only *in vivo*. Rabbit syphilis is uncommon in the research environment. The organism is serologically and morphologically similar to *T. pallidum*, which causes syphilis in humans. *T. paraluiscuniculi*, however, is nonpathogenic for humans. The disease is characterized by the presence of raised, crusted, occasionally hemorrhagic, ulcerative foci on the external genitalia, perineal region, and face. The disease may be confused with lesions caused by urine scald, ear mites, and sarcoptic mange. Asymptomatic infections are common in infected colonies.

The disease is transmitted by direct contact, usually venereally but occasionally by extragenital routes. Affected rabbits should be isolated during the acute disease. Treponematosis can be differentiated from other cutaneous diseases by dark-field examination of scrapings from the lesions. A *T. pallidum* ELISA can be used to diagnose *T. paraluiscuniculi* infections. Three doses of benzathine procaine penicillin intramuscularly administered at weekly intervals is reportedly effective in eliminating the infection. Penicillin derivatives should be used with great caution because of the possibility of inducing a fatal enterotoxemia.

Viral Diseases

Myxomatosis

Rabbit myxomatosis is caused by a poxvirus, the myxoma virus. Multiple viral strains exist with differing associated levels of pathogenicity. The myxoma virus is endemic on four continents: Australia, Europe, South America, and North America. The natural host in the United States is wild cottontails (*Sylvilagus* spp.), although it is also enzootic in wild *Oryctolagus* in the western United States in which severe disease with a high fatality rate is observed. Transmission is usually by arthropod vectors (e.g., mosquitoes and fleas) but can be by direct contact. Aerosol transmission has been suspected in at least one outbreak. The

initial clinical signs are growth of gelatinous, occasionally indistinct subcutaneous masses. Several days later, a mucopurulent conjunctivitis with generalized subcutaneous edema develops, which causes the rabbit's eyes to swell shut and the ears to droop. Diagnosis of active infection is by virus isolation or PCR of infected tissues. Disease survivors can be detected through multiple serologic assays. A vaccine is available in Europe but is not approved for use in the United States.

Papillomatosis

Rabbit oral papillomavirus infection can stimulate papillomas (small white growths) on the underside of the tongue and elsewhere within the oral cavity. The domestic rabbit, *Oryctolagus*, is the natural host, with disease transmission through abraded oral mucosa such as from rough, contaminated feed. Lesions are usually seen in rabbits 2–18 months of age. Papillomas spontaneously regress within a few weeks.

Rotavirus

Rotavirus causes enteritis with mild to severe diarrhea in young rabbits. Weanlings are particularly susceptible. Fecal–oral transmission occurs although aerosol transmission is also suspected. Outbreaks occur in naive colonies, with high morbidity and mortality; infections tend to be acute and self-limiting in animals that survive. Maternal antibodies play a significant role in protecting suckling rabbits against the disease. Diagnosis is based on clinical signs, histologic findings, viral detection, and serological assays. The only treatment is supportive therapy.

Rabbit Hemorrhagic Disease (RHD)

The etiologic agent of rabbit hemorrhagic disease is a calicivirus. It is highly contagious and is spread by direct contact or aerosols. It has been reported in Asia, Europe, Africa, Australia, New Zealand, the United States, and Mexico. Mexico has since instituted an effective eradication campaign and its rabbit populations are no longer endemically infected with the virus.

The disease is seen in rabbits older than 2 months; younger rabbits are not clinically affected. Transmission is by direct and fecal–oral contact, through fomites, and possibly through insects. Onset is acute; however, symptoms may include nervous system signs such as shaking, incoordination, and prostration. Morbidity and mortality range from 80% to 100%. Gross lesions include diffuse hemorrhage of the trachea, lungs, liver, spleen, kidneys, thymus, and peritoneum. The cause of death is most commonly disseminated intravascular coagulation (DIC) with deep venous thromboses. The disease can be presumptively diagnosed by clinical signs, pathological findings, and outbreak characteristics. Definitive diagnosis can be made through ELISA testing or PCR of infected tissues and rabbit inoculation. The virus cannot be reliably isolated *in vitro*. Infected colonies should be culled. An inactivated viral vaccine is available that provides up to 6 months of protection and is most frequently used to protect valuable colonies.

Miscellaneous Viral Diseases

Rare reports of other viruses in rabbits include adenovirus, parvovirus, herpesvirus, and coronavirus. These viruses have been identified to cause disease only in very isolated cases and are not yet considered to be common pathogens of rabbits.

Mycotic Infections

Dermatophytosis

Ringworm is rarely encountered in laboratory rabbits. Infections with multiple dermatophytes have been documented; however, ringworm is most frequently caused by the zoonotic agent *Trichophyton mentagrophytes*. As *T. mentagrophytes* does not fluoresce under ultraviolet light, microscopic examination or culturing of hair or skin scrapings is usually required to establish a diagnosis. Lesions are pruritic, crusty, and hairless and in most cases first appear on the head, ears, or toes. Lesions may be treated with topical antifungal creams applied twice daily or by administration of griseofulvin orally.

Encephalitozoonosis

Encephalitozoonosis is usually a latent disease of rabbits caused by the fungal organism *Encephalitozoon cuniculi*. This agent was once considered a protozoal organism and is uncommon in rabbits produced for biomedical research. *Encephalitozoon* organisms are capable of infecting a wide range of mammals, including humans—where it is most frequently identified in immunocompromised individuals. In the rabbit, transmission most commonly occurs through ingestion of spores in the urine. Intrauterine and aerosol transmission have also been documented.

Infections are typically asymptomatic with mild lesions noted only on postmortem examination. While the organism can affect multiple organs and systems, the three most frequently affected are the kidneys, the central nervous system, and the eye. Infection of the kidneys may induce chronic renal failure but more frequently causes fibrosis and pitting of the renal cortex with no notable loss of kidney function. A pyogranulomatous encephalitis may develop with related clinical signs such as head tilt, seizures, depression, ataxia, swaying, and posterior paresis. Ocular lesions (e.g., uveitis and cataracts) may develop. Recognizing lesions induced by *E. cuniculi* is particularly important so that they are not mistakenly identified as experimental sequelae.

A tentative clinical diagnosis can be made with positive serology (e.g., multiplex fluorescent immunoassay, ELISA, IFA) or PCR of kidney tissue or urine. Definitive diagnosis is made with histologic observation of the organism in affected tissues. Acute infections of *E. cuniculi* have been successfully treated with fenbendazole-medicated pellets. Treatment of chronic infections is frequently unsuccessful.

Parasitic Diseases

Ear Mites

Psoroptes cuniculi, the rabbit ear mite, is a nonburrowing mite that chews on the epidermal skin of the inner ear and causes an intense inflammatory response. A dry, brown, crusty material accumulates on the inner surface of the ear. This condition is common in rabbits raised for the agricultural or pet industries but is exceedingly rare in research colonies.

Severe mite infestations induce inflammation and intense pruritus of the inner pinnae. Affected rabbits may shake their heads and intensely scratch their ears, possibly resulting in self-mutilation. Crusty lesions may be observed on the back of the neck from scratching. Rarely, a secondary bacterial infection occurs, leading to otitis media or interna. The mites

can easily be seen with the aid of an otoscope or by placing the crusts in mineral oil on a slide and examining with a microscope. All life cycle stages of the mite are found on the rabbit; mites may survive in the environment for up to 3 weeks.

There are several options for treatments, including ivermectin, moxidectin, or selamectin. Cleaning of the pinnae and ear canal in severe cases is controversial. While removal of the crusty material will decrease the mite load, ear tissues and blood vessels may inadvertently be damaged. The use of analgesics should be considered in severely affected animals.

Fur Mites

Cheyletiella parasitovorax, the dandruff mite, causes thinning of the fur and scaly lesions, usually over the scapulae. The term "walking dandruff" has been used to describe the easily visualized mites that remain in contact with the keratin layer of the skin to feed on tissue fluid. Pruritus is not associated with infestation as the mite does not burrow. Transmission occurs through direct contact and fomites; adults may survive up to 10 days in the environment. Treatment is accomplished through multiple subcutaneous doses of ivermectin or by topical application of selamectin, permethrin, and carbaryl products. *C. parasitovorax* can infest multiple species, including dogs, cats, and humans.

Mange Mites

Although rabbits are susceptible to *Notoedres cati* and *Sarcoptes scabiei*, these mites do not commonly cause infestation. Clinical signs may include pruritus, alopecia, hyperemia, and epidermal crust formation. Pruritus may be so severe as to result in self-mutilation and trauma. Diagnosis is by observation of the mites on deep skin scrapings. Ivermectin, doramectin, and moxidectin can be used to treat mange mites.

Fleas

Rabbits can be infested with multiple species of fleas, including the rabbit flea (*Spilopsyllus cuniculi*) and the dog and cat fleas (*Ctenocephalides canis* and *C. felis*). Their presence must be strongly considered with wild-caught rabbits due to the possible transmission of multiple zoonotic agents (e.g., plague, Rocky Mountain spotted fever) as well as agents of special concern to rabbit colonies (e.g., myxoma virus, rabbit hemorrhagic disease). Effective treatments have included use of imidacloprid, selamectin, and lufenuron. The environment must also be treated to eliminate the parasite.

Cuterebra

Myiasis (from the Greek word for "fly," *myia*) is defined as the infestation of live vertebrate animals with fly larvae, which feed on the host's dead or living tissue or liquid body substances. Such infestation is rarely seen in laboratory rabbits but may be found in rabbits housed outdoors. *Cuterebra* spp., commonly called bot flies, parasitize rabbits and rodents to cause myiasis. The larvae live in the subcutaneous tissue and appear as large lumps with perforated breathing holes, usually on the neck or upper extremities. They grow up to 1 inch in length. Application of a heavy oil or occlusive ointment interrupts the larva's respiration, forcing it to migrate to the surface of the lesion, where it can be removed easily. Surgical treatment consists of incising the skin and removing the larvae. Care must be taken to avoid crushing the larvae, which can result in acute shock and death in the rabbit.

Pinworms

The rabbit pinworm, *Passalurus ambiguus*, is a whitish hairlike worm that can be seen in the rabbit's cecal contents and colon. Pinworms are generally nonpathogenic to rabbits, and infections are typically asymptomatic. There is no zoonotic potential for humans. Transmission is fecal–oral. Embryonated eggs pass in the feces and are immediately infective. Once ingested, the eggs hatch in the small intestine and larvae molt. Adult worms are found in the cecum. Definitive diagnosis requires identification of the typical egg in the feces. They can be treated with piperazine citrate or fenbendazole. Environmental decontamination is recommended to eliminate infectious adults and ova.

Tapeworms

Rabbits can be infested with tapeworms such as the rabbit tapeworm, *Cittotaenia ctenoides*. Rabbit tapeworms require the presence of an intermediate host (mite), therefore are virtually nonexistent in rabbit research colonies.

Coccidiosis

Coccidiosis is much less common in laboratory rabbits today but is still commonly found in pet rabbits. Two forms of coccidiosis have been identified in *Oryctolagus cuniculus*: intestinal and hepatic (Box 10.4). The intestinal form is most prevalent and is caused by at least 11 species of *Eimeria*, including *E. magna*, *E. perforans*, *E. media*, and *E. irresidua*. The hepatic form is caused by *E. stiedae*.

Box 10.4

Two forms of coccidiosis have been identified in Oryctolagus cuniculus*: intestinal and hepatic.*

Hepatic coccidiosis: Adult rabbits usually show no clinical signs from infection with *E. stiedae*, but heavy infections in young animals may cause weight loss, diarrhea, a potbellied appearance (due to marked hepatomegaly), liver failure, and death. Following fecal–oral transmission, the organism migrates either hematogenously or via the lymphatic system from the intestinal tract to the bile duct in which it reproduces. At necropsy, hepatomegaly with irregularly shaped, slightly raised yellow–white foci are noted. Microscopically, bile duct hyperplasia and inflammation are apparent.

Intestinal coccidiosis: Some species of intestinal coccidia are nonpathogenic while others can induce varying degrees of disease and target different regions of the intestine. Concurrent infections with multiple species are common. In mild cases of intestinal coccidiosis, few if any clinical signs are evident. In moderate to severe cases, weight gain may be poor and an odiferous, profuse, and soft to watery diarrhea with traces of blood may develop. Intussuception may be noted. Mortality is more common in young animals.

Eimeria has a direct life cycle. Fecal–oral transmission occurs most commonly through ingestion of sporulated oocysts in contaminated food or water, or via contact with

contaminated fomites. Sporulation can occur in as little as 2 days if the ambient temperature and humidity are optimal. Reingestion of the soft nighttime fecal pellets does not allow time for sporulation; therefore, infection by this route is unlikely. With both intestinal and hepatic coccidiosis, oocysts appear in the feces except in animals that die very soon after onset of illness. Oocysts remain infectious in the environment for several months. The yeast *Saccharomycopsis guttulatus* is commonly found in rabbit feces and should not be incorrectly identified as a coccidial oocyte.

Prevention and control of coccidiosis is achieved by (1) strict cleaning and disinfection procedures; (2) use of cages with nonsolid flooring with a catch pan, J-type feeders, and water bottles or automatic watering devices to reduce autoinfection; (3) medicated feed or water; and (4) in some cases, culling of infected animals. There is no effective treatment for elimination of *E. stiedae* from the liver. Drugs used for treating coccidiosis include sulfamethazine, sulfadimethoxine, sulfaquinoxaline, amprolium, monensin, robenidine, decoquinate, and lasalocid.

Neoplasia

The most common neoplasm of domestic rabbits is uterine adenocarcinoma, with a high incidence in does 5 years of age and older (Box 10.5). A doe's reproductive performance generally declines several months before the tumor can be detected clinically. Other clinical signs may include a bloody vaginal discharge or bloody urine. Masses may be found by gentle palpation of the abdomen. Metastasis to the lungs and other organs occurs frequently. Because of the frequency of uterine neoplasia in mature does, an ovariohysterectomy should be considered for laboratory rabbits that are held long-term and not used for breeding.

Box 10.5

The most common neoplasm of domestic rabbits is uterine adenocarcinoma.

Other neoplasias commonly identified in the rabbit, in decreasing order of frequency, are as follows: lymphoma/lymphoid leukemia; mammary papilloma and adenocarcinoma; and neoplasms of the skin, including trichoblastoma and collagenous hamartoma. Other neoplasias occasionally reported in the literature include uterine leiomyosarcoma, embryonal nephroma, and pituitary adenoma. Prolactin-secreting pituitary adenomas have been described in aged, nulliparous NZW rabbits. The tumors secrete prolactin and are associated with mammary gland abnormalities, including enlarged, nonpainful teats that are frequently engorged with fluid.

Miscellaneous Conditions

Buphthalmia

Buphthalmia, also known as congenital glaucoma, is one of the more common inherited diseases of domestic rabbits and is commonly seen in NZW laboratory rabbits. It is associated with an abnormal production and removal of aqueous fluid from the anterior chamber

and may occur unilaterally or bilaterally. Clinical signs include an increase in the size of the anterior chamber, corneal opacity, and increased prominence of the eyeball. Vision will eventually be reduced and rabbits may show a slight head tilt to allow better visualization from a normal eye. The condition has a slow onset but, with continued progression, can result in a painful condition due to corneal ulceration and self-trauma. No treatment is necessary in the early stages, although enucleation is an option. Affected animals should not be bred.

Lumbar Spinal Fracture or Luxation

Fractures or luxations of the caudal lumbar spine are common, particularly at the L7–S1 junction (Box 10.6). The condition is strongly suggested by the sudden onset of posterior paralysis and is usually caused by a handler's failure to support an animal's hindquarters or when a rabbit falls or is dropped. The prognosis after back injury depends on the location and severity of the cord lesion but is usually unfavorable. Therapeutic criteria are similar to those for dogs and cats and may include steroids, frequent rotation of the animal to prevent pressure sores, and at least twice-daily manual expression of the bladder. Rarely, an animal will recover with cage rest, but in most cases posterior paralysis with urinary and fecal incontinence persists and euthanasia is recommended.

Box 10.6

Fractures of the caudal lumbar spine are common, particularly at the L7–S1 junction.

Hairballs (Gastric Trichobezoars)

Gastric hairballs should be suspected in rabbits that suddenly stop eating and drinking and pass no fecal material but otherwise appear alert and in good health. The presence of a hairball can usually be detected by palpation, but in some cases a radiograph may be necessary to confirm the diagnosis. Care must be taken in attributing clinical signs to the presence of hairballs as significant-sized hairballs are frequently present in the stomach of asymptomatic animals. Hairballs may be the result of excessive self-grooming secondary to boredom or low-fiber feeds, but dehydration, stress, pain, and *ad libitum* feeding have been suggested as contributing causes.

Restoring and maintaining hydration is of utmost importance. Mineral oil administered by a stomach tube and metoclopramide may be effective. Metoclopramide use must be approached with extreme caution due to the chance of gastric rupture. Another suggested but unproven therapeutic regimen includes the oral administration of fresh pineapple juice (10 mL once daily or divided every 12 hours for 5 days). The pineapple juice contains the enzyme papain, which may help digest the hair in the stomach. Papain may also be purchased in nutrition stores. Administration of bromelin enzyme may be of some value as a treatment and preventive. Most treatments are ineffective, but medical treatment is usually preferred over surgical intervention, which is associated with a high mortality rate. Death following blockage of the stomach by a hairball is usually attributable to ketosis.

Feeding a high-fiber (up to 20%) pelleted diet in restricted amounts is one of the best ways to prevent hairballs (Box 10.7). Providing roughage such as alfalfa or timothy hay may also be beneficial in prevention.

Box 10.7

A high-fiber (up to 20%) pelleted diet is one of the best ways to prevent hairballs.

Heat Stroke

Rabbits are highly susceptible to overheating. Young rabbits in nest boxes, as well as older, obese, or pregnant rabbits, are particularly prone to heat prostration. Other predisposing factors include a high ambient temperature over 29.5°C (85°F), high humidity (70% or higher), poor ventilation, and crowding. Treatment is aimed at quickly reducing the body temperature and providing supportive care such as steroids and intravenous fluids. Prognosis is guarded to poor.

Ketosis (Pregnancy Toxemia)

Ketosis occasionally occurs in rabbits and in most cases is seen in obese does a few days prior to parturition. It is analogous to ketosis in guinea pigs and sheep in terms of etiology, treatment, and prevention. See the discussion on ketosis in Chapter 8, "Guinea Pigs."

Malocclusion

Tooth overgrowth results when open-rooted teeth are misaligned and therefore do not appropriately wear against other teeth or food items. Overgrowth is most commonly seen in the incisors. Mandibular prognathism, a common genetic condition in which the maxilla is abnormally short relative to the length of the mandible, causes the lower incisors to grow anterior instead of posterior to the large, primary upper incisors. Other causes of malocclusion include trauma and tooth root infection. Overgrowth of the cheek teeth may be underdiagnosed because of the difficulty in viewing these teeth. Use of an otoscope in a conscious rabbit or a dental bite block in a sedated rabbit allows improved visualization. A motorized dental bur or Dremel drill can be used to trim overgrown teeth. The use of nail clippers is best avoided because it can cause tooth fractures. Repeat trimming is indicated as needed, typically twice monthly to monthly. Culling of an affected animal and its offspring is the only successful means of eradication in a breeding group.

Moist Dermatitis

A moist dermatitis may occur in rabbits affecting either the area ventral to the dewlap or the anogenital region. Moisture from malocclusion-induced hypersalivation or the use of water bowls, and prolonged contact with urine or diarrhea in obese animals lends to colonization by a variety of opportunistic bacteria. Clipping and cleaning the affected areas and correction of environmental contributors will produce an uneventful resolution.

Splay Leg or Hip Dysplasia

Splay leg is an abnormality in which one or more limbs is abducted and cannot be maintained in a normal position. It may be caused by a recessive inherited trait although environmental conditions, including inappropriate flooring, can cause or contribute to the condition. The most common form is subluxation of the hip. Affected animals sit with their legs splayed out sideways. There is no effective treatment. Provision of flooring with good traction during the postnatal period is critical in preventing development of hip dysplasia in rabbits. Culling animals carrying this trait can help to reduce the incidence in a breeding colony.

Ulcerative Pododermatitis

Ulcerative pododermatitis is commonly called sore hock, but it actually involves the ventral metatarsal region rather than the hock. It usually occurs in heavy rabbits maintained on rounded wire-mesh floors rather than on flat-bar floors or in animals housed on solid floors that become urine soaked. The lesion consists of a circumscribed ulcerated area of the skin covered by a dry crusty scab. Abscesses, caused by organisms such as *Staphylococcus aureus*, may form under the scab. Affected individuals may lose weight and sit in a hunched position or shift weight on their hind feet.

Treatment consists of providing soft, clean, dry bedding in a solid-bottom cage or providing a commercially available resting board. Animals should be limit-fed to prevent obesity, a contributing factor to the condition. Lesions may be treated by application of a topical ointment such as zinc oxide. A bandage affords protection against further trauma. If abscesses are present, broad-spectrum systemic antibiotics are indicated.

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CHAPTER 10 REVIEW

Multiple Choice

1. The principal anatomic feature differentiating rabbits from rodents is the presence of
   A. unipapillate kidneys
   B. two pairs of upper incisors
   C. a sacculus rotundus
   D. an undivided stomach
2. Spinal fractures most commonly occur at this site.
   A. C1–C2
   B. L1–L2
   C. C5–C6
   D. L7–S1
3. Small breed of rabbit
   A. Dutch
   B. Flemish
   C. New Zealand White
   D. Californian
4. Etiologic agent of snuffles
   A. *Bordetella bronchiseptica*
   B. *Klebsiella pneumoniae*
   C. *Pasteurella multocida*
   D. *Streptococcus pneumoniae*
5. Ear mite of the rabbit
   A. *Psoroptes*
   B. *Otodectes*
   C. *Cheyletiella*
   D. *Sarcoptes*
6. False statement regarding rabbit urine
   A. Its color varies from yellow to orange.
   B. It has a pH 7.2.
   C. It may appear cloudy.
   D. Crystals are frequently seen.
7. Location of the sacculus rotundus
   A. tip of cecum
   B. ileocecal junction
   C. pyloric region
   D. cardia of stomach

(*Continued*)

8. False statement regarding female rabbit
   A. The female is called a doe.
   B. The female has a prominent dewlap.
   C. The female has 4–5 pairs of mammary glands.
   D. The female rabbit has a bicornate uterus and a single cervix.
9. Rabbits mark their territory using this gland.
   A. chin
   B. inguinal
   C. ventral sebaceous
   D. hip
10. Lymphoid organs associated with the gastrointestinal tract
   A. spleen and sacculus rotundus
   B. liver and vermiform process
   C. vermiform process and sacculus rotundus
   D. sacculus rotundus and spleen
11. Common pathogen associated with enteritis in 4- to 8-week-old rabbits
   A. *Clostridium difficile*
   B. *Clostridium spiroforme*
   C. *Clostridium tetani*
   D. *Clostridium perfringens*
12. Causes blue-green discoloration of skin
   A. *Fusobacterium necrophorum*
   B. *Staphylococcus aureus*
   C. *Pasteurella multocida*
   D. *Pseudomonas aeruginosa*
13. Location of the vermiform process
   A. tip of cecum
   B. ileocecal junction
   C. pyloric region
   D. cardia of stomach
14. *Treponema paraluis cuniculi* causes lesions on
   A. external genitalia and face
   B. feet and anus
   C. ovaries and uterus
   D. chest and face
15. Nighttime feces are called
   A. autotrophs
   B. vermiforms
   C. cecotrophs
   D. coliforms

16. Pinworm of the rabbit
    A. *Oxyuris*
    B. *Passalurus*
    C. *Syphacia*
    D. *Aspiculuris*
17. Disease that has zoonotic potential
    A. rabbit syphilis
    B. tularemia
    C. snuffles
    D. myiasis
18. Causes hepatic coccidiosis
    A. *Eimeria magna*
    B. *Eimeria perforans*
    C. *Eimeria media*
    D. *Eimeria stiedae*
19. Common tumor of older domestic rabbits
    A. bile duct adenoma
    B. lymphosarcoma
    C. uterine adenocarcinoma
    D. embryonal nephroma
20. Yeast commonly seen in rabbit feces
    A. *Saccharomycopsis guttulatus*
    B. *Encephalitozoon cuniculi*
    C. *Taenia pisiformis*
    D. *Notoedres cati*

Critical Thinking

21. A new investigator is about to receive six New Zealand White rabbits in the 2.2–2.4 kg weight range. Rabbit cages with the following dimensions are available in the storage room: 21″ long, 18″ wide, and 14″ high. Calculate the floor space available in square feet. Can these cages house the incoming rabbits?

11 Ferrets

The European ferret belongs to the order Carnivora and the family Mustelidae. The scientific name of the domestic ferret is *Mustela putorius furo*, which appropriately translates into "stinky weasel thief." The domestic ferret is believed to be derived from the wild European polecat and is related to the skunk, weasel, otter, badger, and mink. The domestic ferret should not be confused with the native North American black-footed ferret, *Mustela nigripes*. There are two predominant varieties of domestic ferret based upon coat color and pattern: fitch (also known as sable or wild-type) and albino. The fitch, most commonly used in biomedical research, has a buff-colored coat with black mask, limbs, and tail. The albino, due to a recessive mutation, lacks all pigmentation and is therefore white with pink eyes. Male ferrets are referred to as hobs, females as jills, and the young as kits. A spayed female is a sprite; a neutered male is a gib; a group of ferrets is called a business. Adult male ferrets weigh 1–2 kg; females are much smaller, weighing 0.45–1.35 kg (600–950 g).

USES

Although not used in large numbers for research, ferrets are valuable in a number of areas of study, including reproductive physiology, virus-induced neoplasms, bacterial infections, and viral diseases of humans and other animals. They have been particularly useful for studies of canine distemper, vesicular stomatitis, *Helicobacter pylori* gastritis, human influenza, and severe acute respiratory syndrome (SARS)–associated coronavirus. Ferrets have been used as an alternative carnivore for drug studies, replacing dogs and cats. Ferrets have a strong emesis response and thus are used to screen compounds for emesis potential. Because they

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have an oropharyngeal anatomy similar to that of human infants, ferrets are also commonly used for neonatal intubation training.

BEHAVIOR

Ferrets are inquisitive, extroverted, and playful by nature. Most are docile and easily handled, particularly if acclimated to gentle handling when young, but may bite if excited, frightened, or hungry, or if handled only occasionally. Sexually intact males will fight with other males during the breeding season (periods of extended daylight hours), and females may defend their young. Ferrets are crepuscular and nocturnal, spending 15–20 hours per day sleeping; they are often slow to rouse when awakened. Similar to other mustelids, they engage in burrowing and digging behavior and prefer to use a nest box for seclusion and sleep. A corner or two of the cage is selected for urination and defecation; thus they may be trained to use a litter pan if this is preferred. Vocalizations include a chattering or screech, which occurs during playful activity; a hiss, which is used as a warning; and a whine, which may indicate discomfort. Ferrets will also emit a high-pitched scream in response to pain. During normal ambulation, the back is carried in an arched position, similar to other mustelids; a more horizontal carriage reflects clinical illness or pain, and it may result in mild ataxia. A shivering behavior may be noted, which is not clearly understood; however, it occurs most frequently when the ferret is awoken or excited and is regarded as normal behavior.

ANATOMIC AND PHYSIOLOGIC FEATURES

Ferret biologic and reproductive data are listed in Table 11.1. Ferrets have long, thin bodies with short legs, a medium-length tail, and thick, soft fur. Five clawed digits are present on all limbs; the nails are not retractable. Substantial seasonal fluctuations in weight are due to the accumulation of subcutaneous fat during the autumn months and its subsequent loss in the spring and summer. The albino coat color becomes tinged yellow as the ferret matures due to sebaceous secretions from the apocrine skin glands; these secretions are primarily responsible for the distinct musky odor. The ferret also has paired anal glands, one on either side of the anus, that are used for scent marking. If the animal becomes frightened, angry, or amorous, the glands may discharge their odiferous contents. It should be emphasized that descenting ferrets may reduce odor, especially in males, but will not eliminate it as the skin still produces the sebaceous secretions (Box 11.1).

Box 11.1

Descenting ferrets may reduce odor, especially in males, but will not eliminate it as the skin still produces the sebaceous secretions.

Normal rectal temperature is in the range 38°–39°C (100°–102°F), but excitement may raise it to 40°C (104°F). Well-developed sweat glands are absent, and as a result these animals

Table 11.1. Ferret biologic and reproductive values

Adult body weight: Male	1–2 kg
Adult body weight: Female	600–950 g
Life span	5–11 y
Body temperature	37.8°–40°C (100°–104°F)
Heart rate	200–400 beats per minute
Respiratory rate	33–36 breaths per minute
Food consumption	140–190 g/d (semimoist diet)
Water consumption	75–100 mL/d
Breeding onset: Male	8–12 mo
Breeding onset: Female	7–10 mo
Estrous cycle length	Continuous until intromission
Gestation period	42 + 2 d
Postpartum estrus	Occasional
Litter size	8, avg. (1–18 range)
Weaning age	6–8 wk
Breeding duration	2–5 y
Chromosome number (diploid)	40

Source: Adapted from Fox (1998).

are prone to heat exhaustion at temperatures approaching 32°C (90°F). Heat is dissipated primarily through the respiratory system.

The dental formula, 2(I 3/3, C 1/1, P 3/3, M 1/2), is unique among carnivores, with three premolars per quadrant rather than the typical four. Supernumerary teeth are common in adults. They have a simple stomach similar to that of humans. The intestinal tract is short, having a transit time of 3–4 hours. There is no cecum, appendix, taeniae coli, or ileocolonic sphincter.

Ferrets have a very long trachea and large-diameter airways, which results in lower airway resistance. The lungs are relatively large compared with other small mammals, with approximately three times the lung capacity relative to their body size. Their lungs have two left lobes and four right lobes. Small, pale yellow, slightly elevated lesions may be seen on the surface of the lungs at necropsy; these are foci of alveolar histiocytosis and are of no known clinical significance. A single, brachiocephalic artery arising from the aortic arch is present in place of the bilateral common carotid arteries and may represent a functional adaptation to ensure cerebral blood flow when the head rotates 180°.

The reproductive systems of both males and females are similar to those of other carnivores, with a few exceptions. The female has a bicornate uterus with only one cervical os. Females have four pairs of mammary glands. Males retain open inguinal canals throughout their lives. During the nonbreeding season, August to November, spermatogenesis ceases, testicular size decreases, and the testes retract into the abdomen. Males lack seminal vesicles and a bulbourethral gland but do have a prostate gland and an os penis. Sexing of both adult and neonatal ferrets is accomplished without difficulty as the penis is situated on the ventral abdomen and the anogenital distance is greater in the male. The female vulva is readily visible just ventral to the anus.

There are several distinct characteristics of the physiologic and laboratory profiles of ferrets. Hematocrit values, hemoglobin levels, erythrocyte counts, and reticulocyte counts tend to be somewhat higher in ferrets than in other species of laboratory animals. The average values for males are slightly higher than for females in all except reticulocyte counts. Female ferrets in estrus tend to have lower platelet and leukocyte counts. Hematologic values may be altered by isoflurane anesthesia for both sexes, which should be a consideration in the interpretation of samples collected during or shortly after anesthesia. During the nonbreeding season, males have reduced plasma testosterone levels. The normal urine pH ranges between 6.5 and 7.5. Slight to moderate proteinuria is commonly found in ferrets. The naturally dark urine of the male may present difficulty in interpretation of urine chemistry tests when colorimetric methods are used. Hematologic and biochemical parameters for the ferret are listed in Appendix 1, "Normal Values."

BREEDING AND REPRODUCTION

Ferrets normally reach sexual maturity in the spring after their birth or at approximately 9–12 months of age. The breeding season is light-dependent and in the Northern Hemisphere usually lasts from March to August for females and from December to July for males. Artificial illumination (16 hours light and 8 hours dark) can be used to produce an earlier onset of estrus for intensive breeding purposes.

Females are seasonally polyestrous and are induced ovulators. Marked swelling of the vulva, two to three times larger than the anestrous diameter, signals the start of estrus. Female ferrets that are not bred may remain in estrus for up to 6 months, which is characterized by weight loss, increased susceptibility to uterine infections, and aplastic anemia. Male sexual activity precedes female activity to allow for sperm maturation. The enlargement and descent of the testicles into the scrotum marks the period of sexual activity in the male.

Mating should occur 2 weeks after the onset of vulvar swelling. The female should be taken to the male's cage and observed for fighting. Mating in ferrets is frequently noisy and energetic, with rolling, jumping, and the female submitting to being dragged around by the scruff of the neck. Copulation lasts from 1 to 3 hours; part of this time is spent in achieving intromission because the males are not very coordinated. Both neck restraint and intromission are required for ovulation, which occurs 30–40 hours after mating, with release of 5–13 ova. The female's enlarged vulva will regress to normal size quickly if she is pregnant, but slowly (5–6 days) if breeding was unsuccessful. Unlike other mustelids, delayed implantation does not occur in the ferret. The average gestation length is 42 days. Nonfertile matings result in a pseudopregnancy, which also lasts 42 days. Fetuses can be palpated by 14 days, detected radiographically at 30 days, or detected via ultrasound as early as 10 days. Females in estrus may be induced to ovulate via stimulation by other females, by vasectomized males, or by injections of human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone (GnRH).

Jills should be single housed and provided a nest box with bedding within 2 weeks of delivery. Appropriate bedding includes pieces of soft fabric or shredded paper. Litter size ranges from 1 to 18 kits with an average litter of 8. The young weigh on average 8–10 g at birth and are altricial. Deciduous teeth erupt at 14 days; eyelids and external ear canals open

at 28–35 days of age; and permanent canines erupt between 47 and 52 days. Kits begin eating solid food at about 3 weeks of age. They are weaned at 6–8 weeks and reach their adult weight at about 4 months. Weaned ferrets should be group housed until sexually mature. Differences in size of males and females, due to sexual dimorphism, are apparent by week 7 and persist throughout life.

Depending on the time of year, the female will return to estrus about 2 weeks after the young are weaned or the following March. Jills should be either rebred or administered hCG to terminate estrus. Ferrets can produce two to three litters per year if maintained on a stimulatory photoperiod and provided adequate nutrition.

HUSBANDRY

Housing and Environment

Ferrets can be housed in a variety of caging suitable to other small mammals, such as cats or rabbits. Care must be taken to modify caging to ensure secure containment of these smaller animals, known to be great escape artists. Solid-bottom cages are more suitable for their small feet than are those with slotted or wire mesh flooring. If wire mesh must be used, it should be 0.25 inch, and a sanitizable, commercially available resting board or platform can be placed in the cage to help protect the feet.

Adequate space must be provided to permit the animal to move around freely; a floor area of 0.27 m^2 (3.0 ft^2) with a height of at least 36 cm (14 in.) is acceptable for an adult ferret. Hardwood chips or recycled paper products can be used in solid-bottom cages; a waste pan is employed under suspended cages with wire-grid floors. Ferrets seem to prefer a den or nest box with a small entry hole and will use soft fleece materials commercially produced for the laboratory animal environment. Large-diameter PVC elbows and huts may also be used to provide an area of seclusion or as enrichment. Ferrets tolerate low temperatures without difficulty. A temperature range of 4°–18°C (39°–64°F) is generally optimal, but the young should not be kept at temperatures below 15°C (59°F). A humidity range between 40% and 65% and at least 10–15 air changes per hour are recommended. Photoperiods should be controlled through timed lighting and provide a 12:12 cycle of light and dark, with 14 hours of light for breeding animals.

Environmental Enrichment and Social Housing

Social housing is optimal for compatible animals of the same sex. Commercially available enrichment devices for ferrets include shaded polycarbonate huts, hammocks (Figure 11.1), certified items for gnawing or carrying, and balls that may be manipulated or filled with treat foods designed for laboratory animal consumption. Due to their proclivity for gnawing and object ingestion, these items should be limited to those expressly designed for use with the ferret in a laboratory setting. Positive interaction with human handlers is also a viable form of enrichment. Release into a safe enclosure for periods of exercise is also a valuable addition to an enrichment program. Ferrets will readily use a shallow pool fashioned from common facility items (e.g., shoebox cages) for swimming activity; supervision should be provided.

Fig 11.1. Wild-type (fitch) variety ferret in Leisure Lounge hammock enrichment device. (Photo courtesy of Marshall BioResources.)

Feeding and Watering

Ferrets are obligate carnivores and require a diet high in protein and fat and low in fiber. Commercially prepared, extruded diets intended for all life cycles are produced for use with laboratory ferrets and provide a guaranteed analysis of at least 38% protein (usually derived from chicken), 20.5% fat, and not more than approximately 4% fiber. These diets are available in a certified (screened for common environmental contaminants) or an irradiated form. Due to their high energy requirements, a documented range of 200–300 kilocalories per kilogram of body weight is required daily. Feeding is usually *ad libitum* and provided via a J-type feeder; these may require modification to prevent escape. Adult animals will consume approximately 6% of their body weight in feed daily. Due to their short gastrointestinal tract transit time, fasting over 3 hours is generally contraindicated and may result in hypoglycemia. Ferrets consume about 75–100 mL of water per day. They should have fresh, potable water available *ad libitum* via water bottle or automatic watering system.

Sanitation

Ferrets habitually use one or two corners of the cage for defecation and urination, and they can learn to use a litter pan, which should be cleaned daily and sanitized 2 to 3 times weekly. If used, direct bedding should be replaced as often as is necessary to keep the animals clean and dry; primary enclosures should be sanitized at least every 2 weeks. Water bottles are sanitized at least weekly and feeders every 1–2 weeks. Cages, water bottles, and feed hoppers

should be disinfected with chemicals, hot water 61.6°–82.2°C (143°–180°F), or a combination of both. Detergents and chemical disinfectants enhance the effectiveness of hot water, but they must be thoroughly rinsed from all caging surfaces with fresh water. Refer to Chapter 4, "Mice," for a more detailed description of disinfection methods.

TECHNIQUES

Handling and Restraint

Frequent, gentle handling of ferrets will promote tractability. They can be picked up from behind with one hand placed under the chest and the other supporting their hindquarters (Figure 11.2). Ferrets may be "tucked" into the crook of one's elbow for noninvasive procedures such as obtaining a rectal temperature.

Most ferrets can be manually restrained using exam gloves; gentle restraint is usually adequate for physical examinations. For more invasive procedures, one method is to scruff the loose skin at the nape of the neck with one hand and suspend the body (Figure 11.3), which appears to relax and immobilize ferrets. This restraint method will often induce a yawning behavior, which is considered a normal response. Most simple procedures, such as nail clipping, ear cleaning, chest auscultation, intramuscular and subcutaneous injections, and abdominal palpation can be performed using this technique. Another method is the placement of a hand across the shoulders with the thumb under the chin, the forefinger around the neck, and the other fingers around the chest caudal to the forelimbs. The other

Fig 11.2. Restraint for carrying a ferret. (Photo courtesy of C. Medina.)

Fig 11.3. Restraint of the ferret by scruffing.

hand can be used to support the lower back. If a more secure hold is required, the other hand is placed around the pelvis cranial to the hind limbs. Ferrets should not be "stretched out" as doing so may cause them to vigorously resist. Ill animals or females with young may bite and should be handled with caution.

Identification

Cage cards may be used as a general means of identifying caged ferrets. Ear tags, ear punches, tattoos, or a subcutaneous microchip may be used for permanent identification of individual animals.

Blood Collection

Approximate blood volumes for ferrets are listed in Table 11.2. Assuming the animal is mature, healthy, and on an adequate plane of nutrition, the blood volume of most species averages 7% of the body weight in grams. A typical rule of thumb for blood withdrawal suggests that up to 10% of the circulating blood volume can be withdrawn every 2–3 weeks from normal, healthy animals with minimal adverse effect. Small amounts of blood, approximately 0.5 mL, can be obtained from the lateral saphenous or cephalic veins and can be accessed with a 25-gauge needle and tuberculin syringe. The superficial veins are not readily

Table 11.2. Adult ferret blood volumes

	Volume (mL)
Total blood	60–120
Single sample	6–12
Exsanguination	24–60

Source: Adapted from Hillyer and Brown (1994).
Note: Values are approximate.

discernible and, for best success, the hair should be clipped and a small elastic tourniquet used.

The superficial caudal tail artery on the ventral side of the tail can be used to obtain blood from a restrained or sedated ferret. The hair should be clipped and the vessel dilated with warm water. Using a 22- to 25-gauge needle attached to a 3 mL syringe, the needle is inserted at a 30° angle to the skin about 2–3 mm caudal to the anus. The needle is advanced until it reaches the vertebra and then slowly withdrawn while aspirating until blood flows; 1–3 mL of blood may be collected from this site.

The jugular vein is the most accessible site for collection of larger blood volumes; up to 10 mL may be obtained via this route. For jugular access, the ferret can be restrained in the standard cat restraint approach, with the forelimbs held over the end of a table and the chin restrained upward. Alternatively, the caudal approach can be used: with the animal in dorsal recumbency, grasp the scruff to extend the neck with one hand and use the other to restrain the forelimbs. Exceptionally tractable animals may tolerate a gentle "towel and scruff" restraint while distracted with a nutritional supplement paste (Ferretone, Nutrical). For fractious ferrets, light sedation may be necessary. Clipping the fur and occluding the jugular at the thoracic inlet optimizes visualization of the vein. The cranial vena cava is one more site that can be used to collect larger amounts of blood in a sedated or anesthetized ferret, with an assistant to facilitate restraint. The animal is held in dorsal recumbency, with the head extended and the forelegs directed caudally. The needle is inserted between the first rib and manubrium at a 45° angle to the body and directed toward the opposing rear limb, nearly to the hub. Aspirate as the needle is slowly withdrawn until blood enters and fills the syringe. This site carries a higher risk of hemorrhage and should be performed with caution.

Refinements in blood collection techniques include the use of a surgically placed vascular access port or indwelling catheter. Although these have not been used extensively with ferrets, the technology is available and results in less repeated stress to ferrets and handlers. As with any intravascular device, aseptic technique and maintenance of patency with an appropriate anticoagulant flush are important.

Cardiac puncture can be used to collect a large terminal blood sample in anesthetized ferrets. The needle should be directed at the point of maximal heart beat intensity by palpation; it can be inserted either intercostally through the lateral chest wall or just lateral to the xiphoid process and directed craniodorsally at an approximately 30° angle into the heart.

Blood transfusions may be necessary for ferrets suffering from anemia of chronic disease, estrogen toxicity, or blood loss due to trauma or severe ulceration. Transfusions are usually indicated when the packed cell volume (PCV) falls below 15% for acute blood loss, below

12% with chronic disease, or below 25% for estrogen-induced anemia. There is a low risk of transfusion reaction, and there is no need to cross-match blood donors and recipients because there are no detectable blood groups. Large male ferrets should be used as donors. Administer atropine IM, then anesthetize via face mask or induction chamber with isoflurane or sevoflurane. Approximately 10 mL of blood may be collected from an average male via the jugular vein or cranial vena cava. Blood should be collected into a syringe with an anticoagulant; 1 mL of CPDA-1 for 6 mL of blood works well. Fresh whole blood, 13–22 mL/kg, may be administered immediately at a rate of 0.25–0.5 mL/min.

Urine Collection

Urine may be collected using a metabolism cage, urinary catheter, cystocentesis, or by free catch. Urinary catheters can be placed in all hobs and most jills. A 3.5-French catheter is recommended, and sterile technique must be used. The urethral orifice of hobs is located on the ventral surface of the penis. Catheterization is easily accomplished but may require sedation or light anesthesia. To place a catheter in a jill, a vaginal speculum or otoscope is used to help visualize the urethral opening, which is about 1 cm cranial to the clitoral fossa.

The cystocentesis approach is similar to that used in cats, using ultrasound visualization, a 22-gauge needle, and 3 mL syringe. The needle is introduced at a 90° angle just cranial to the pubic bone. Excited ferrets will often void on the table and samples can be used for simple dipstick urinalysis but cannot be used for culture.

Nail Trimming

Ferret nails are quite sharp, grow continuously, and should be kept trimmed to reduce the likelihood of forearm scratches during restraint.

Drug Administration

Maximum dosing volumes are listed in Table 11.3. Oral dosing with palatable solutions is easy to accomplish by placing a syringe at the corner of the ferret's mouth and slowly

Table 11.3. Maximum dosing volumes in ferrets

Route	Amount (mL/kg)
IP	10.0
SC	20.0
IM	0.5/site
IV	2.0 (bolus)/10.0 (infusion)[a]
PO	40.0

Source: Adapted from Harkness and Wagner (1995) and Lukas (1999).

[a]Infusion volumes are extrapolated from the R.M. Hull article (Hull, 1995) on guideline limit volumes, which states that generally the volume administration on a single occasion will be less than 10% of the circulating blood volume.

depositing the liquid on the back of the tongue. As ferrets lack a cough reflex, caution is warranted in administration of compounds in a struggling animal. Unpalatable drugs can be given by an orogastric tube using a commercial bite block. The proper placement of the tube should always be verified by aspirating stomach contents or by placing the external end of the tube into a cup of water. If air is easily drawn into the syringe or bubbles are observed in the cup containing the submerged tube, it is likely in the trachea rather than the stomach. A dose of 50 mL can be given orally to an adult ferret without adverse effects. The maximum oral dose is 40 mL/kg.

Subcutaneous (SC) injections with a maximum volume of 20 mL/kg can be administered under the loose skin over the dorsum. Intramuscular (IM) injections with a maximum volume of 0.5 mL are given as in the dog or cat, in the semimembranosus or quadriceps thigh muscles. IM injections are easily administered when a ferret is scruffed or wrapped in a towel with a leg exposed. During the fall, allowance should be made for the thicker layer of subcutaneous fat, which may require a longer needle to deliver an injection. Placing a small amount of nutritional supplement paste (Nutrical or Ferretone) on their abdomen may provide a distraction for injections. Intraperitoneal (IP) injections with a maximum volume of 10 mL/kg can be administered just off of the midline in the lower abdominal quadrant. Care must be taken to aspirate before injecting the solution to ensure that the needle has not entered the bladder, bowel, or other organ. The cephalic or lateral saphenous veins can be used to deliver small volumes, 2 mL/kg, intravenously. Larger volumes with a maximum of 10 mL/kg as a slow infusion should be administered either through a temporary indwelling catheter into the cephalic or saphenous veins or through a surgically placed subcutaneous vascular access port with an indwelling catheter into the jugular vein.

Immunization

Only monovalent vaccines labeled for use in ferrets are recommended. Ferrets must be routinely protected against canine distemper by vaccination with the canarypox vectored recombinant vaccine, PUREVAX (Merial). The PUREVAX vaccine lacks adjuvants and complete distemper virus, reducing or eliminating postvaccination reactions, has a wide margin of safety, and is effective in prevention of the disease. Kits should be vaccinated starting at 8 weeks of age, with 2 additional boosters given at 3-week intervals until 14 weeks, then annually. Hypersensitivity reactions ranging from skin erythema and pruritus to vomiting/diarrhea to anaphylaxis have been reported. As a precaution, it is prudent to observe the ferret for at least 15–20 minutes after vaccination. Diphenhydramine at 0.5–2.0 mg/kg IV or IM can be given prior to vaccination to prevent a repeat reaction or in response to clinical symptoms. Epinephrine (20 µg/kg IV, IM, or SC) and a short-acting corticosteroid such as dexamethasone sodium phosphate at 1–2 mg/kg IV or IM may also be used.

A killed rabies vaccine, Imrab-3 (Merial), labeled for use in ferrets is available and should be administered at 3 months of age and annually thereafter. Vaccine reactions have also been observed but the incidence is markedly lower than with the canine distemper vaccine. Ferrets do not appear to be natural hosts for feline panleukopenia, canine parvovirus, canine parainfluenza virus, mink enteritis virus, transmissible mink encephalopathy, respiratory syncytial virus, or pseudorabies, although they have been experimentally infected with these agents.

Anesthesia, Surgery, and Postoperative Care

Agents commonly used for anesthesia and tranquilization in ferrets are listed in Table 11.4. Ferrets should not be fasted for longer than 3–6 hours due to rapid gastrointestinal tract transit time (3–4 hours) and physiology (Box 11.2). A number of injectable agents can provide adequate anesthesia for short procedures. Atropine at 0.05 mg/kg IM or SC or glycopyrrolate at 0.01 mg/kg IM or SC should be administered as a preanesthetic to reduce salivation and gastrointestinal secretions and to prevent bradycardia. The ketamine–xylazine combination has been associated with a significant number of premature ventricular contractions and death; the ketamine–acepromazine combination seems to work well. It is advisable to use inhalant anesthetics rather than injectable agents in ill or debilitated animals. For optimal care a small induction chamber can be used to anesthetize a clinically ill animal, quickly intubate it, administer oxygen, and place an IV catheter to provide fluid support during an anesthetic procedure.

Box 11.2

Ferrets should not be fasted for longer than 3–6 hours due to rapid gastrointestinal tract transit time and physiology.

Inhalants, including isoflurane and sevoflurane, are safe and commonly used in ferrets; they are preferred for most surgical procedures as they provide rapid induction and recovery. Ferrets can be placed in a small induction chamber or masked for anesthetic induction. Premedication with acepromazine or other suitable sedative allows for an easier induction if a mask is used. Animals should be intubated with a cuffed or uncuffed tube (2.0–3.5 mm internal diameter) for longer procedures. A degree of jaw tension is retained (and thus is not a good indicator of anesthetic depth); gauze may be used to retract the jaws to facilitate intubation. A topical anesthetic spray such as 2% lidocaine can be applied to help prevent laryngospasm. Due to their small body size, a nonrebreathing system should be used. Rate and depth of respiration, heart rate, and pedal and palpebral reflexes are used to monitor the depth of anesthesia.

Commercial breeders neuter and descent ferrets at 3–4 weeks of age. Surgical approaches used in cats can be used in ferrets. Scrotal incisions with an open or closed technique can be used for castration. When performing an ovariohysterectomy (OHE), note that the ovarian pedicles normally contain large amounts of fat. Neutering reduces aggression in males and diminishes the musky scent released from the sebaceous skin glands by the action of estrogen and testosterone. An OHE prevents aplastic anemia caused by a prolonged state of estrus in the female. As in all animals, use of aseptic technique, prevention of hypothermia, and attention to hydration status increase the number of successful postoperative outcomes.

Analgesics should be administered for all surgical procedures unless there is scientific justification to withhold them. Providing analgesics preoperatively is more efficacious in achieving pain relief in humans and other mammals. Acceptable analgesic agents for ferrets are listed in Table 11.5. Butorphanol is indicated to control mild postoperative discomfort. Buprenorphine works well to control acute or chronic visceral pain but causes more sedation. For musculoskeletal pain and inflammation, nonsteroidal anti-inflammatory drugs

Table 11.4. Anesthetic agents and tranquilizers used in ferrets

Drug	Dosage	Route	Reference
Inhalants			
Isoflurane	2%–5% to effect	Inhalation	Brown (1993)
Sevoflurane	To effect	Inhalation	Morrisey and Carpenter (2011)
Injectables			
Acepromazine	0.1–0.5 mg/kg	SC, IM	Morrisey and Carpenter (2011)
Diazepam	0.5–2 mg/kg	IM, IV	Morrisey and Carpenter (2011)
Fentanyl–droperidol	0.15 mL/kg	IM	Flecknell (1987)
Fentany/fluanisone	0.5 mL/kg	IM	Flecknell (2009)
Ketamine	30–60 mg/kg	IM	Fox (1998)
Ketamine	20–35 mg/kg	SC, IM	Hillyer and Brown (1994)
+ acetylpromazine	0.2–0.35 mg/kg	SC, IM	
Ketamine	25–35 mg/kg	IM	Brown (1993)
+ diazepam	2–3 mg/kg	IM	
Ketamine	4–8 mg/kg	IM	Flecknell (2009)
+ medetomidine	0.05–0.1 mg/kg	IM	
Ketamine	5 mg/kg	IM	Evans and Springsteen (1998)
+ medetomidine	0.08 mg/kg	IM	
+ butorphanol	1 mg/kg	IM	
Ketamine	5–10 mg/kg	IM, IV	Morrisey and Carpenter (2011)
+ midazolam	0.25–0.5 mg/kg	IM, IV	
Ketamine	10–25 mg/kg	IM	Hillyer and Brown (1994)
+ xylazine	1–2 mg/kg	IM	
Medetomidine	0.1–0.2 mg/kg	SC, IM	Morrisey and Carpenter (2011)
Medetomidine	0.08 mg/kg	IM	Ko and Heaton-Jones (1997)
+ butorphanol	0.1–0.2 mg/kg	IM	
Midazolam	0.25–0.5 mg/kg	SC, IM, IV	Morrisey and Carpenter (2011)
Pentobarbital	30–36 mg/kg	IP	Fox (1998)
Propofol	3–6 mg/kg	IV	Morrisey and Carpenter (2011)
Tiletamine–zolazepam	12–22 mg/kg	IM	Payton and Pick (1989)
Tiletamine–zolazepam	3 mg/kg	IM	Ko et al. (1996)
+ ketamine	2.4 mg/kg	IM	
+ xylazine	0.6 mg/kg	IM	
Xylazine	1 mg/kg	SC, IM	Fox (1998)

IM = intramuscular; IP = intraperitoneal; IV = intravenous; SC = subcutaneous.

(NSAIDs) such as aspirin, flunixin, and carprofen work well in ferrets, but all NSAIDs may cause gastrointestinal upset and should be used with caution. It is particularly important that postprocedural care for ferrets ensure a rapid return to food and water intake due to rapid gastrointestinal tract transit time and to prevent a hypoglycemic state. Warmed lactated Ringer's and the use of a circulating water heating blanket will ensure hydration and preservation of euthermia. A list of select critical care products that may be used in ferrets is available in Appendix 3.

Table 11.5. Analgesic agents used in ferrets

Drug	Dosage	Route	Reference
Acetylsalicylic acid	0.5–22 mg/kg q8–24h	PO	Hillyer and Brown (1994)
Buprenorphine	0.01–0.03 mg/kg q8–12h	SC, IM, IV	Flecknell (2009)
Butorphanol	0.1–0.5 mg/kg q4–6h	IM, SC, IV	Cantwell (2001)
Carprofen	1 mg/kg q12–24h	PO	Morrisey and Carpenter (2004)
	4 mg/kg	SC	Flecknell (2009)
Flunixin meglumine	0.5–2 mg/kg q12–24h	SC	Flecknell (2009)
Ibuprofen	1 mg/kg q12–24h	PO	Morrisey and Carpenter (2004)
Ketoprofen	1–3 mg/kg q12–24h	PO, SC, IM	Morrisey and Carpenter (2011)
Meperidine	5–10 mg/kg q2–4h	SC, IM, IV	Heard (1993)
Meloxicam	0.1–0.2 mg/kg	SC, PO	Flecknell (2009)
Morphine	0.5–2 mg/kg q6h	SC, IM	Flecknell (2009)
Nalbuphine	0.5–1.5 mg/kg q2–3h	IM, IV	Heard (1993)
Oxymorphone	0.05–0.2 mg/kg q8–12h	IM, IV, SC	Heard (1993)
Pentazocine	5–10 mg/kg q4h	IM	Heard (1993)
Tramadol	5 mg/kg q12–24h	PO	Morrisey and Carpenter (2011)

IM = intramuscular; IV = intravenous; PO = per os; SC = subcutaneous.

Imaging Techniques

Great advances have been made in rodent and small animal imaging over the last decade. Many imaging modalities once used only in humans [e.g., digital x-ray imaging, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), dual-energy x-ray absorptiometry (DEXA) scan, and ultrasound] have been adapted for use with these species, producing images of exquisitely fine detail. In addition, novel imaging techniques have been developed for use in animal research, including *in vivo* optical imaging. Some x-ray units (e.g., Faxitron animal imaging and irradiation systems) have been designed for use specifically with small animals. These units produce a highly magnified and clear resolution image of the field of interest.

An x-ray machine with high milliamperage, such as a 200- or 300-mA unit with the capability for low settings of peak voltage delivered (kVp) and small incremental changes, is ideal. High-detail film–screen combinations work well. Short exposure times of 1/40 second or less should be used to decrease the chance of motion artifact. Suggested exposure factors are 46–52 kVp, 300 mA, 1/40 second, 40 SID, 7.5 mA·s. Alternatively, nonscreen film can be used with standard x-ray or dental units. Nonscreen film produces high-detail radiographs, but longer exposure times are needed. A brief period of isoflurane- or sevoflurane-induced anesthesia will provide adequate chemical restraint and reduce the likelihood of motion artifact. The unanesthetized animal may be restrained using a plastic cylinder or stockinette.

Euthanasia

The most common and acceptable method of euthanasia for ferrets is the administration of an IV or IP overdose of a barbiturate or commercial euthanasia solution. Other acceptable

methods include an overdose of inhalant anesthetic or IV administration of a supersaturated solution of potassium chloride (1–2 mmol/kg) bolus in conjunction with full anesthesia to induce cardiac arrest. All euthanasia methods must be in compliance with the current *AVMA Guidelines on Euthanasia* (AVMA, 2013).

THERAPEUTIC AGENTS

Antimicrobial and antifungal agents and recommended dosages for ferrets are listed in Table 11.6. Antiparasitic agents for use in ferrets can be found in Table 11.7, and miscellaneous drugs and dosages for ferrets are listed in Table 11.8.

INTRODUCTION TO DISEASES OF FERRETS

Improvements in environmental quality as well as advanced understandings of both preventive medicine and husbandry programs for ferrets have resulted in healthier laboratory ferret colonies. Most ferrets today are relatively free of the variety of agents that cause clinical disease. However, a decreased incidence does not mean that infections will not occur, so it is imperative to be able to diagnose, treat, and control the spread of pathogenic agents if they are present. Presented here is a general overview of common bacterial, viral, fungal, and parasitic agents of ferrets and some standard diagnostic and treatment modalities.

Bacterial Diseases

Proliferative Bowel Disease

Lawsonia intracellularis is an intracellular campylobacter-like organism that causes proliferative bowel disease (PBD) in numerous species, including ferrets, rabbits, hamsters, and pigs. Transmission is likely fecal–oral. The disease in ferrets, primarily seen in the young, is characterized by protracted intermittent diarrhea of greater than 6 weeks' duration, severe weight loss, rectal prolapse, and dehydration. Diarrhea is often green in color and may contain mucus or blood. In severe cases, ataxia and muscle tremors may occur.

Diagnosis is based upon clinical signs, a palpably thickened colon, and colonic biopsy with identification of the intracellular organisms via a silver stain. Mucosal thickening and glandular epithelial hyperplasia of the colon are characteristic histologic findings in ferrets, in contrast to the hamster and pig, in which it is primarily in the ileum.

Several reports indicate that ferrets respond well to treatment with chloramphenicol at 50 mg/kg twice daily or metronidazole at 20 mg/kg twice daily for 2 weeks. Ferrets must also receive supportive care, including parenteral fluids and nutritional support for the best outcome. Severe cases may occasionally result in death despite supportive care.

Helicobacter spp. Infection

Ferrets infected with *Helicobacter mustelae* are prone to develop gastritis and gastroduodenal ulcers and develop a disease condition similar to that seen in humans infected with *H. pylori*. Studies have shown that nearly 100% of ferrets are infected with *H. mustelae* shortly

Table 11.6. Antimicrobial and antifungal agents used in ferrets

Drug	Dosage	Route	Reference
Amikacin	10–15 mg/kg q12h	SC, IM	Brown (1993)
Amoxicillin	20 mg/kg q12h	PO, SC	Hillyer and Brown (1994)
Amoxicillin	10 mg/kg q12h	PO	Hoefer and Bell (2004)
+ metronidazole	20 mg/kg q12h	PO	
+ bismuth subsalicylate	1 mL/kg q12h for 14 d	PO	
Amoxicillin–clavulanate	13–25 mg/kg q8–12h	PO	Hillyer and Brown (1994)
Amphotericin B	0.4–0.8 mg/kg q7d (total dose 7–25 mg)	IV	Besch-Williford (1987)
Ampicillin	5–30 mg/kg q8–12h	SC, IM, IV	Morrisey and Carpenter (2004)
Cefadroxil	15–20 mg/kg q12h	PO	Brown (1995)
Cephalexin	15–30 mg/kg q8h	PO	Hillyer and Brown (1994)
Cephaloridine	10–15 mg/kg q24h for 5–7 d	SC, IM	Brown (1993)
Clarithromycin	12.5–25 mg/kg q12h	PO	Morrisey and Carpenter (2011)
Clarithromycin	12.5 mg/kg q8h	PO	Marini et al. (1999)
+ ranitidine	24 mg/kg q8h for 14 d	PO	
Chloramphenicol	50 mg/kg q12h	PO, SC, IM, IV	Hillyer and Brown (1994)
Ciprofloxacin	10 mg/kg q12h	PO	Brown (1995)
Clindamycin	5.5–10 mg/kg q12h	PO	Brown (1995)
Cloxacillin	10 mg/kg q6h	PO, IV, IM	Johnson-Delaney (1996)
Enrofloxacin	5–10 mg/kg q12h	PO, SC, IM	Brown (1995)
Erythromycin	10 mg/kg q6h	PO	Besch-Williford (1987)
Gentamicin	2–4 mg/kg q12h	SC, IM	Brown (1999)
Griseofulvin	25 mg/kg q24h	PO	Hillyer and Brown (1994)
Ketaconazole	10–30 mg/kg q8h	PO	Besch-Williford (1987)
Lincomycin	11 mg/kg q8h	PO	Besch-Williford (1987)
Metronidazole	50 mg/kg q24h	PO	Collins (1995)
Neomycin	10–20 mg/kg q6h	PO	Besch-Williford (1987)
Oxytetracycline	20 mg/kg q8h	PO	Besch-Williford (1987)
Penicillin, procaine	40,000–44,000 IU/kg q24h	SC	Morrisey and Carpenter (2004)
Sulfadimethoxine	30–50 mg/kg q12–24h	PO	Collins (1995)
Sulfamethazine	1 mg/mL drinking water		Collins (1995)
Tetracycline	25 mg/kg q12h	PO	Morrisey and Carpenter (2011)
Trimethoprim–sulfa	15–30 mg/kg q12h	PO, SC	Hillyer and Brown (1994)
Tylosin	10 mg/kg q8–12h	PO	Collins (1995)
	5–10 mg/kg q12h	IM, IV	Collins (1995)

IM = intramuscular; IV = intravenous; PO = per os; SC = subcutaneous.

Table 11.7. Antiparasitic agents used in ferrets

Drug	Dosage	Route	Reference
Amitraz	0.3% solution q7–14d for 3–6 treatments	Topical	Morrisey and Carpenter (2004)
Amprolium	19 mg/kg q24h	PO	Brown (1999)
Carbaryl (5%)	Once weekly for 3–6 wk	Topical	Fox (1998)
Fenbendazole	20 mg/kg q24h for 5 d	PO	Morrisey and Carpenter (2004)
Fipronil	1 pump of spray or 1/5 of cat tube q60d	Topical	Morrisey and Carpenter (2011)
	0.2–0.4 mL q30d		Williams (2000)
Imidacloprid	1 cat dose q30d	Topical	Morrisey and Carpenter (2011)
Ivermectin	0.4 mg/kg, repeat in 2–4 wk	PO, SC	Hillyer and Brown (1994)
	0.05 mg/kg/mo	PO	Hillyer and Brown (1994)
	0.5 mg/kg, half dose in each ear, repeat in 2 wk	Topical	Hillyer and Brown (1994)
Lime sulfur dip 2.5%	Dilute 1:40 with water, dip q7d for 6 wk	Topical	Morrisey and Carpenter (2011)
Lufenuron	30–45 mg/kg q30d	PO	Morrisey and Carpenter (2011)
Melarsomine dihydrochloride	2.5 mg/kg once, repeat in 30 days with 2 treatments 24 h apart	IM	Brown (1999)
Metronidazole	15–20 mg/kg q12h for 2 wk	PO	Brown (1993)
Milbemycin oxime	1.15–2.3 mg/kg/mo	PO	Johnson-Delaney (1996)
Piperazine citrate	50–100 mg/kg repeat in 14 d	PO	Morrisey and Carpenter (2011)
Praziquantel	5–10 mg/kg, repeat in 10–14 d	SC, PO	Morrisey and Carpenter (2011)
Pyrantel pamoate	4.4 mg/kg repeat in 2 wk	PO	Brown (1993)
Pyrethrin powder	Once weekly for 3 wk	Topical	Fox (1998)
Selmectin	6 mg/kg	Topical	Morrisey and Carpenter (2004)
Sulfadimethoxine	30–50 mg/kg q12–24h	PO	Collins (1995)

PO = per os; SC = subcutaneous.

after weaning. Fecal–oral transmission is likely, as Koch's postulates have been fulfilled by oral inoculation of naive ferrets. The organism can infect young ferrets as early as 5–6 weeks of age and is highly prevalent in the adult population. Most infected ferrets are asymptomatic. Vomiting, chronic weight loss, low hematocrit, and melena (black, tarry stools) are associated with gastric or duodenal ulcers. Gastric adenocarcinoma may occur secondary to infection and present with the clinical signs of vomiting, anorexia, and weight loss.

Diagnosis is challenging but can be accomplished by mucosal biopsy and endoscopy. *H. mustelae* can be cultured in a microaerobic atmosphere to allow identification of the organism by Gram-stain and morphology, as well as resistance or sensitivity to antibiotics. There are a few treatment regimens that require multidrug, multiday dosing including a 7–10 day regimen of triple therapy: oral amoxicillin (30 mg/kg) and metronidazole (20 mg/kg) combined with bismuth-subsalicylate (17.5 mg/kg) three times a day for 3–4 weeks. Cimetadine

Table 11.8. Miscellaneous agents used in ferrets

Drug	Dosage	Route	Reference
Activated charcoal	1–3 g/kg	PO	Richardson and Balabuszko (2001)
Aminophylline	4.4–6.6 mg/kg q12h	PO, IM	Hillyer and Brown (1994)
Anastrazole	0.1 mg/kg q24h	PO	Weiss et al. (1999)
Apomorphine	5 mg/kg	SC	Besch-Williford (1987)
Atenolol	6.25 mg per animal q24h	PO	Brown (1995)
Atipamezole	1 mg/kg	SC, IP, IV	Flecknell (1997)
Atropine	0.04 mg/kg	SC, IM, IV	Hillyer and Brown (1994)
	5–10 mg/kg (organophosphate toxicity)	SC, IM	Brown (1995)
Azathiprine	0.9 mg/kg q24–72h	PO	Burgess and Garner (2002)
Barium (20%)	15 mL/kg	PO	Hillyer and Brown (1994)
Bicalutamide	5 mg/kg q24h	PO	Weiss and Scott (1997)
Bismuth-subsalicylate (Pepto-Bismol)	0.25 mL/kg q4–6h	PO	Hillyer and Brown (1994)
Calcium EDTA	20–30 mg/kg q12h	SC	Morrisey and Carpenter (2004)
Captopril	1/8 of 12.5 mg tablet per animal q48h, increase to q12–24h	PO	Hillyer and Brown (1994)
Chlorpheniramine	1–2 mg/kg q8–12h	PO	Hillyer and Brown (1994)
Cimetidine	10 mg/kg q8h	PO, IV	Hillyer and Brown (1994)
Cisapride	0.5 mg/kg q8–12h, increase to 1 mg/kg if needed	PO	Morrisey and Carpenter (2011)
Deoxycorticosterone pivalate	2 mg/kg q21d	IM	Goett and Degner (2003)
Dexamethasone	0.5–2 mg/kg	SC, IM, IV	Brown (1995)
Diazoxide	10 mg/kg/d or divided q8–12h	PO	Hillyer and Brown (1994)
Digoxin	0.005–0.01 mg/kg q12–24h	PO	Brown (1995)
Diltiazem	3.75–7.5 mg per animal q12h	PO	Brown (1995)
Diphenhydramine	0.5–2 mg/kg q8–12h	PO, IM, IV	Brown (1995)
Doxapram	5–10 mg/kg	IV, IM, IP	Flecknell (2009)
Enalapril	0.25–0.5 mg/kg q24–48h	PO	Brown (1995)
Epinephrine	0.02 mg/kg	SC, IM, IV, IT	Morrisey and Carpenter (2011)
Epoetin alfa	50–150 IU/kg	PO, IM	Brown (1995)
Famotidine (Pepcid)	0.25–0.5 mg/kg q24h	SC, PO, IV	Brown (1999)
Flutamide	10 mg/kg q12–24h	PO	Brown (1999)
Furosemide	2 mg/kg q8–12h	PO, SC, IM, IV	Hillyer and Brown (1994)
Glycopyrrolate	0.01 mg/kg	IM	Heard (1993)
Gonadotropin-releasing hormone	20 μg/animal, repeat in 2 wk prn	SC, IM	Hillyer and Brown (1994)
Hairball laxative, feline	1–2 mL/animal q48h	PO	Brown (1993)
Heparin	200 IU/kg q12h for 5 d	SC, IM	Hillyer and Brown (1994)
Human chorionic gonadotropin	100 IU per animal, repeat in 1–2 wk prn	IM	Hillyer and Brown (1994)

(*Continued*)

Table 11.8. (*Continued*)

Drug	Dosage	Route	Reference
Hydrocortisone sodium succinate	25–40 mg/kg	IV	Besch-Williford (1987)
Hydroxyzine	2 mg/kg q8h	PO	Morrisey and Carpenter (2004)
Insulin, NPH	0.5–6 IU/kg or to effect	SC	Besch-Williford (1987)
Iron dextran	10 mg/kg once	IM	Morrisey and Carpenter (2011)
Kaolin–pectin	1–2 mL/kg q2–6h prn	PO	Johnson-Delaney (1996)
Lactulose syrup	0.15–0.75 mg/kg q12h	PO	Brown (1999)
Leuprolide acetate	0.1 mg/animal <1 kg q4–6 wk	IM	Johnson-Delaney (1999)
	0.2 mg/animal >1 kg q4–6 wk	IM	Johnson-Delaney (1999)
Loperamide	0.2 mg/kg q12h	PO	Brown (1999)
Megestrol acetate	Do not use, predisposes ferret to pyometra		Johnson-Delany (1996)
Melatonin	0.5–1 mg/animal q24h prn	PO	Paul-Murphy (2001)
Metaclopramide	0.2–1 mg/kg q6–8h	PO, SC, IM	Morrisey and Carpenter (2011)
Mitotane	50 mg per animal q24h for 7 d, then q72h	PO	Hillyer and Brown (1994)
Naloxone	0.04–1 mg/kg	IM, SC, IV	Dobromylskyj et al. (2000)
Nitroglycerin 2% ointment	1/16–1/8 inch per animal q12–24h		Brown (1993)
Omeprazole	0.7 mg/kg q24h	PO	Fox (1998)
Oxyglobin	6–15 mL/kg over 4h	IV	Orcutt (2001)
Oxytocin	0.2–3 IU/kg	SC, IM	Brown (1993)
Pentobarbital elixir	1–2 mg/kg q12h	PO	Brown (1995)
Phenobarbital	1–2 mg/kg q8–12h	PO	Lewington (2000)
Prazosin	0.05–0.1 mg/kg q8h	PO	Pollock (2004)
Prednisone	0.6 mg/kg q24h, gradually taper dose	PO	Besch-Williford (1987)
Prednisolone sodium succinate	22 mg/kg	IV	Hillyer and Brown (1994)
Propanolol	0.2–1 mg/kg q8–12h	PO	Hillyer and Brown (1994)
Prostaglandin	0.1–0.5 mg per animal	IM	Brown (1999)
Ranitidine bismuth	24 mg/kg q8h	PO	Burke (1988)
Ranitidine HCl	3.5 mg/kg q12h	PO	Burke (1988)
Stanozolol	0.5 mg/kg q12h	PO, SC	Brown (1993)
Sucralfate	1/8 of 1 g tablet per animal q6h	PO	Hillyer and Brown (1994)
Theophylline elixir	4.25 mg/kg q8–12h	PO	Johnson-Delaney (1996)
Thyroxin	0.2–0.4 mg/kg q12–24h	PO	Johnson-Delaney (1996)
Vitamin B complex	1–2 mg/kg prn	SC, IM	Morrisey and Carpenter (2011)
Yohimbine	0.2–1 mg/kg	IM, IV	Morrisey and Carpenter (2011)

IM = intramuscular; IV = intravenous; IP = intraperitoneal; PO = per os; SC = subcutaneous.

and sucralfate may also be administered to increase stomach pH and coat ulcers, respectively. Sucralfate requires an acidic pH to function properly and therefore must be administered at least 2 hours prior to cimetidine administration. Alternatively, ranitidine bismuth (24 mg/kg) and clarithromycin (12.5 mg/kg) dosed orally three times per day for 2 weeks has resulted in eradication of the organism. Another reportedly efficacious regimen includes oral clarithromycin (50 mg/kg) once daily and oral amoxicillin (35 mg/kg) once daily for 2 weeks. In addition, both omeprazole (0.7 mg/kg, orally, once daily) and cimetidine (10 mg/kg, orally, three times per day) can be given to ferrets to reduce acid secretions. Ferrets proven free of *H. mustelae* infections are available commercially.

Campylobacteriosis

Campylobacter jejuni is a Gram-negative, spiral bacterium associated with enteritis and diarrhea in humans and many different animals, including dogs, cats, pigs, cows, goats, sheep, and ferrets. The organism, however, has also been isolated from asymptomatic ferrets. *C. jejuni* has been identified in zoonotic disease outbreaks in people recently in contact with young puppies or kittens that had diarrhea. Transmission is fecal–oral through direct contact or by ingestion of contaminated food and water. Experimental oral inoculation of the organism to young ferrets resulted in a self-limiting, mild to watery, mucinous diarrhea, with anorexia, dehydration, and/or tenesmus developing in some animals. Experimental inoculation of pregnant females has resulted in reproductive failure. Diagnosis depends on clinical signs and culture of affected animals. *C. jejuni* has special culture requirements, including thermophilic and microaerophilic conditions. Ferrets with known *C. jejuni* infection should be isolated to control spread, provided supportive care, and given antibiotics based on culture and sensitivity.

Salmonellosis

Salmonella spp. are Gram-negative rod-shaped bacteria associated with bloody diarrhea in humans and animals. Transmission is fecal–oral through direct contact or by ingestion of contaminated feed or water. Clinical cases have been rare, but several serotypes have been isolated from the feces of ferrets in a research colony. Typical clinical signs include melena anorexia, dehydration, and weight loss; abortion may be induced in pregnant females. Diagnosis is based on clinical signs and culture of the organism. Treatment includes antibiotic and fluid therapy, nutritional support, and reduction of stress. Due to the public health significance of this zoonotic organism, infected animals must be handled with caution and may be culled in lieu of treatment.

Tuberculosis

Ferrets are highly susceptible to certain avian, bovine, and human strains of the Gram-positive, acid-fast *Mycobacterium* spp. Tuberculosis in ferrets in U.S. research colonies is highly unlikely today due to its relatively low incidence in humans and agricultural animals. Most reports of this disease occurred in the early 1900s and were probably associated with the feeding of infected raw meat. The organism can be transmitted by inhalation, ingestion, or wound infection. The body system affected (e.g., gastrointestinal, respiratory, lymphatic) is heavily influenced by the infecting *Mycobacterium* spp. Tuberculosis should be suspected in cases of lymphomegaly, especially mesenteric. Definitive diagnosis requires isolation and

identification of the organism. A subcutaneous tuberculin test regimen has not been defined for ferret use. The current status of tuberculosis as a reemerging disease in humans may lead to an increased incidence of the disease in ferrets. Infected ferrets should be euthanized due to the zoonotic potential.

Mastitis

Staphylococcus aureus, *Streptococcus* spp., and hemolytic *Escherichia coli* are frequently associated with mastitis in nursing jills. Transmission is most likely associated with a contaminated environment, trauma from nursing kits, and stress of lactation. The acute form occurs within the first few weeks following parturition. Clinical signs include swollen, darkened, painful glands that may become gangrenous and proceed to septicemia and death. The chronic form develops as a sequela to the acute form or approximately 3 weeks after parturition and is characterized by firm mammary glands. Initial treatment includes the administration of analgesics as well as a broad-spectrum antibiotic (e.g., enrofloxacin, 2.5 mg/kg orally twice a day) that may then be refined based on culture and antibiotic sensitivity testing of the milk. Surgical debridement of the affected glands may be necessary for gangrenous lesions. Kits may need to be supplemented with milk replacer or cross-fostered because jills are often too painful to nurse. Antibiotic treatment of the kits is also warranted.

Pneumonia

Ferret pneumonia has been associated with a primary or secondary infection of multiple bacterial organisms, including *Streptococcus zooepidemicus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Noninfectious primary causes of pneumonia include megaesophagus or the accidental dosing of substances into the lungs during oral gavage. Clinical signs may include nasal discharge, dyspnea, increased lung sounds, anorexia, and pyrexia. Diagnosis is made by a combination of clinical signs, hematology, radiographs, and culture of tracheal wash. Treatment of this potentially fatal disease includes appropriate antibiotics, oxygen, fluids, and nutritional support.

Miscellaneous Bacterial Infections

Vaginitis is common in the female during estrus, especially when housed on hay or straw as the bedding may stick to the swollen vulva or enter the vagina and act as a nidus for a secondary bacterial infection. *Staphylococcus* spp. and *Streptococcus* spp. have been cultured from bite wound abscesses resulting from fighting or mating. Treatment of localized infections includes the administration of antibiotics (based on culture and sensitivity assays) and may require lancing.

Mycotic Diseases

Dermatophytosis (Ringworm)

Dermatophytosis caused by both *Microsporum canis* and *Trichophyton mentagrophytes* has been reported in ferrets, with cases involving *M. canis* predominating. Both zoonotic and interspecies disease transmission can occur. In ferrets, this fungal infection primarily affects young or older, immunosuppressed animals. Infection is influenced by geographic location and/or climate as cases are more frequently reported in the southern United States. Trans-

mission is by direct contact or via fomites, such as infected bedding. Ferrets housed in overcrowded or unsanitary conditions are predisposed to developing the disease. Clinically, ferrets may exhibit the typical ringlike lesions of alopecia and inflammation on any part of their body. Crusting and erythema may be evident, and pruritus may lead to scratching and secondary bacterial infections.

A tentative diagnosis may be based on typical clinical signs, but a fungal culture of the skin or hair or examination of a skin scraping partially digested with 10% KOH is needed to confirm the diagnosis. Use of a Wood's lamp is of limited diagnostic value as less than 50% of *M. canis* isolates and no *T. mentagrophytes* isolates fluoresce under UV light. Treatment consists of shaving the hair around the lesions, cleaning the affected animal with keratolytic baths and povidone iodine scrubs, and then applying topical antifungal ointments. Griseofulvin may also be administered per os at 25 mg/kg/day for 21–30 days, but this treatment is rarely needed. Environmental decontamination is important.

Viral Diseases

Canine Distemper

Distemper is a significant disease of ferrets due to their high susceptibility to canine distemper virus and an approximately 100% case mortality rate (Box 11.3). Transmission of the paramyxovirus is usually by aerosols or fomites, with an average incubation period of 7–9 days. Two disease phases may develop: a catarrhal phase and a neurological phase. In the catarrhal phase, infected ferrets initially develop anorexia, a serous-to-mucopurulent nasal and ocular discharge, and a rash beneath the chin and in the inguinal region. Edematous, hyperkeratotic footpads and anal prolapse may later develop. Ferrets that survive the catarrhal phase may die in a neurotropic episode with hyperexcitability, hypersalivation, muscular tremors, convulsions, and coma. Treatment is usually unsuccessful. Death commonly occurs within 2–5 weeks of disease exposure. Euthanasia is frequently elected over symptomatic supportive therapy. A suggestive diagnosis is based on clinical observations, known or suspected viral exposure, and a history of no prior vaccination. Confirmatory diagnosis is based on the observation of eosinophilic, intracytoplasmic, and intranuclear inclusions of tracheal, bronchial, epithelial, and bile duct tissues with Pollack's trichrome stain. Young ferrets should be routinely vaccinated for the disease at a frequency recommended by the vaccine manufacturer. Animal caretakers who have young puppies or work with dogs not vaccinated against canine distemper virus should not be assigned to care for ferrets.

Box 11.3

Distemper is a significant disease of ferrets due to their high susceptibility to the disease and an approximately 100% case mortality rate.

Human Influenza

Ferrets are susceptible to several strains of human influenza virus, an orthomyxovirus. Influenza viruses are transmitted by aerosols, are highly infectious, and may cause initial

signs similar to those of distemper. Within 48 hours of exposure, ferrets become listless and anorexic and develop a sharp rise in rectal temperature. Sneezing attacks occur and may be accompanied by a mucoserous nasal discharge. Ferrets may also develop conjunctivitis, photosensitivity, and otitis. Uncomplicated clinical disease usually largely resolves in approximately 4–5 days although disease complicated by a secondary bacterial pneumonia or in an immunocompromised animal may persist for multiple weeks. Recovery by day 4 differentiates uncomplicated influenza infection from the early stages of distemper. Congestion may be relieved by use of antihistamines. Cough suppressants may also be helpful for symptomatic relief. Influenza viruses may be freely transmitted between humans and ferrets. Animal caretakers with upper respiratory infections should wear respiratory protection and gloves when handling ferrets, and exercise extreme caution with neonatal kits, which are especially susceptible to the disease.

Aleutian Disease

Ferrets are susceptible to Aleutian disease virus (ADV), the parvovirus that causes Aleutian disease (AD) in mink. In contrast to the disease in mink, AD usually progresses slowly in ferrets. Infection may be subclinical for years; infected animals may serve as a source of infection for other ferrets during this time. ADV induces an immune-mediated disease in which a robust humoral immune response is stimulated, generating an overwhelming number of antigen–antibody complexes that then deposit in tissues, resulting in systemic vasculitis. Transmission is by direct contact, fomites, or via aerosol of saliva, urine, blood, or feces. Clinical signs may include a slow wasting of body condition, melena, splenomegaly, episodic fevers, posterior paresis, tremors, and eventually death. A presumptive diagnosis is made based on clinical signs and the finding of a characteristic hypergammaglobulinemia. Diagnosis is confirmed by polymerase chain reaction (PCR), immunofluoresence assay (IFA), and histologic findings, although an increase in gammaglobulins greater than 20% of total proteins is often considered diagnostic. Typical histopathologic lesions include lymphocytic and plasmacytic infiltrates of the kidney, liver, spleen, and meninges. There is no treatment or vaccine to prevent AD. Ferrets with clinical disease should be culled and their environment thoroughly disinfected.

Rabies

Ferrets are susceptible to infections with rabies virus, a rhabdovirus. There is an exceptionally low risk of rabies infection in laboratory ferrets unless they are allowed to roam freely outdoors in a rabies endemic area. There are a few reports of rabies in pet ferrets in the United States. Central nervous signs predominate with anxiety, lethargy, and posterior paresis. If vaccination is indicated based on the risk of rabies exposure and applicable regional and state regulations, ferrets should be administered a USDA-approved killed rabies vaccination at 3 and 12 months of age and then annually. There is no treatment for rabies. Ferrets that bite humans may be subject to euthanasia and a rabies screen depending on the state regulations.

Epizootic Catarrhal Enteritis

Epizootic catarrhal enteritis (ECE) is a coronaviral disease with up to 100% morbidity but low mortality. Transmission is likely fecal–oral. Ferrets become dehydrated secondary to

fluid lost through vomiting and diarrhea. Most deaths occur in older animals with concurrent illnesses. Clinical signs include projectile vomiting, diarrhea, dehydration, and depression. The stool changes through the course of the infection. Initially it may appear bright green and slimy because of abundant mucus, or it may skip that phase and appear as a yellow–gold, bubbly diarrhea. During the recovery phase, stools look like golden jelly filled with "bird seeds," an indication of intestinal malabsorption. In addition, teeth grinding, indicative of gastrointestinal ulcers and/or pain, may be observed.

Diagnosis is based on the typical clinical signs and histologic findings. Some ferrets may have elevated liver enzymes. Histologically, intestinal villar tip necrosis with moderate-to-marked crypt hyperplasia is observed. Treatment is primarily supportive, including aggressive fluid therapy to combat the dehydration and feeding a soft, palatable food to sustain a positive energy balance. One milliliter of sucralfate liquid (or other gastrointestinal protectant) given one hour prior to feeding has been found to encourage eating in ferrets that may be suffering from gastrointestinal ulcers.

Ferret Systemic Coronavirus

Ferret systemic coronavirus (FRSCV) is a newly recognized fatal disease of ferrets that closely resembles the dry form of feline infectious peritonitis and is closely related to the ferret epizootic catarrhal enteritis virus. Clinical signs are often nonspecific and include lethargy, weight loss, anorexia, diarrhea, vomiting, and neurologic signs (e.g., ataxia, tremors, head tilt, and seizures). However, palpable abdominal masses are frequently reported that may involve the mesentery, mesenteric lymph nodes, and abdominal viscera. Pyrexia is occasionally observed. Transmission is suspected to be fecal–oral with disease quickly spreading between grouped animals. As such, environmental decontamination is important in preventing disease spread. Diagnosis is by histology (characteristic granulomatous inflammation), immunohistochemistry (to identify the specific coronavirus antigen), and PCR. Prognosis is poor and currently no highly effective treatment has been identified. Current treatment efforts are centered on the provision of supportive care while multiple drugs are being evaluated for their effectiveness in suppressing or controlling the body's immune system response to the virus.

Miscellaneous Viral Infections

Rotaviruses may cause diarrhea in 1- to 4-day-old kits. Secondary bacterial infections may cause more severe diarrhea. Mortality is reduced if kits continue nursing and are given appropriate supportive care.

Parasitic Diseases

Sarcoptic Mange

Sarcoptes scabiei induces a generalized or pedal mange. In the generalized condition, focal or widespread alopecia with intense pruritus develops; in pedal mange, the feet may become hyperemic, edematous, variably pruritic, and ulcerated from irritation and chewing. Although rare in the research setting, the organism can be transmitted by direct contact or contact with contaminated fomites. Diagnosis is by observation of the organism in skin scrapings or crusts partially digested in 10% KOH. Treatment consists of subcutaneous ivermectin (0.2–0.4 mg/kg) administered every 2 weeks until the mite is no longer detectable. Soaking

or shampooing the feet in warm water may help relieve foot pruritus. Alternative therapies include 2% lime sulfur dips provided weekly until clinical signs have abated for at least 2 weeks. Antibiotics may be indicated if a secondary bacterial infection develops. All animals showing clinical signs or in contact with an affected animal should be treated and the environment decontaminated. The mite may transiently infect humans.

Demodectic Mange

Demodex spp. mites have been reported rarely in ferrets. Clinical signs include pruritus, alopecia, and discoloration of the skin. Diagnosis is by deep skin scraping. Treatment with amitraz dips, three times at weekly intervals, successfully eradicates the infestation.

Fleas

Ctenocephalides spp., especially the cat or dog flea, are common in pet ferrets but are rarely found in research colonies. If present, topical preparations used for elimination of fleas in cats (e.g., imidacloprid, fipronil, or selamectin) may be used for the effective and safe treatment of ferrets.

Ear Mites

Otodectes cyanotis infection is a common problem in ferrets that causes an accumulation of dark, waxy ear exudate, and inflammation of the ear canal. Mites can be acquired from, or transmitted to, dogs, cats, and other ferrets. The life cycle, from egg to mature mite, is about 3 weeks. Diagnosis is made by direct microscopic observation of the adult mites or their eggs in ear exudate. Mite eggs are resistant to treatment; thus one treatment kills only the mature mites and multiple weekly treatments are required to eliminate infection. Ears should be cleaned of exudate prior to treatment. Topical and subcutaneous treatments of ivermectin have been used successfully, but topical treatment appears to be more effective. For topical treatment, 1% ivermectin diluted 1:10 in propylene glycol at a dosage of 400 µg/kg, divided between both ears and repeated in two weeks, is most effective.

Heartworms

Ferrets are susceptible to natural and experimental infection with *Dirofilaria immitis*. Heartworm disease is unlikely to be found in a laboratory setting but is becoming more prevalent in pet ferrets, especially in the southern United States. Microfilaremia is characteristically of low concentration and transient in nature, similar to that seen in heartworm-infected cats. Ferrets seemingly have little ability to tolerate the presence of adult parasites in the heart without lethal consequences. Clinical signs of heartworm infection in ferrets may include lethargy, anorexia, dyspnea, pale mucous membranes, and ascites. A definitive diagnosis can be made with a combination of assays that detect heartworm antigen or antibodies, and echocardiography.

Coccidiosis

The most common parasite identified in ferrets is coccidia (*Eimeria* spp. or *Isospora* spp.). While most infections are subclinical, young or stressed animals as well as intensively managed colonies are prone to develop clinical disease to include diarrhea, lethargy, dehydration, rectal prolapse, and death. Disease is diagnosed through high-magnification fecal exam-

ination for detection of coccidial oocysts; however, fecal shedding is often intermittent and may be difficult to identify. Treatment with sulfadimethoxine at 50 mg/kg orally once and then 25 mg/kg daily for 9 days may be effective although altered doses and treatment duration may be required. Environmental cleaning and good husbandry practices should lower the oocyst burden and reduce the likelihood of spread through the ingestion of sporulated oocysts.

Cryptosporidiosis

Cryptosporidium spp. are protozoans that can infect a wide range of species, including ferrets, cattle, dogs, cats, and humans. To date, both immunocompetent and immunodeficient ferrets diagnosed with *Cryptosporidium* infection have failed to develop clinical signs. Transmission is by ingestion of sporulated oocysts in the feed, water, or environment. Autoinfection can also occur. Diagnosis is through observation of the small, spherical oocysts in a stained fecal preparation. There is no known treatment. *Cryptosporidium* spp. pose a zoonotic risk; thus children and immunosuppressed individuals should be restricted from access to infected ferrets. Good hygiene after handling any animal should also help to reduce the potential for human infection.

Miscellaneous Helminth Intestinal Parasites

Many types of intestinal parasites have been reported, including *Toxascaris leonina*, *Toxocara cati*, *Ancylostoma* spp., *Dipylidium caninum*, *Mesocestoides* spp., and *Filaroides* spp. Identification of specific parasites is established by fecal examination. Treatments recommended for elimination of intestinal parasites are generally the same as for cats and dogs and include ivermectin and praziquantel.

Neoplasia

Pancreatic Beta Cell Tumors (Insulinomas)

Pancreatic beta cell tumors, also called insulinomas, are the most commonly reported neoplasm of ferrets. Four- to five-year-old ferrets are most commonly affected, although disease has been reported in 2-year-old ferrets. The cancerous cells produce high levels of insulin that drive glucose into the cells, resulting in a severe hypoglycemia, below the normal fasting blood glucose of 90–125 mg/dL. Clinical signs are secondary to hypoglycemia and include intermittent and progressive episodes of weakness, posterior paresis, ataxia, stupor or "star gazing" (short trancelike state), head bobbing, lethargy, weight loss, and vomiting. Ptalism (drooling) and pawing at the mouth may be observed as these are signs of nausea in the ferret. In severe cases, ferrets may develop seizures, become comatose, and die.

Diagnosis is based on clinical signs and blood glucose and blood insulin levels. Ferrets with 4-hour fasting blood glucose values between 60 and 85 mg/dL are suspect, whereas blood glucose less than 60 mg/dL may be diagnostic. Blood insulin levels greater than 350 pmol/L aids in the diagnosis. Small, well-defined tumor nodules are often palpable within the pancreas at necropsy or during surgical exploration. Tumor metastasis rarely occurs. Surgical debulking with partial pancreatectomy is the treatment of choice as it often produces a disease-free state and is associated with increased survival time. Unfortunately, about a 40% recurrence of the tumor over 10 months is reported. Medical treatment involves using drugs such as prednisone that inhibit glucose uptake and stimulate gluconeogenesis.

Alternatively, diazoxide suppresses insulin secretion as well as inhibits glucose uptake and stimulates gluconeogenesis. Prognosis depends on whether metastasis has occurred and on how aggressively treatment is pursued. Most cases are chronic and usually fatal, but proper treatment can prolong the life of the ferret.

Adrenal Tumors

Adrenal cortical tumors are the second most common neoplasia of ferrets. Ferrets between 3 and 6 years of age are most commonly affected, with females more commonly affected than males. While adrenocortical adenomas and adenocarcinomas can both occur, adenomas are most common. Metastasis can occur. Adrenocortical hyperplasia is extremely common in ferrets greater than 4 years of age and induces the same clinical signs as are seen with neoplasia. It is notable that cortisol is rarely increased in ferrets although an excess production of adrenal steroids does occur.

Typical clinical signs include alopecia, swollen vulva in spayed females, prostatic enlargement in males, and increased sexual behavior in neutered ferrets. Pruritus is occasionally observed. In addition, hind-limb weakness may be apparent in chronic cases. Hair loss is the most common sign observed in ferrets with adrenal disease. Bilaterally symmetrical alopecia often begins at the tail and progresses forward over the hindquarters and abdomen until hair is present only on the neck, head, and extremities. Typical signs associated with hyperadrenocorticism in dogs, such as polyuria, polydypsia, polyphagia, pot-bellied appearance, and thin skin, are rarely seen in ferrets.

The clinical signs may be so characteristic that additional diagnostics may not be necessary. Ultrasound can be used to detect adrenal gland enlargement. Surgical removal of the abnormal adrenal gland is usually curative although a 10%–12% mortality is associated with the surgical intervention. The left gland is affected 50% more often than the right. If both adrenals are enlarged, removal of the larger adrenal plus a subtotal adrenalectomy on the other gland is usually effective. A medical treatment regimen using mitotane has been described but is not specific for the tumor cells and thus causes unwanted side effects. A commercially available, subcutaneous implant of deslorelin acetate (Suprelorin F, Virbac Animal Health, Fort Worth, TX) is also available that provides approximately 12 months of nonsurgical management of the disease.

Lymphoma

There is a high incidence of lymphoma reported in ferrets, with two typical clinical presentations. The first form, juvenile lymphosarcoma, is seen in young ferrets less than 2 years of age that develop an acute lymphoblastic disease, frequently without lymph node enlargement. Large, immature lymphocytes quickly infiltrate the viscera, including the thymus, spleen, liver, and many other organs. One of the more common clinical signs is dyspnea due to a rapidly growing thymic mass that compresses the lungs. This condition is often misdiagnosed as cardiomyopathy or pneumonia. Less commonly, extensive infiltration of the liver by neoplastic lymphocytes may result in marked increases in hepatic enzymes and icterus. Lymphoblastic lymphosarcoma should always be on the differential list for young ferrets presenting with serious illness. Clusters of lymphoma cases have been reported that suggest a potential infectious etiology (e.g., an unidentified retrovirus or Aleutian disease virus variant), but this possibility has not been confirmed.

The second form of lymphoma, "classic" lymphosarcoma, is seen in ferrets older than 3 years. These ferrets have a slowly progressive but chronic lymphocytic disease with lymphadenopathy. Classic lymphosarcoma is usually insidious, resulting in little clinical debility until extensive infiltration of visceral organs has occurred, which ultimately results in organ failure and death. Leukemia, increased numbers of circulating lymphocytes in the blood, can occur with either form but is uncommon. Clinical signs are nonspecific and include anorexia, weight loss, and lethargy.

Diagnosis should rely on microscopic evaluation of a lymph node aspirate or biopsy by an individual experienced with cell cytology. Biopsies or aspirates of visceral organs may also help in the diagnosis, with the spleen yielding the most consistent number of positive specimens. An elevated lymphocyte count on a complete blood count (CBC) is not definitive for lymphosarcoma and must not be used as the sole criterion for initiating treatment.

Prognosis is usually poor but depends on the stage at diagnosis and response to therapy. Treatments vary but include the administration of prednisone at 1 mg/kg orally once daily. This may initially lead to clinical improvement, but within 4–6 weeks, the majority of ferrets will develop disseminated disease that is refractory to further treatment. A more aggressive treatment protocol includes multiple-week (e.g., 14-week) IV administration of a combination of chemotherapeutic agents (e.g., vincristine, asparaginase, cyclophosphamide, and doxorubicin), often following treatment protocols developed for treatment of dogs and cats. Radiation therapy has also been used in the treatment of ferret lymphoma.

Mast Cell Tumors

Ferrets frequently develop mast cell tumors. The masses intermittently appear and are associated with alopecia and variable pruritus. Tumors are described as firm, small (2–10 mm), tan or erythematous, slightly raised, and circumscribed. A black, crusty exudate is often present around the mass. Most tumors are benign, in contrast to the more malignant mast cell tumors described in other species. Complete surgical excision is usually curative.

Miscellaneous Neoplasia

Other types of spontaneous neoplasms are uncommon in ferrets. Ovarian leiomyomas, chordomas, squamous cell carcinomas, adenocarcinomas, and malignant megakaryocytic myelosis have been reported.

Miscellaneous Conditions

Estrus-Associated Aplastic Anemia

Female ferrets that are not bred and remain in persistent estrus will develop an aplastic anemia attributable to prolonged estrogenic exposure that suppresses both red and white blood cell production in the bone marrow. The common practice of spaying female ferrets at commercial vendors prior to their sale has greatly decreased the incidence of this condition over time. Clinical examination of affected animals typically reveals pale mucous membranes, vulvar enlargement, alopecia, petechial hemorrhages, anorexia, and marked depression. Hematologic findings include severe anemia, thrombocytopenia, granulocytopenia, and hypocellularity of the bone marrow. Death is usually due to thrombocytopenia with resultant uncontrolled hemorrhage or severe leukopenia with secondary bacterial infections.

An ovariohysterectomy is the treatment of choice for ferrets diagnosed early in the condition and with normal blood parameters. If a ferret is anemic or thrombocytopenic, then one or two injections of 100 IU of human chorionic gonadotropin or 20 µg of gonadotropin-releasing hormone (GnRH) can be given to induce ovulation and return the ferret to anestrus. B vitamins, iron, and blood transfusions may help to correct the anemia and stabilize the patient. Once blood parameters have returned to normal, the ferret should be spayed. Prognosis is usually based on the severity of the anemia, with a PCV of less than 20% indicating a guarded prognosis and a PCV of less than 14% considered a poor prognosis. Supportive care that includes the use of steroids, force feeding, and vitamin supplementation may improve the chances of survival. Because of the high incidence of this disease in unmated female ferrets and the poor response to even the most vigorous therapy, ovariohysterectomy prior to the first estrus is advisable for females not intended for breeding.

Cardiomyopathy

There are two types of cardiomyopathy: dilatative and hypertrophic. The dilatative form is the most common in ferrets, usually affecting middle-aged or older ferrets. With dilatative cardiomyopathy, the heart muscles become flaccid, weak, and thin with the condition progressing until the heart can no longer effectively pump blood. Hypertrophic cardiomyopathy consists of progressively thickened heart muscles that also become ineffective in pumping blood. The cause of cardiomyopathy is unknown in ferrets. It has been shown that, in cats and dogs, dilatative cardiomyopathy may be linked to insufficient amounts of select dietary amino acids, specifically taurine for cats and carnitine for dogs. It had been proposed, but not proven, that a dietary insufficiency may contribute to this disease in ferrets.

Regardless of the form, whether dilatative or hypertrophic cardiomyopathy, both produce similar clinical signs due to failing cardiac function and resultant pleural effusion and/or ascites. Lethargy, exercise intolerance, inappetence, weight loss, dyspnea, and respiratory distress are commonly seen. Coughing and abdominal enlargement (secondary to ascites) may also be present.

Diagnosis of cardiomyopathy can be made with an x-ray and ultrasound examination. While observation of cardiomegaly on x-ray is indicative of cardiomyopathy, cardiac ultrasound is required to determine the type of cardiomyopathy and the degree of damage present. Additional tests, such as an electrocardiogram, blood profiles, and thoracocentesis (using a thin needle to obtain fluid from the chest for microscopic examination) enable a more definitive diagnosis.

Treatment of cardiomyopathy involves medication to remove accumulated fluid, such as the diuretic furosemide. Vasodilators such as nitroglycerin or captopril to modulate blood pressure are also often used. Digitalis derivatives can be helpful to increase the strength and efficiency of the failing heart. The long-term prognosis for ferrets with cardiomyopathy is poor, but treatment can effectively reduce the symptoms and increase the quality of life for a period of many months. If diagnosed early and treated aggressively, ferrets can be stabilized for several months or years.

Dystocia

Dystocia is common in ferrets. Problems are typically caused by positional abnormalities or oversized feti. Congenitally malformed kits may also cause a delayed onset of labor. Kits

remaining *in utero* beyond 43 days of gestation typically die. Medical intervention is indicated when a small litter size is the cause of delayed parturition. Parturition may be induced by injection of a prostaglandin (0.5–1.0 mg Lutalyse) followed by 0.3 mL of oxytocin (6 U) 3 hours later. If the jill does not deliver within 8 hours of this regimen, a cesarean section should be performed. Ideally, an inhalation anesthetic regimen should be used, but if an injectable regimen is required, ketamine (35 mg/kg) and xylazine (5 mg/kg) IM works well. Yohimbine IV or atipamezole IM can be given to partially reverse the xylazine.

Pregnancy Toxemia

Pregnancy toxemia is a life-threatening condition that may develop in late-term, pregnant females that undergo a negative energy balance most frequently due to a large fetal load (>10 kits), feeding of an inadequate diet, or decreased food consumption as may occur following a change in diet. Primiparous or stressed jills are at highest risk. Hyperlipidemia, hypoglycemia, ketosis, and hepatic lipidosis are common sequelae. Clinical signs include lethargy, recumbency, altered mentation, dehydration, and melena. Fur may fall out in clumps when the animal is handled, and hypothermia is often evident. Toxemia should be suspected based on history and clinical signs. Additional diagnostics include CBC, chemistry, and urinalysis that may reveal a low blood glucose (<50 mg/dL), azotemia, and ketonuria. The only characteristic postmortem change is a fatty yellow liver. Treatment consists of IV fluids containing glucose, oral glucose, or high caloric supplement. Subcutaneous or intraosseous fluids may be given if an IV line cannot be placed. The ferret must be kept warm using a circulating water blanket, but caution must be taken not to cause heat prostration by overwarming the ferret. A cesarean section should be performed immediately under gas anesthesia. Postoperatively, the jill should be provided supportive care including nutritional support, fluid therapy, and provision of an appropriate heat source. Prognosis is poor if liver damage is significant. The prognosis improves for animals that survive the first 24 hours after surgery. Supplemental feeding or cross-fostering of the kits may be required as agalactia of the jill is common.

Eosinophilic Gastroenteritis

There have been several reports of eosinophilic gastroenteritis in ferrets. It is most commonly seen in young male ferrets less than 14 months of age. Clinical signs are nonspecific and include anorexia, weight loss, and diarrhea. Eosinophilia may or may not be present. Intestines and mesenteric lymph nodes may be palpably enlarged. Diagnosis is based on the identification of diffuse eosinophilic infiltration on gastric, intestinal, or lymph node biopsy and may be supported by the finding of eosinophilia on a CBC. No etiologic agent has been identified in this syndrome. Ivermectin at a dose of 0.4 mg/kg SC and repeated in 2 weeks as well as lifelong treatment with corticosteroids have reportedly been effective in disease control. Feeding of hypoallergenic diets has been suggested as an alternative therapy.

Gastrointestinal Foreign Bodies

Because of their propensity to chew on anything, ferrets frequently ingest inappropriate items that then obstruct the stomach or intestines. It has been reported that a gastrointestinal foreign body is the most common diagnosis in young, inappetent, or anorexic pet ferrets although foreign bodies may also occur in a research setting where animals are provided

enrichment items or are allowed to exercise in inappropriate or unsupervised areas. Additionally, ferrets may develop trichobezoars that can also obstruct the gastrointestinal tract. Clinical signs may include scant feces, a palpable mass in the stomach or intestines, depression, anorexia, dehydration, and sometimes vomiting. Diagnosis is made on history, clinical signs, physical examination with palpation, and abdominal radiographs. Contrast studies may help to pinpoint the location of the obstruction. Removal of items by use of an endoscope is possible in some cases. If not possible, surgical intervention should be taken.

Hair Loss

Alopecia in ferrets is common and can be associated with a number of conditions, including hyperadrenocorticism, biotin deficiency, ovarian tumor, persistent estrus, seasonal molting, flea bite hypersensitivity, and high environmental temperature and humidity. A bilaterally symmetrical pattern of hair loss is seen with endocrine-based alopecia. A random or patchy hair loss is seen with most other types of alopecia. Females usually molt following the first ovulation of the season, and males molt in October or November. At times, ferrets may lose their hair for undetermined reasons.

Infant Mortality

High infant mortality is a common problem for ferret breeders, with contributing causes including maternal lactation failure, maternal neglect, and inappropriate or unsanitary nesting boxes. Many of the deaths are attributable to failure of lactation. Spontaneous congenital malformations, including neuroschisis, gastroschisis, absence of limbs, and corneal dermoids, are also relatively common.

Lactation Failure

Postparturient females may not be able to produce enough milk to feed their kits. There are a number of reasons for this failure, including genetic predisposition, poor management, inadequate nutrition, systemic disease, or mastitis. Jills that are unable to nurse their kits frequently stop lactating quickly; thus for at least 5 days after parturition, jills and their kits should be left in a quiet environment. Food and water should be within reach of a jill's nesting area so she can rest, nurse, and provide warmth to her kits. A diet with insufficient fat (25%–30%) or protein also leads to lactation failure as does metritis, cystitis, lymphosarcoma, or mastitis. If a nursing jill does become ill, providing food mixed with warm water or high-calorie supplements such as Nutrical can help support her nutritional requirements to maintain lactation.

Megaesophagus

A dilated esophagus is an uncommon but frequently fatal disorder of unknown etiology in ferrets. It is more commonly seen in middle-aged to older ferrets. Death is usually the result of aspiration pneumonia. Diagnosis is based on history, clinical signs, and radiographic evidence of an enlarged esophagus.

As a symptomatic treatment, ranitidine HCL (15 mg/mL) can be administered (0.1 mL twice daily, 30 minutes before feeding) to reduce gastric acid reflux into the esophagus. Cisapride may also help by enhancing gastrointestinal motility. When feeding the ferret with megaesophagus, it is important to elevate the head and maintain the neck in a straight align-

ment so that food can pass unobstructed into the stomach. Alternate positioning increases the risk for aspiration pneumonia.

Renal Cysts

Renal cysts are relatively common in ferrets: one report gives an incidence of 10%–15%. The cysts may be single or multiple and may affect one or both kidneys. The identification of cysts is usually an incidental finding on physical examination or ultrasound with no induced clinical signs, but their presence may lead to renal failure if the cysts are large or numerous and impinge upon normal renal tissue. A CBC, serum chemistry, and urinalysis will help to assess renal function if polycystic kidneys are identified.

Splenomegaly

Splenomegaly is an extremely common physical exam finding in ferrets that is nonspecific and may be associated with several diseases, including neoplasia, adrenal disease, infection, and congestion secondary to cardiac disease (Box 11.4). The vast majority of enlarged spleens are benign conditions that do not threaten the ferret's health and are caused by the occurrence of extramedullary hematopoiesis (a massive proliferation of red and white blood cell precursors external to the bone marrow) in the spleen. About 5% of enlarged spleens are attributed to tumors, the most common being lymphosarcoma. Splenomegaly may also transiently develop in the anesthetized ferret due to splenic sequestration of red blood cells.

Box 11.4

Splenomegaly is an extremely common physical exam finding in ferrets that is nonspecific and may be associated with several diseases, including neoplasia, adrenal disease, infection, and congestion secondary to cardiac disease.

Diagnosis of splenomegaly includes abdominal palpation. On occasion, the splenomegaly is so pronounced that the outline of the spleen is visible when the ferret is dorsally recumbent. Additionally, blood work and fine needle splenic aspirates can help to differentiate hematopoiesis from lymphosarcoma. Hematology parameters are within normal limits in ferrets with benign splenomegaly. A splenectomy is recommended only if the ferret has become lethargic due to the presence of the enlarged spleen, and an infection is not identified.

Urolithiasis

Urolithiasis is not uncommon in ferrets, with neutered males most frequently affected. Magnesium ammonium phosphate (struvite) uroliths are most common and diet plays a large role in the production of stones. Animal protein diets result in acidic urine in which struvite is soluble. Ferrets fed a diet with plant protein have alkaline urine, and struvite tends to precipitate at this higher pH. The normal urine pH of ferrets should be around 6.0 when maintained on a high-quality, meat-based diet.

Clinical signs include frequent urination, licking of the perineum, urinary incontinence, hematuria, and dysuria. If obstructed, a ferret may show signs of straining to urinate and may vocalize in pain. If not promptly corrected, obstruction leads to life-threatening metabolic disturbances that result in coma and death. Treatment is similar to that for other mammals with this disease and includes reestablishment of urinary excretion, fluid therapy, and dietary management. Surgery is usually the treatment of choice for obstruction or if the uroliths in the bladder are of significant size. Long-term dietary management is based on a gradual switch to a meat-based diet to prevent stone formation. Diets used to decrease stone formation in dogs and cats generally have lower protein levels than is recommended for ferrets; therefore, their use is not advised.

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CHAPTER 11 REVIEW

Match Up

Match the following with their respective descriptions:

A. Hob
B. Kit
C. Clowder
D. Jill
E. Business
F. Gib
G. Pack
H. Sprite

1. _____ Group of ferrets
2. _____ Male ferret
3. _____ Neutered male
4. _____ Young ferret
5. _____ Female ferret
6. _____ Spayed female

Multiple Choice

7. Gestation is _____ days.
   A. 21
   B. 30
   C. 63
   D. 42
8. Ferrets are unusual in that they have _____ premolars per quadrant.
   A. 4
   B. 3
   C. 2
   D. 0
9. Ferrets reach sexual maturity at this age.
   A. 3–4 months
   B. 5–7 months
   C. 9–12 months
   D. 1–2 months
10. The ferret is a/an
    A. carnivore
    B. strict herbivore
    C. omnivore
    D. vegetarian

11. A species of bacteria associated with gastritis and ulcers
    A. *E. coli*
    B. *Salmonella*
    C. *Helicobacter*
    D. *Streptococcus*
12. Ferrets must be routinely vaccinated against
    A. feline panleukopenia
    B. canine parvovirus
    C. feline rhinotracheitis
    D. canine distemper
13. A female ferret that is not bred and has a protracted estrus cycle can develop
    A. splenomegaly
    B. aplastic anemia
    C. eosinophilic gastroenteritis
    D. lymphoma
14. Causal organism of a pedal mange
    A. *Demodex*
    B. *Ctenocephalides*
    C. *Sarcoptes*
    D. *Otodectes*
15. A parvovirus that affects ferrets
    A. Aleutian disease virus
    B. human influenza virus
    C. canine distemper
    D. rabies
16. Ferrets lack this segment of the gastrointestinal tract.
    A. duodenum
    B. cecum
    C. jejunum
    D. stomach
17. The wild-type variety is called
    A. agouti
    B. buff
    C. albino
    D. fitch
18. Resembles the "dry form" of feline infectious peritonitis
    A. proliferative bowel disease
    B. Aleutian disease
    C. ferret systemic coronavirus
    D. epizootic catarrhal enteritis

(*Continued*)

19. Most commonly reported neoplasm of ferrets
    A. insulinoma
    B. lymphoma
    C. adrenal cortical tumor
    D. mast cell tumor
20. Extremely common nonspecific physical exam finding in ferrets
    A. splenomegaly
    B. dry skin
    C. megacolon
    D. cough

Critical Thinking

21. Ferrets are frequently used in toxicology testing. Why?

12 Primates

Nomenclature and classification of nonhuman primates are subject to periodic change. Nevertheless, individuals concerned with the medical care or research use of these animals should be able to recognize the more frequently seen species and categorize them correctly. Nonhuman primates can be differentiated by their unique biologic characteristics, regions of origin, environmental and nutritional requirements, and disease susceptibility. There is a vast range in size among different species of nonhuman primates from the smallest, the pygmy mouse lemur (*Microcebus myoxinus*) at 30–109 g, to the largest ape, the gorilla, weighing more than 200 kg (440 lb).

TAXONOMY

Taxonomic classification of nonhuman primates is an ever-changing field. There is a long-standing debate regarding classification of certain nonhuman primates (NHPs), such as tarsiers, and both current and obsolete references are found in the literature. For the purposes of this book, we adopt the approach of classifying NHPs into two suborders: Strepsirrhini ("wet-nosed") and Haplorrhini ("dry-nosed"). Strepsirrhini translates from Greek into "having a curved nose" and includes lemurs, lorises, and galagos. Haplorrhini translates from Greek into "simple-nosed" and includes tarsiers, monkeys, and apes. This chapter focuses on the more prominently used research species within the Haplorrhini suborder.

Strepsirrhini (Lemurs, Lorises, and Galagos)

Many strepsirrhine species superficially resemble dogs or rodents more than true primates. The snouts are generally elongated and the moist nose (rhinarium) joins with the upper lip,

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which attaches to the gum, limiting their ability to make facial expressions. The brain-to-body ratio is smaller than that of haplorrhines. The olfactory lobes are large, indicating a greater reliance on smell. All lemuriformes (lemurs), with the exception of the aye-aye, have a dental comb made up of grouped incisors and canines for grooming purposes; another grooming adaptation is a claw on the second toe. The most medial digit of the foot is widely separated from the others, allowing a tight grip for locomotion. Most species are nocturnal and have sensitive hearing for night hunting. Their natural diet consists primarily of insects, but some species prefer a fruit diet and some are carnivorous. Strepsirrhines have a breeding season rather than a cycle, as is typical of haplorrhines. All species in this suborder possess a bicornate uterus, have one or two pairs of mammary glands in varying locations, and produce 1–3 offspring. Strepsirrhine species include lemurs, lorises, and galagos. All species of lemurs are classified as endangered; very few are used in biomedical research.

Haplorrhini (Tarsiers, Monkeys, and Apes)

No single anatomic feature distinguishes haplorrhines from other animals. Because the upper lip is not directly connected to the nose or gum, these primates can make a wide range of facial expressions. The brain-to-body ratio is significantly greater than that of the strepsirrhines. Vision is the sense most relied on, and most species have some degree of dichromatic or trichromatic vision. A majority of the species are diurnal, or active in the daytime, with the exception of tarsiers and owl monkeys. The hands and feet are adapted more generally for locomotion. Some additional important characteristics are the presence of a simplex uterus (tarsiers have a bicornate uterus), paired pectoral mammae, a pendulous penis, scrotal testes, a clavicle, and a hallux (most medial toe of the foot). The suborder Haplorrhini is divided into nine families: Tarsiidae, Callitrichidae, Cebidae, Aotidae, Pitheciidae, Atelidae, Cercopithecidae (Old World monkeys), Hylobatidae (lesser apes), and Hominidae (great apes).

New World Primates (Family—Callitrichidae)

Marmosets and tamarins are among the smallest of the New World (Central and South American) primates. They have soft, silky hair and long tails that are not prehensile. Representative species are common marmosets, pygmy marmosets, golden lion tamarins, and cotton-top tamarins. The common marmoset, *Callithrix jacchus*, is the callitrichid species that is most commonly used in research and has an average body weight of 300–350 g. The cotton-top tamarin, *Sanguinus oedipus*, is endangered and thus can be used only in research that benefits the species. The average weight is 450–550 g. Callitrichids have several unique characteristics: possessing claws rather than nails; having axillary rather than anterior mammary glands; and being biovulatory—thus twinning is normal. Marmosets and tamarins are used in a variety of research applications, including infectious disease, viral oncology, pharmacokinetics, and behavioral and reproductive studies.

New World Primates (Family—Cebidae)

Squirrel monkeys, *Saimiri sciureus*, have short, dense hair coats varying in color from orange to gray; a dark, round muzzle; and white hairless patches around their eyes (Figure 12.1). They have long tails that are partially prehensile, meaning the tail can aid in locomotion and balance but cannot manipulate objects. Squirrel monkeys typically weigh 500–1500 g and

Fig 12.1. Squirrel monkey (*Saimiri sciureus*). (Image courtesy of Christian Abee.)

are one of the most common neotropical primates used for research due to their small size, tractable nature, and tendency to breed well in captivity. Sexual dimorphism is marked primarily by body weight, with males weighing 25%–30% more than females. Both males and females undergo seasonal reproductive changes. They have been used in a variety of research applications and are particularly important in the study of atherosclerosis. Squirrel monkeys are widely distributed throughout the rain forests of South and Central America. Animals from different geographic areas are generally distinguished by having a "gothic arch" (*Saimiri sciureus* group comprising three subspecies) or "Roman arch" (*Saimiri boliviensis* group comprising two subspecies) in reference to the slightly differing color and shape characteristics of the hair around the eyes.

Sapajus, formerly known as cebus monkeys, have also been referred to as capuchins because their coloration likens them to the Capuchin order of monks. The six extant species of *Sapajus* have medium-length, dense hair coats ranging from dark brownish-black to white and long, partially prehensile tails. Adults weigh on average 1–3 kg. *Sapajus apella*, the black-capped capuchin, has a unique appearance, with long, dark sideburns and tufts of dark hair arising from its brow. *Sapajus apella* are occasionally used in research.

New World Primates (Family—Atelidae)

Spider monkeys, *Ateles* spp., are aptly named because of their long, gangly arms and legs and thick, rounded abdomens. These monkeys are anatomically unique, possessing four fingers but no thumb, and long, prehensile tails. In the wild, they are strictly arboreal. Adults weigh 5–7 kg. Females have an unusually long clitoris that can be mistaken for a penis. Spider

monkeys are native to the rain forests of South and Central America. They are not commonly used in research.

New World Primates (Family—Aotidae)

Owl monkeys, *Aotus* spp., are one of only two nocturnal haplorrhine primates (the second are the tarsiers). They have short, dense hair coats varying in color from gray to red; a small, dark muzzle; large, owl-like eyes; and white crescents of hair around each eye. They have long, nonprehensile tails, weigh 900–1200 g, and are arboreal and monogamous. These primates are not as hardy as squirrel monkeys and do not adapt as well to the laboratory. Owl monkeys are one of the most important models for studies of human malaria and viral oncology and are of special importance in vision research due to a unique eye structure. There are eight different species of owl monkeys with diploid chromosome numbers ranging from 46 to 56.

Old World Monkeys (Family—Cercopithecidae)

Rhesus monkeys, *Macaca mulatta*, are medium-sized, Old World, Asian monkeys that have short, reddish-brown hair coats and medium-length tails. The close-cropped head hair accentuates an expressive face. There is moderate sexual dimorphism; males are larger and weigh 6–11 kg, females weigh 4–9 kg. Similarly, males develop large canines, whereas females do not. Rhesus monkeys tend to be one of the more aggressive macaque species. They are frequently used for vaccine testing, pharmacology and toxicology studies, and infectious disease research. *M. mulatta* are found across a wide range of Central Asia from Afghanistan to China. Due to destruction of their natural habitat as well as religious and political factors, the supply of rhesus monkeys from the wild is restricted.

Cynomolgus monkeys, *Macaca fascicularis*, also called crab-eating and long-tailed macaques, are slightly smaller than rhesus and have long, nonprehensile tails and medium-length, olive hair coats on the dorsum, with white to gray hairs on the ventrum and around the face. The hair around the face is longer and tends to form a small mane. Males have long, sharp, canine teeth and are slightly larger than females. They tend to be less aggressive than rhesus monkeys. Cynomolgus are frequently used in drug testing and infectious disease research. They are found primarily in Southeast Asia; however, a population of *M. fascicularis* was introduced onto the island of Mauritius located in the Indian Ocean, just east of Madagascar. Of the baboons, *Papio anubis*, the olive baboon, and *Papio cynocephalus*, the yellow baboon, are the species most commonly used in biomedical research. They are large, weighing 15–30 kg as adults. They have long hair coats, long nonprehensile tails, and a long, prominent muzzle that gives them a dog-faced appearance. There is marked sexual dimorphism, with males exhibiting a shoulder mane; having longer, daggerlike canine teeth; and weighing 50% more than females. They are found over a wide range of Africa and tend to be agricultural pests. The baboon is occasionally used for surgery, for reproductive physiology research, and as an alternative model for acquired immunodeficiency syndrome (AIDS).

African green monkeys, *Chlorocebus aethiops*, formerly *Cercopithecus aethiops*, are small monkeys, with brownish-green hair coats and long nonprehensile tails. African greens are a species of guenon and are also commonly known as vervet or grivet monkeys. They weigh 2–6 kg. Males of all species have bright blue scrotal areas contrasting with red penises. This striking coloration, when combined with the white fur of their undersides, is highlighted in

what is called the "red, white, and blue display." They are occasionally used in biomedical research.

Mangabeys, *Cercocebus* spp. and *Lophocebus* spp., are slender animals with long legs and tails. *Cercocebus atys atys*, or sooty mangabeys, are susceptible to the organism that causes leprosy, *Mycobacterium leprae*, and act as asymptomatic carriers of the simian immunodeficiency virus (SIV). They have been used in leprosy and AIDS research, but their status as an endangered species now limits their use.

Lesser Apes (Family—Hylobatidae)

Lesser apes comprise several genera of gibbons, including *Hylobates* spp., *Hoolock* spp., *Nomascus* spp., and the largest gibbon or siamang, *Symphalangus* spp. These animals have exceptionally long arms and no tail; locomotion involves brachiating from branch to branch. They are monogamous and mate for life. They are arboreal, sleeping in a fork between branches, and principally eat fruit and vegetables. Gibbons are notorious for loud vocalizations, which are used in the wild to warn off other members of this species from their territory. They are listed as endangered.

Great Apes (Family—Hominidae)

The chimpanzee, genus *Pan*, is the highest form of nonhuman primate once used in significant numbers in biomedical research. Adults grow quite large, with average weights of 40 kg for females and 50 kg for males. They are endangered, and none have been imported into the United States from the wild for years; some chimpanzees are available from former United States breeding colonies. They are used for testing hepatitis B and C virus and AIDS vaccines, and for psychobiology research. In 2000, the Chimpanzee Health, Improvement, Maintenance, and Protection (CHIMP) Act (PL 106-551) was signed into law to provide support for the construction of chimpanzee sanctuaries and for the lifetime care of the animals used in government research through funding from the National Institutes of Health (NIH).

Orangutans, genus *Pongo*, are found in Borneo and Sumatra and appear to be approaching extinction. These animals are not used in biomedical research. Gorillas, genus *Gorilla*, are native to equatorial Africa. In the wild, the usual adult weight is 74–180 kg. These primates are endangered and used only rarely in studies that are not detrimental to their health, such as for learning and behavioral research.

USES

Nonhuman primates share an unparalleled anatomic and physiologic proximity to humans, consequently serving a critical role in biomedical research. Historically, nonhuman primates have been primary models in the study of viral diseases, including smallpox and poliovirus. Today, they continue to be important models for the study of viral diseases such as AIDS, toxicological investigations, behavior, learning, neurological diseases such as Parkinson's and Alzheimer's, dentistry, reproduction, and infectious diseases.

The greatest numbers of nonhuman primates used in biomedical research are the macaques, primarily the cynomolgus and rhesus species (Box 12.1). These two species of macaques

Box 12.1

The greatest numbers of nonhuman primates used in biomedical research are the macaques, primarily the cynomolgus and rhesus species.

constitute more than 50% of the total number of nonhuman primates used in research. Squirrel monkeys, marmosets, baboons, African green monkeys, mangabeys, owl monkeys, cebus monkeys, and tamarins are used in moderate to small numbers. Chimpanzees are used in extremely limited numbers. Overall, nonhuman primates constitute less than 2% of the total number of animals used in biomedical research each year. Conservation of species has been a major concern; threatened and endangered species are no longer removed from the wild. Research investigations using captive animals have led to a greater knowledge base of their biologic requirements and methods to enhance conservation.

National Primate Research Centers

Seven National Primate Research Centers (NPRCs) are located throughout the country and provide animals, facilities, and expertise in all aspects of nonhuman primate biology and husbandry. These facilities and resources enable collaborative research among NPRC staff scientists, NPRC host institutions, and other NIH-funded researchers. Major areas of research include AIDS, avian flu, Alzheimer's disease, Parkinson's disease, diabetes, asthma, and endometriosis. The centers' specialized resources are also used by investigators funded by other federal, state, and local agencies, as well as by research foundations and the private sector. The primate centers house more than 28,000 nonhuman primates of 20 different species; the majority are macaques. More information about these resources, including access criteria, is available through the Office of Research Infrastructure Programs, National Primate Research Centers (http://dpcpsi.nih.gov/orip/cm/primate_resources_researchers.aspx).

BEHAVIOR

Due to complexity and species diversity, it is beyond the scope of this chapter to provide a detailed description of the behavior of nonhuman primates. Rather, this section provides an overview to familiarize the reader.

A majority of nonhuman primate species are extremely social animals. The most common social organization is that of a troop containing from 20 to 100 animals; however, some are primarily solitary or live in small family groups. Baboons and macaques aggregate in sizeable troops. Within these groups exists a definite hierarchical arrangement: one male is the dominant or alpha male and one female is the dominant or alpha female. Next in the hierarchy are a small number of males, followed by a group of high-ranking females, and finally a group of low-ranking males, females, and younger animals. An animal's rank has a high correlation to the rank of its mother. The hierarchical position of a female in a troop normally

remains quite stable, whereas the position of a male seems to be more transitory, with changes in leadership every 4–5 years. Depending on the stability of the hierarchy and the species, fighting frequently occurs in social groupings. Subordinate members lipsmack and present their hindquarters to more dominant animals as a sign of submission. Direct eye contact is perceived as a threat and will often elicit aggressive behavior, including yawns, which reveal their large canines, as well as threatening facial expressions and postures. Nonhuman primates learn reproductive and social behaviors from adults; thus, they must be reared in a reasonably representative social situation to develop normal behavioral patterns. Hand-reared and isolated animals frequently develop behavioral abnormalities and rarely mate.

As highly social animals, grooming is a critical component of social bond formation. Primates usually exhibit fewer incidences of stereotypical behavior when housed in social groups and provided with environmental enrichment; this fosters the development of normal reproductive and social behaviors. Primary disadvantages to group housing include increased trauma from fighting, the inability to determine adequate food and fluid intake of individuals, and the inability to easily isolate individuals for treatment. Despite potential drawbacks, social housing is preferred for the psychological well-being of these animals; single housing must be scientifically justified by the principal investigator and approved by the IACUC.

ANATOMIC AND PHYSIOLOGIC FEATURES

Physical characteristics that distinguish Old World (OW) from New World (NW) primates are outlined in Table 12.1. OW primates are also classified as catarrhines, a Latin derivative for "narrow-nosed"; NW monkeys are also classified as platyrrhines, or Latin for "flat-nosed." Most nonhuman primates used in research are so similar to humans morphologically that human anatomy and surgery references can serve as excellent guides.

Rhesus and some other OW species have cheek pouches in which food is collected to be chewed and swallowed later. In a number of species, including macaques and baboons, males have formidable canine teeth that may serve as vicious weapons. Some OW species possess ischial callosities on their buttocks; these hard, keratinized pads serve as protection for the bony prominence of the ischium.

Table 12.1. Monkey physical characteristics: New World vs. Old World

New World Monkeys (Platyrrhines)	Old World Monkeys (Catarrhines)
Prehensile tails in some species	No prehensile tails
No ischial callosities	Ischial callosities in some species
Broad-nosed	Narrow-nosed
Require dietary vitamin D_3	Do not require dietary vitamin D_3
No cheek pouches	Cheek pouches in some species
No opposable thumbs	Opposable thumbs in all species
Three premolar teeth per quadrant	Two premolar teeth per quadrant

There is marked sexual dimorphism of body structure, weight, hair coat, and size of the teeth in some species. Females have a simplex-type uterus, similar to the type found in humans. Most have placentas that are monodiscoid (one lobe) or bidiscoid (two lobes). Sexing is usually simple except in a few species such as spider monkeys; the females have a long clitoris that resembles a penis but may be recognized by the absence of a urethral opening. All female OW primates have menstrual bleeding as a feature of their sexual cycle. This is absent in NW species. Twinning is extremely rare in OW monkeys, but it is the norm in some species of NW monkeys.

Most mammals can synthesize vitamin C, but primates require a dietary source (Box 12.2). NW primates cannot utilize vitamin D_2 and must be provided with a dietary source of vitamin D_3. NW species also require a higher percentage of protein in their diets than do OW species.

Box 12.2

Most mammals can synthesize vitamin C, but primates require a dietary source.

Blood parameters vary to some extent among species and may also vary depending on the methods used for sampling and on the housing conditions. It has been reported that baboon and chimpanzee hematologic and clinical chemistry values compare favorably with normal clinical values established for humans. Selected hematologic and biochemical values for several species of nonhuman primates are listed in Appendix 1, "Normal Values."

BREEDING AND REPRODUCTION

General biologic and reproductive data are listed in Table 12.2. The breeding and the reproductive biology of nonhuman primates is quite varied among the different species. General breeding and reproductive indices, as well as some peculiarities for some of the more common nonhuman primates, are presented.

Puberty is marked in OW monkeys by the onset of menstrual cycling, or menarche, in the female and increased testicular size and spermatogenesis in the male. The average age of initiation of menarche in cynomolgus and rhesus macaques is between 2 and 3 years. The average age of sexual maturity of the female is between 2.5 and 3.5 years in these species, with the first birth usually occurring between 3 and 5 years. Males generally mature 1–2 years later than females.

Although most OW species are nonseasonally polyestrous (spontaneous ovulators), the rhesus macaque is a seasonal breeder, with most fertile cycles occurring in the winter months. The menstrual cycle lasts an average of 28–30 days in most OW species. Baboons, rhesus, and chimpanzees present with edema and color changes of the perineal or sex skin that cycles with the hormonal changes. In the rhesus, the sex skin has a corrugated appearance, whereas the sex skin of the baboon is smoother. Maximum turgescence and pinkish-red color intensity are associated with the follicular phase of the menstrual cycle, estrus, and ovulation.

Table 12.2. Biologic and reproductive data for select nonhuman primate species

	Rhesus	Cynomolgus	Baboon	Squirrel
Adult body weight: Male	6–11 kg	6 kg	22–30 kg	700–1100 g
Adult body weight: Female	4–9 kg	4 kg	11–15 kg	500–1000 g
Life span	30+ y	37 y	40–45 y	20 y
Body temperature	36°–40°C (96.8°–104°F)	36°–38°C (96.8°–100.4°F)	36°–39°C (96.8°–102.2°F)	33.5°–38.8°C (92.3°–101.8°F)
Heart rate	150–333 beats per minute	107–215 beats per minute	80–200 beats per minute	225–350 beats per minute
Respiratory rate	10–25 breaths per minute	32–44 breaths per minute	29 breaths per minute	20–50 breaths per minute
Breeding onset: Male	38 mo	42–60 mo	73 mo	60 mo
Breeding onset: Female	34–43 mo	46 mo	51–73 mo	36–46 mo
Estrous cycle length	28 d	28 d	31–36 d	18 d
Gestation period	167 d	162 d	175–180 d	170 d
Weaning age	210–425 d	365–547 d	180–456 d	182 d
Chromosome number (diploid)	42	42	42	44

Source: Adapted from Johnson-Delaney (1994) and Bennett et al. (1995).

Deturgescence and decreased color intensity occur rapidly after ovulation and are associated with the luteal phase of the menstrual cycle. Optimal mating time can be determined for individuals who have regular cycle patterns by averaging three to four of their menstrual cycle lengths and mating them 3–4 days prior to deturgescence.

Many OW species, including the cynomolgus and rhesus macaques as well as chimpanzees, have a "placental sign" or vaginal bleeding. This occurs approximately the same time as menstruation would appear, but it is actually hemorrhage associated with implantation of the blastocyst. Gestation lengths for some of the more common species are listed in Table 12.2. Parturition in macaques frequently occurs in the late evening or early morning hours, with labor lasting 2–3 hours. Head-first presentation of the fetus is normal. Infants begin nursing immediately after birth and nurse frequently throughout the day. Infant macaques and baboons may begin ingesting solid food around 2–3 months of age. Weaning is quite variable between the species and occurs at approximately 1 year of age.

HUSBANDRY

Housing and Environment

The most suitable housing system for nonhuman primates depends on species, use, climatic conditions, and a number of other factors. In research laboratories, animals are often housed in stainless steel cages with slotted or grid floors. The Animal Welfare Act (AWA) regulations and the *Guide for the Care and Use of Laboratory Animals* (the *Guide*; ILAR, 2011) give cage size specifications for nonhuman primates. Most macaque monkeys are less than 15 kg

and can be housed in 0.56 m^2 (6.0 ft^2) cages with a height of at least 81.28 cm (32 in.). A cage with a built-in squeeze design is desirable when animals are to receive frequent injections or blood collections. Cage pans are frequently used beneath individual cages to collect feces and urine. Wood-chip bedding may be placed in the pans to help absorb moisture; alternatively, there may be a sloped floor beneath a row of cages that diverts the urine and feces into a sewage drain. Nonhuman primates are highly intelligent and frequently learn how to open cage doors; thus, a sufficiently secure lock, such as a padlock, should be used.

In housing nonhuman primates for research purposes, rigid control of room temperature, relative humidity, ventilation, and lighting are essential. A temperature range of 18°–29°C (64°–84°F), relative humidity range of 30%–70%, and 10–15 air changes per hour are recommended. Ventilation from nonhuman primate housing should be filtered and fully exhausted to prevent contamination of personnel areas. Typically, 12–14 hours of light per day should be provided, controlled by automatic timers.

Breeding colonies can be housed in a variety of ways, including indoor pens connected to outdoor runs, covered cylindrical enclosures called corn cribs, outdoor corrals with some form of protection from extreme weather conditions, and free range on islands.

Environmental Enrichment and Social Housing

The AWA regulations mandate that primates used in research must be provided with environmental enrichment to promote psychological well-being. An excellent reference on this topic, *The Psychological Well-Being of Nonhuman Primates*, is published by the Institute for Laboratory Animal Research (ILAR, 1998; see www.nap.edu). A detailed, written plan for environmental enhancement must be developed, documented, and implemented (Box 12.3); these should be reviewed and reapproved by the IACUC periodically. It is well documented that social interaction is the best form of enrichment, although if animals are incompatible, fighting and trauma may occur. Thus, compatibility must be determined by trial and observation. The *Guide* states, "Group housing is critical and numerous species specific factors such as age, behavioral repertoire, sex, natural social organization, breeding requirements, and health status should be taken into consideration when forming a group. In addition, due to conformational differences of animals within groups, more space or height may be required to meet the animals' physical and behavioral needs." Newly grouped animals should never be left unattended for long periods. Pair or group housing is most successful when animals are placed together at a young age, and this promotes normal behavior such as grooming. Numerous cage designs allow side-by-side or top and bottom cages to become one large social cage by simply pulling dividing panels. If individual primates are strongly socialized toward humans, positive human interaction can also be considered an additional form of enrichment.

Box 12.3

A detailed, written plan for environmental enhancement must be developed, documented, and implemented.

Fig 12.2. C.H.O.I.C.E. Smart Cage and social wheel viewing access. (Images courtesy of Britz and Company.)

In addition to social housing, other forms of environmental enrichment should be provided to promote species typical activities. Newer caging designs are available that allow occupants to select audio and video recordings and to alter lighting and temperature, and that provide a "social wheel" to permit or exclude visualization or contact with other animals (Figure 12.2). Portable exercise rooms are also available that allow for periods of more aerobic activity (Figure 12.3). Grooming and foraging boards and more complex problem-solving devices such as puzzle feeders or video games can be used to encourage species-typical behavior. A simple piece of wood with bark or coconut shells enables important foraging and grooming behavior. For arboreal species, a swing or perch is important to allow the animals to engage in climbing activities. Food treats such as fruit, peanuts, granola, flavored ice cubes, vegetables, popcorn, and seeds may also be used. Animals are frequently observed watching television with great interest. Finally, manipulanda such as mirrors, balls, and chains can be offered as toys, but they must be nontoxic, safe, durable, and designed for laboratory primate use. It is best to offer a varied schedule of enrichments so that the objects remain novel. Formal approval should be secured from the principal investigator, the IACUC, and the facility veterinarian prior to the introduction of any enrichment items.

Feeding and Watering

Commercially milled, pelleted diets produced for specific types of laboratory primates adequately meet the nutritional needs if properly stored and if fed within 180 days of milling.

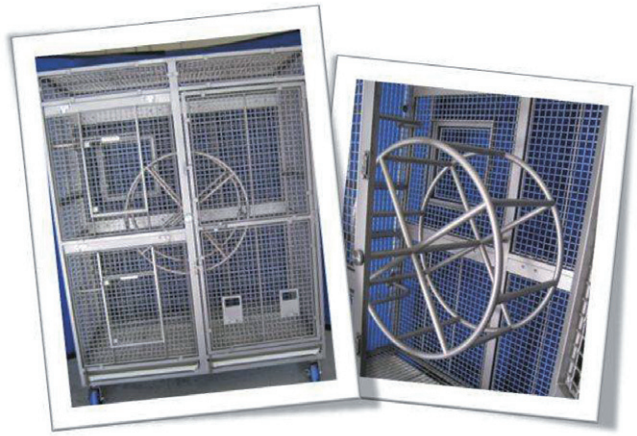

Fig 12.3. Play cage with wheel in the middle. (Image courtesy of Britz and Company.)

Primates will consume an average of 3%–5% of their body weight in dry feed per day, but there is considerable waste; splitting portions into 2 or 3 daily feedings may reduce waste and more closely approximates natural behavior. Commercial diets prepared for NW primates usually contain 25% protein and 9% fat, supplemented with vitamin D_3; OW diets typically contain 15% protein and 5% fat. Some nutritionists recommend a 25% protein diet for pregnant, lactating, or juvenile OW monkeys. Protein-deficient animals are more susceptible to pneumonia, bacterial enteritis, and other illnesses. Diets may be supplemented with fresh fruits and vegetables, primarily as a form of environmental enrichment. Feeders or feeding stations should be physically distanced to prevent dominant animals from monopolizing the food supply.

Fresh, potable water should be provided *ad libitum* either through an automatic watering system or in water bottles attached to the cage. Bottles are more labor intensive but provide a route for the administration of medications or dietary supplements. Automatic watering systems can malfunction and should be manually checked by the animal care staff daily to ensure the availability of water to each cage. System malfunction should be suspected and investigated if otherwise healthy animals become anorexic.

Sanitation

Due to concerns regarding the potential transmission of zoonotic disease discussed later in this chapter, it is important to note that all work involving nonhuman primates must employ universal precautions, including a gown or other protective outer layer, splash-proof eye protection (or full face shield), cap, mask, shoe covers, and exam gloves. This protection is particularly important when working with soiled caging and other implements used with NHPs, particularly when spray may aerosolize biological material.

Housing requires a minimum of once-daily cleaning. In certain situations, such as group housing in a gang cage, a sufficient amount of urine and feces is produced to justify twice-daily cleaning. Generally, cage pans are removed, and the urine and feces are disposed of

through a drain system or collected as contaminated waste. If suspended pans are not used, then the feces and urine must be rinsed from beneath the cages and to the drain. Care must be taken to avoid wetting the occupants when this method is used. Floors should be thoroughly rinsed or mopped to remove any adherent debris. Pans can be either replaced with clean pans or washed thoroughly and replaced. Racks, cages, pans, water bottles, and feed hoppers should be sanitized at least once every 2 weeks with chemicals, hot water 61.6°–82.2°C (143°–180°F), or a combination of both. Refer to Chapter 4, "Mice," for more specific information on disinfection of caging.

TECHNIQUES

Handling and Restraint

One of the most striking features of primates is their great strength and agility. Chemical restraint is recommended for safe handling of all larger nonhuman primates. Individuals who handle NHPs must be adequately trained in order to minimize stress to the animal and to maximize safety for the handler. Personnel handling primates must wear protective clothing including a gown or other protective outer layer, splash-proof eye protection, cap, mask, shoe covers, and exam gloves (Figure 12.4). Great care must be taken with macaques as scratches from the animals or their caging, aerosolized biological material, or bites have the potential to transmit *Macacine herpesvirus 1* (B virus), which induces a fatal encephalomyelitis in humans. The video "Working Safely with Nonhuman Primates," produced by the

Fig 12.4. Protective clothing for working with macaque monkeys. (Photo courtesy of C. Medina.)

NIH Office of Animal Care and Use and available through the Office of Laboratory Animal Welfare, provides instruction and training in personal protection and primate behavior.

For primates weighing more than 2 kg, chemical restraint is strongly recommended. Administration of injections is greatly facilitated by restraint in a squeeze-back cage by staff who are trained to use this equipment. With patience and the use of positive reinforcement, animals can be trained to present themselves for an injection and greatly reduce the stress on both personnel and animals. Ketamine hydrochloride is the drug most frequently used for chemical restraint. Individual animals may have different responses to ketamine and may regain purposeful movement; a second dose should be available for quick response to these situations.

Manual restraint can be used for animals weighing less than 10 kg, but it should ideally be performed with at least two handlers. The risk for personal injury increases with manual restraint; in addition to protective clothing, full-length leather gloves should be worn but may not be impervious to sharp canine teeth. Manual restraint can be accomplished by squeezing the primate to the front of the cage to immobilize it; the cage may be opened and the upper arms just above the elbows can be grasped. The animal can then be removed by holding the arms behind the back so that the elbows nearly touch. The second handler is required to release the squeeze mechanism, to operate the cage door, and, obviously, for safety reasons. Primates restrained in this manner should be held away from the handler's body to avoid being grabbed or scratched by the animal's feet. The restraint of a small primate is depicted in Figure 12.5.

Another option for restraint is the pole-and-collar method. A small, lightweight plastic or aluminum collar with two small handles is secured around the neck of the anesthetized

Fig 12.5. Manual restraint of marmoset (*Callithrix jacchus*). (Photo courtesy of C. Medina.)

Fig 12.6. Pole-and-collar restraint of macaque (*Macaca fasciularis*). (Photo courtesy of C. Medina.)

animal several days to weeks before restraint is actually necessary to permit acclimation. Depending on the primate's size and temperament, pole-and-collar restraint can be accomplished with one or two handlers as depicted in Figure 12.6. Once the animal is squeeze-restrained in the cage, the collar handles are grasped with poles and the primate is assisted from its cage and placed in a restraint chair or on a restraint table, where the neck collar is secured. Primates should be trained to this type of restraint with positive reinforcement and repetition. They adapt well to this routine and will often walk to the restraint device. Animals placed in a restraint device should never be left unattended.

Restraint devices are specifically mentioned in the AWA regulations with the following specifications: (1) restraints are not to be considered a normal method of housing; (2) restraints must be approved by the institutional animal care and use committee (IACUC); (3) restraint of animals should be kept to an absolute minimum amount of time; (4) restraints should not be used merely as a convenience but must be necessary to achieve research goals; and (5) restraints may require temporary or permanent removal of animals that become ill, injured, or behaviorally altered from the prolonged restraint. In instances where restraint over 12 hours is required, the animal must be provided the opportunity for daily unrestricted activity for at least one continuous hour during the period of restraint, unless continuous restraint is justified in the research proposal and approved by the IACUC.

Identification

Cage cards are used as a means of identifying individually caged animals but are never used as the sole means. Additional methods include chest or thigh tattoos, neck tags, ear tags, or

Table 12.3. Adult nonhuman primate blood volumes (mL)

	Rhesus	Cynomolgus	Baboon	Squirrel
Total blood	320–880	320–480	880–2400	40–120
Single sample	40–110	40–60	110–300	5–15
Exsanguination	120–330	120–180	190–330	15–45

Source: Adapted from Dysko and Hoskins (1995) and Johnson-Delaney (1994).

Notes: Values are approximate.

electronic microchips. For group-housed animals, it is best to be able to identify individuals from a distance by means of a large identification tag placed around the neck. A unique pattern of clipped hair or marker or dye on the coat or skin can be used as methods for temporary identification.

Blood Collection

Approximate blood volumes for adults of several nonhuman primate species are listed in Table 12.3. Assuming the animal is mature, healthy, and on an adequate plane of nutrition, the blood volume of most species averages 7% of the body weight in grams. A typical rule of thumb for blood withdrawal suggests that up to 10% of the circulating blood volume can be withdrawn from normal, healthy animals every 2–3 weeks with minimal adverse effect. If larger volumes are required, collection intervals should be increased, hematocrits should be monitored, and fluid replacement therapy should be instituted to offset the volume depletion.

Blood samples can easily be obtained from percutaneous venipuncture of the femoral vein or artery. Sedation is required for larger animals, and the femoral triangle must be cleaned with alcohol or other antibacterial. The needle should be introduced just medial to the femoral pulse to obtain a venous sample. Individuals performing this technique must be familiar with the typical anatomy of the femoral triangle. If an arterial sample is taken, direct pressure must be applied for a minimum of 3–5 minutes to ensure adequate hemostasis.

Other collection sites include the cephalic and saphenous veins. Primates can be trained using positive reinforcement (operant conditioning) to offer their arms or legs for blood collection, but this requires a dedicated staff that can provide consistent training. The use of training is encouraged in the 2011 edition of the *Guide*, which states, "Habituating animals to routine husbandry or experimental procedures should be encouraged whenever possible as it may assist the animal to better cope with a captive environment by reducing stress associated with novel procedures or people. The type and duration of habituation needed will be determined by the complexity of the procedure. In most cases, principles of operant conditioning may be employed during training sessions, using progressive behavioral shaping to induce voluntary cooperation with procedures."

Surgically placed vascular access ports (VAPs) and tether systems allow for long-term blood collection and chronic dosing. The VAP can be maintained for many months with proper use of anticoagulants and sterile technique.

Table 12.4. Maximum dosing volumes (mL/kg) in select species of nonhuman primates

Route	Macaque	Marmoset	Baboon
IP	10.0	20.0	5.0
SC	5.0	5.0	3.0
IM	0.5 (3.0 mL max)	0.5 (1.0 mL max)	0.5 (3.0 mL max)
IV	2.0 (bolus)/10.0 (infusion)	2.5 (bolus)/10.0 (infusion)	2.0 (bolus)/5.0 (infusion)
PO	15.0	15.0	15.0

Source: Adapted from Lukas (1999).

Urine Collection

Urine can be collected by cystocentesis, placement of a urinary catheter, or free-catch. When performing cystocentesis, the bladder should first be palpated to ensure urine is present and the abdomen cleaned with alcohol and disinfectant. Utilizing ultrasound guidance, the needle should enter perpendicular to the abdomen just cranial to the pubic bone. To use the free-catch method, a metabolism cage or a collection pan with a wire grid to separate the urine and the feces both work well.

Drug Administration

Maximum dosing volumes for the various routes are listed in Table 12.4. Drug administration routes for NHPs are similar to those of most other large mammals. Oral dosing can be accomplished by placing the drug in a piece of fruit or on a piece of bread covered with peanut butter and jelly. Tablets can also be crushed or capsules opened to mix the drug in a favorite food. Primates, however, are adept at picking out the drug or eating around it. Many will accept drugs when mixed into a flavored yogurt or flavored syrup or juice; alternatively, animals can be trained to take juice from a syringe. Commercial vendors incorporate drugs into fruit-flavored tablets upon receipt of a prescription from a veterinarian. These tablets are well received and ensure accurate enteral dosing. Unpalatable drugs can be given to a lightly sedated animal through a nasogastric tube or orogastric tube using a bite block. The proper placement of the stomach tube should always be verified before administering drugs.

Subcutaneous (SC) injections can be administered under the loose skin over the dorsal cervical area. Intramuscular (IM) injections are usually given in the thigh muscles, using care to avoid the sciatic nerve. The triceps and gluteal muscles can also be used for intramuscular injections of larger monkeys.

The cephalic, jugular, or saphenous veins can be used to deliver drugs intravenously. Larger volumes should be administered through an indwelling catheter. Surgical implantation of a vascular access port is another alternative for long-term dosing. The port is implanted subcutaneously, usually on the back between the shoulder blades. Primates can be trained to present their port for injections into the port, or they can be restrained via a pole-and-collar system. Alternatively, the vascular system can be accessed through a tether system, which consists of a jacketed backpack pump or cage-top pump that allows for continuous infusion of drugs or saline. The vascular catheter exits through the skin on the back

of the animal and enters the backpack pump or through a swivel that protects the catheter as it courses up to the cage-top pump. Osmotic minipumps may also be implanted for extended drug delivery.

Anesthesia, Surgery, and Postoperative Care

Due to a higher basal metabolic rate, NW primates often require higher dosages of anesthetics per kilogram of body weight compared with OW species. Due to the great diversity in nonhuman primate species, a discussion of anesthesia for the many different species is beyond the scope of this book. Presented here is a general overview of anesthesia, surgery, and postoperative care for the most common species used in biomedical research, the macaque (specifically *M. fascicularis*, *M. mulatta*). Agents commonly used for anesthesia and tranquilization are listed in Table 12.5. Macaques are usually anesthetized with an injection of ketamine hydrochloride at 10 mg/kg IM to remove them from the cage safely. This dose may be adequate for endotracheal intubation; the animal can then be maintained by inhalation anesthesia. If the initial dose is inadequate, a small intravenous bolus of ketamine or propofol can be given to achieve intubation. Inhalants, including isoflurane and sevoflurane, are safe and commonly used in nonhuman primates. Animals should be premedicated with atropine or glycopyrrolate to minimize salivation and bradycardia.

Ketamine alone is not satisfactory for major surgery, since it does not provide adequate analgesia. The ketamine/xylazine combination, however, can be used for minor, short-term procedures. This injectable regimen usually provides about 30–45 minutes of anesthesia. Propofol at 5–10 mg/kg when given intravenously through an indwelling catheter provides a smooth induction and can be used to maintain anesthesia by constant-rate infusion, but it will induce a brief period of apnea and will not provide adequate analgesia for very invasive procedures. The depth of anesthesia is best gauged by the rate and depth of respiration, heart rate, and degree of jaw tension. The palpebral and pedal reflexes may also be used. Intraoperatively, body temperature should be maintained by use of a circulating water heating blanket. Warmed intravenous fluids are also helpful in preventing hypothermia. Placement of an indwelling catheter is important for long-term anesthesia, allowing for administration of fluids and emergency drugs. Sterile technique is imperative.

Some of the routine surgeries performed on nonhuman primates include finger and tail amputations and laceration repair due to fight injuries. The use of a subcuticular suturing pattern is recommended as it usually prevents the animal from removing the sutures and eliminates the need for suture removal. Postoperatively, the animal should be placed in a recovery cage (or in the home cage if housed singly); supplemental heat such as a heat lamp should be provided to help prevent hypothermia. Frequent observation is required until the primate is able to maintain an upright position.

It is absolutely essential that pain be minimized through the use of analgesics in nonhuman primates that undergo painful procedures. Provision of analgesics prior to the painful stimulus, or preemptive analgesia, is the most effective method of pain management. The dosage and frequency of administration of all analgesic agents must be tailored to the animal, procedure, and likely level of pain. Table 12.6 lists analgesics for use in macaques. Buprenorphine is recommended for control of acute or chronic visceral pain but can cause sedation. Butorphanol is recommended for mild postoperative discomfort. Nonsteroidal anti-inflammatory agents such as carprofen (2–4 mg/kg SC) and ketoprofen (2 mg/kg) provide

Table 12.5. Anesthetic agents and tranquilizers used in macaques

Drug	Dose	Route	Reference
Inhalants			
Isoflurane	3%–4% induction, 1.5%–2.0% maintenance	Inhalation	Feeser and White (1992)
Sevoflurane	4%–8% induction, 1.25%–4% maintenance	Inhalation	Murphy et al. (2012)
Injectables			
Acepromazine	0.5–1 mg/kg	IM, SC, PO	Johnson et al. (1981)
Carprofen	2–4 mg/kg q12–24h	PO, SC	Carpenter (2005)
Chlorpromazine	1–6 mg/kg	IM, PO	Johnson et al. (1981)
Diazepam	0.25–0.5 mg/kg	IM, IV	Ialeggio (1989)
Fentanyl	5–10 μg/kg IV bolus with 10–25 μg/kg/h continuous infusion	IV	Murphy et al. (2012)
Fentanyl patch	25 μg/kg/h × 2 patches	Topical	Carpenter (2005)
Fentanyl–droperidol	0.05–0.1 mL/kg	IM, IV	Johnson et al. (1981)
Fentanyl/fluanisone	0.3 mL/kg	SC, IM	Carpenter (2005)
Ketamine	10–15 mg/kg	IM	Carpenter (2005)
Ketamine	15 mg/kg	IM	Flecknell (2009)
+ diazepam	1 mg/kg	IM	
Ketamine	2.0–5.0 mg/kg	IM	Murphy et al. (2012)
+ medetomidine	0.03–0.05 mg/kg	IM	
Ketamine	15 mg/kg	IV	Carpenter (2005)
+ midazolam	0.05–0.15 mg/kg	IV	
Ketamine	10 mg/kg	IM	Flecknell (2009)
+ xylazine	0.5 mg/kg	IM	
Propofol	5–10 mg/kg	IV	Sainsbury (1991)
	2.5–5.0 mg/kg followed by 0.3–0.4 mg/kg/min	IV	Sainsbury (1991)
Tiletamine–zolazepam	2–6 mg/kg; marked hypothermia	IM	Ialeggio (1989)
Xylazine	0.5 mg/kg	IV	Popilskis and Kohn (1997)

IM = intramuscular; IV = intravenous; PO = per os; SC = subcutaneous.

up to 24 hours of analgesia for mild to moderate pain. Local analgesics such as bupivicaine should also be considered to provide enhanced pain control with a nerve block for highly invasive procedures, such as thoracotomies. Combinations of narcotics and nonsteroidals are commonly used. Select, commercially available supportive and critical care products are listed in Appendix 3.

Euthanasia

All methods of euthanasia must conform to the most current *AVMA Guidelines on Euthanasia* (AVMA, 2013). After sedation with an intramuscular injection of ketamine, primates

Table 12.6. Analgesic agents used in macaques

Drug	Dosage	Route	Reference
Acetaminophen	6 mg/kg q8h	PO	APV (2013)
Acetylsalicylic acid	20 mg/kg q8–12h	PO	APV (2013)
	25 mg/kg suppository	Rectal	Carpenter (2005)
Bupivicaine	1–2 mg/kg locally	Infiltration	Murphy et al. (2012)
Buprenorphine	0.005–0.03 mg/kg q12h	IV, IM, SC	Murphy et al. (2012)
Butorphanol	0.5 mg/kg q8h	IM	APV (2013)
Carprofen	3–4 mg/kg q24h	SC	Flecknell (2009)
Flunixin meglumine	0.5–2 mg/kg q24h	SC, IV	Flecknell (2009)
Ibuprofen	7 mg/kg q12h	PO	Flecknell (2009)
Ketoprofen	2 mg/kg q24h	IV, IM	APV (2013)
Meloxicam	0.1–0.2 mg/kg q24h	SC, PO	Flecknell (2009)
Morphine	1–2 mg/kg q4h	IM, IV, PO, SC	Rosenberg (1991)
Naloxone	0.01–0.05 mg/kg	IV, IM	Flecknell (1987)
Oxymorphone	0.15 mg/kg q4–6h	IM, IV, SC	Rosenberg (1991)

IM = intramuscular; IV = intravenous; PO = per os; SC = subcutaneous.

can be euthanized by an intravenous overdose of a barbiturate or commercial euthanasia solution. Other methods of euthanasia are permissible if the animal is fully anesthetized, such as a bolus of potassium chloride or exsanguination and perfusion with a tissue fixative. Refer to Chapter 4 for more details regarding euthanasia guidelines.

THERAPEUTIC AGENTS

When considering therapeutics for nonhuman primates, it is important to carefully consider the diversity of species. The wide range of body size and types of primates makes extrapolation from one animal to another undependable, and it should be avoided. Whenever possible, the scientific literature or species experts should be consulted to determine the safest drugs and dosages. Antimicrobial and antifungal drug dosages for use with macaques are listed in Table 12.7; antiparasitic agents are listed in Table 12.8, and miscellaneous drugs are listed in Table 12.9.

INTRODUCTION TO DISEASES OF NONHUMAN PRIMATES

Improvements in husbandry, diagnostics, and therapeutics have led to the identification and control of a wide range of pathogenic organisms in closed colonies of nonhuman primates today. When housed in large colonies in outdoor corrals, the incidence of disease tends to increase as there is a greater likelihood of pathogen exposure and often less environmental control. Basic knowledge of the agents that may infect a population is required to quickly

Table 12.7. Antimicrobial and antifungal agents used in macaques

Drug	Dosage	Route	Reference
Amikacin	5 mg/kg q8h	IM	APV (2013)
Amoxicillin	6.7–13.3 mg/kg q8h	IM, PO	APV (2013)
Amoxicillin + clavulanic acid	6.5–13.5 mg/kg q8h	PO	Carpenter (2005)
Amphotericin B	0.25–1 mg/kg q24h	IV	Johnson et al. (1981)
Ampicillin	20 mg/kg q8h	PO, IM, IV	Johnson et al. (1981)
Cefazolin	20 mg/kg q8h	IM, IV	APV (2013)
Cephalexin	30 mg/kg q12h	PO	APV (2013)
Chloramphenicol succinate	33.3 mg/kg q8h	IM	APV (2013)
Ciprofloxacin	10 mg/kg q12h	PO	APV (2013)
Clarithromycin	10 mg/kg q12h for 7d	PO	Carpenter (2005)
Clindamycin	12.5 mg/kg q8h	PO	APV (2013)
Doxycycline	2.5 mg/kg q12h	PO	Johnson-Delaney (1994)
Enrofloxacin	5 mg/kg q24h for 10d (to treat shigellosis)	PO, IM	Banish et al. (1993)
Erythromycin	75 mg/kg q12h for 10d	PO	RPRC-WA (1987)
	15–20 mg/kg q12h	IM	APV (2013)
Fluconazole	2–3 mg/kg q24h for 30d	PO	Carpenter (2005)
Gentamicin	2–4 mg/kg q12h	IM	APV (2013)
Griseofulvin	20 mg/kg q24	PO	APV (2013)
	200 mg/kg once q10d	PO	APV (2013)
Itraconazole	10 mg/kg q24h	PO	Carpenter (2005)
Ketoconazole	5–10 mg/kg q12h	PO	Carpenter (2005)
Lincomycin	5–10 mg/kg q12h	IM	Weller et al. (1992)
Methicillin	50 mg/kg q12h for 7d	IM	RPRC-WA (1987)
Metronidazole	12.5–15 mg/kg q12h	PO	Carpenter (2005)
	50 mg/kg q24h	PO	APV (2013)
Neomycin	50 mg/kg q12h	PO	APV (2013)
Nystatin	100,000 units q8h	PO	APV (2013)
Oxacillin	16.5 mg/kg q8h	SC, IM	Carpenter (2005)
Oxytetracycline	10 mg/kg q24h	SC, IM	Carpenter (2005)
Penicillin G, benzathine	20,000–60,000 IU/kg q24h	IM	APV (2013)
Penicillin G, procaine	20,000–40,000 IU/kg q12h	IM, SC	APV (2013)
Sulfamethazine	66 mg/kg q12h	PO	Carpenter (2005)
Tetracycline	20 mg/kg q8h	PO	APV (2013)
Trimethoprim	4 mg/kg q8h	SC, PO	APV (2013)
Tylosin	5 mg/kg q12h	PO	Carpenter (2005)
Vancomycin	20 mg/kg q12h	IM, IV	Carpenter (2005)

IM = intramuscular; IV = intravenous; PO = per os; SC = subcutaneous.

Table 12.8. Antiparasitic agents used in macaques

Drug	Dosage	Route	Reference
Albendazole	25 mg/kg q12h for 3–5 d	PO	Murphy et al. (2012)
Azithromycin	25–50 mg/kg q24h for 7 d	SC	Carpenter (2005)
Doxycycline	5 mg/kg q12h once, then 2.5 mg/kg q24h	PO	Carpenter (2005)
Fenbendazole	50 mg/kg q24h for 3 d, repeat in 3 wk, repeat in 3 mo	PO	APV (2013)
Ivermectin	0.2 mg /kg	PO, SC	APV (2013)
Levamisole	10 mg/kg	PO	Wolff (1990)
Mebendazole	50 mg/kg q12h for 3 d	PO	APV (2013)
	100 mg/kg q12h for 3 d, repeat in 3 wk (for *Trichuris*)	PO	APV (2013)
Mefloquine	25 mg/kg once	PO	Carpenter (2005)
Metronidazole	35–50 mg/kg divided q12h for 10 d	PO	Wolff (1990)
Paromomycin	10–20 mg/kg q12h for 5–10 d	PO	Marks (1994)
Piperazine	65 mg/kg q24h for 10 d	PO	Russell et al. (1981)
Praziquantel	5 mg/kg for one dose	PO, IM, SC	APV (2013)
Primaquine	0.3 mg/kg/d for 14 d; treat concurrently with chloroquine	PO	Wolff (1990)
Pyrantel pamoate	11 mg/kg for one dose	PO	Wolff (1990)
Quinacrine	2 mg/kg q8h for 7 d	PO	Swenson (1993)
Sulfadimethoxine	50 mg/kg/d for the 1st day, then 25 mg/kg/d	PO	Wolff (1990)
Thiabendazole	75–100 mg/kg, repeat in 3 wk	PO	Fraser (1991)
	50 mg/kg/d for 2 d (for *Strongyloides*)	PO	Wolff (1990)

IM = intramuscular; PO = per os; SC = subcutaneous.

and appropriately respond to a disease outbreak, if it does occur. The most common health problems of nonhuman primates are bacterial enteritis and bacterial pneumonia. These diseases may be latent in animals living in an unstressed state; however, active disease may be precipitated by the stress of transportation, a change in diet, or a new environment. Select vaccinations must be considered for all nonhuman primates, especially those housed in large colonies or outdoor facilities. Vaccination against measles, rabies, and tetanus should be strongly considered for macaques housed under these conditions.

Nonhuman primates should be placed in quarantine and receive a thorough physical examination soon after their arrival at a new facility. Subsequent physical examinations should be conducted at least annually although more frequent examinations would likely be warranted. These animals tend to be quite stoic, making the accurate detection of clinical signs of illness or distress challenging. The examination is performed in a manner similar to that for other species, with attention given to evidence of diarrhea, nasal or ocular discharge, dyspnea, conditions of the skin and hair coat, alertness, and nutritional state. It is equally important that an animal is observed, ideally without the animal's awareness of the observer's presence, prior to disturbing the animal in any way. This provides the observer the opportunity to appreciate changes in an animal's activity, locomotion, and interaction with other nearby animals that may be affected by the observer's presence. Areas that should receive

Table 12.9. Miscellaneous agents used in macaques

Drug	Dosage	Route	Reference
Acetylcysteine	50–60 mL/h for 30–60 min q12h	Inhalation	Johnson et al. (1981)
Aminophylline	25–100 mg/animal q24h	PO	APV (2013)
Antipamezole	0.2 mg/kg	SC, IM, IV	Murphy et al. (2012)
Atracurium	0.09–1.5 mg/kg	IV	Carpenter (2005)
Atropine	0.02–0.04 mg/kg	IM, IV, SC	APV (2013)
Biotin	20 µg per animal q24h	PO	Krasnow (1987)
Bismuth subsalicylate (Pepto-Bismol)	40 mg/kg q8–12h	PO	APV (2013)
Calcium chloride	10–20 mg/kg	IV	Carpenter (2005)
Calcium gluconate	200 mg/kg	SC, IM, IV	Carpenter (2005)
Captopril	1 mg/kg	PO	Carpenter (2005)
Chlorpromazine	1–3 mg/kg	IM	Carpenter (2005)
Cimetadine	10 mg/kg q8h	IM, PO	APV (2013)
Cisapride	0.2 mg/kg q12h with meals	PO	Hotchkiss (1995)
Dexamethasone	≤2 mg/kg	IV, IM, PO	Johnson et al. (1981)
Diphenhydramine	5 mg/kg q6–8h	IV, IM, PO	APV (2013)
Dobutamine	2–10 µg/kg/min	IV	Popilskis and Kohn (1997)
Dopamine	2–5 µg/kg/min	IV	Popilskis and Kohn (1997)
Doxapram	2 mg/kg	IV	Flecknell (1987)
Epinephrine	0.2–0.4 mg/kg diluted in 5 mL sterile water	IT	Fortman et al. (2002)
	0.1–0.5 mg	SC, IM, IV	APV (2013)
Erythropoietin	100 IU/kg	IM	Carpenter (2005)
Etomidate	1 mg/kg	IV	Carpenter (2005)
Flumazenil	0.02 mg/kg	IV	Murphy et al. (2012)
Folic acid	15 µg/kg q24h	PO	APV (2013)
Furosemide	2 mg/kg	PO	Johnson et al. (1981)
Glycopyrrolate	0.005–0.01 mg/kg	IM	Carpenter (2005)
Haloperidol	0.03–0.05 mg/kg q12h	IM	APV (no date)
Insulin, NPH	0.25–0.5 units/kg/d initially and adjust accordingly	SC	Johnson-Delaney (1994)
Isopreterenol	0.01–0.03 µg/kg/min	IV	Carpenter (2005)
Kaolin and pectin	0.5–1.0 mL/kg q2–6h	PO	Johnson et al. (1981)
Levythyroxine	0.01 mg/kg q12h	PO	Carpenter (2005)
Lidocaine	0.7–1.4 mg/kg prn	IV	APV (2013)
Mannitol (25%)	0.25–1.0 g/kg bolus over 20 min	IV	Murphy et al. (2012)
Metoclopramide	0.2–0.5 mg/kg q8–24h	IM	APV (2013)
Nalbuphine	0.5 mg/kg q3–6h	IM, IV	Carpenter (2005)
Naloxone	0.1 mg/kg as needed to reverse opioids	IV, IM, SC	APV (2013)
Neostigmine	0.07–0.08 mg/kg q2–4h	IM	APV (2013)
Nitroprusside	1–4 µg/kg/min	IV	Popilskis and Kohn (1997)
Omeprazole	0.4 mg/kg q12h	PO	Dubois et al. (1998)
Phenylephrine	One spray in each nostril q6h	Intranasal	Johnson et al. (1981)

(*Continued*)

Table 12.9. (*Continued*)

Drug	Dosage	Route	Reference
Prednisolone sodium succinate	10 mg/kg	IM, IV	APV (2013)
Prednisone	0.5–1 mg/kg q12h for 3–5 d, then q24h for 3–5 d, then q48h for 10 d, then half the dose q48h	PO	Isaza et al. (1992)
Protamine	1 mg/80 U heparin	IV	Popilskis and Kohn (1997)
Ranitidine	0.5 mg/kg q12h	PO	Carpenter (2005)
Tetanus antitoxin	1500 units	IV	Siegmund (1979)
Tryptophan	100 mg q12h	PO	Weld et al. (1998)
Vitamin C	25 mg/kg q12h for 5 d	PO	Ratterree et al. (1990)
Vitamin E + selenium	3.75 IU/kg + 1.15 mg/kg q 3d for 30 d	IM	Sainsbury (1991)
Yohimbine	0.1 mg/kg	IM, IV	APV (2013)

IM = intramuscular; IP = intraperitoneal; IV = intravenous; PO = per os; SC = subcutaneous; IT = intratracheal.

particular attention are body weight and body condition, the lymph nodes, the mouth, the palpable reproductive organs, and the digits. Body weights should be frequently monitored and considered in concert with a critical, hands-on examination of body condition so that unexpected changes can be quickly identified and investigated. Superficial lymph node enlargement or drainage can be suggestive of tuberculosis infection. Oral examination should include a thorough dental examination for detection of dental caries and broken teeth. Ulcers in a macaque are suggestive of the fatal zoonotic herpes B virus. Hemorrhage of the gums and gingivitis is characteristic of vitamin C deficiencies and gingivitis is associated with shigellosis in some species. The palpable reproductive organs (e.g., external genitalia, testicles, uterus, and ovaries) should be evaluated for evidence of trauma, neoplasia, and expected hormonal variations; digits should be evaluated for evidence of trauma such as may be incurred through fighting or entrapment in caging.

Zoonotic Diseases

One of the primary concerns in managing nonhuman primates is the protection of personnel from zoonotic, potentially fatal, diseases. All nonhuman primates must be regarded as potential sources of zoonotic disease (Box 12.4). Generally, the more closely related a species is to humans, the greater the danger for zoonotic disease transmission.

Box 12.4

All nonhuman primates must be regarded as potential sources of zoonotic disease.

Some of the significant bacterial diseases transmissible to humans are tuberculosis, shigellosis, salmonellosis, melioidosis, and staphylococcal and streptococcal infections. Among the

most significant viral diseases transmissible to humans from nonhuman primates are *Macacine herpesvirus 1* (herpes B), viral hepatitis, poxviruses, yellow fever, SV-40, poliomyelitis, rabies, and measles.

The most significant zoonotic parasites include protozoan and helminth parasites such as *Entamoeba histolytica* and *Enterobius vermicularis*. Malaria can also be transmitted if a vector is available. Many parasites of OW monkeys are transmissible to humans, while parasites of NW monkeys are more closely related to those of dogs and rodents and are generally not transmissible to humans.

The following sections describe diseases that may likely be encountered in modern research colonies in the United States. They include infectious diseases that may spread quickly through a colony, often with fatal results, or that are caused by a zoonotic or anthropozoonotic (transmissible from humans to animal) organism. In addition, select, common, noninfectious disease conditions are described.

Bacterial Diseases

Pneumonia and Respiratory Diseases

Nonhuman primates are susceptible to the human, bovine, and avian strains of *Mycobacterium* spp. The human strain, *Mycobacterium tuberculosis*, is by far the most frequent cause of simian tuberculosis. Although not the most common disease of nonhuman primates, it is one of the most devastating diseases likely to be encountered.

Tuberculosis is usually transmitted from humans to nonhuman primates in captivity, which underlies the importance of screening employees for inapparent tuberculosis infection. All nonhuman primate species are capable of contracting tuberculosis, but the disease is more prevalent in OW monkeys than in NW monkeys. Young macaques are the most susceptible group; rapid disease dissemination prior to the development of clinical signs is often seen. Disease development in older macaques, baboons, and apes is more similar to that in humans, with a slower progression. Experimentally induced tuberculosis has been observed to progress from initial infection to death within 6 weeks to 12 months. The primary route of transmission is through aerosols via the respiratory tract. Other routes include intestinal tract invasion; cutaneous infection such as by bites or tattoo needles; and exposure to the blood, sputum, excreta, cerebrospinal fluid, and exudates from lesions or tissues of infected animals.

The clinical signs of tuberculosis are usually not striking until the disease is in an advanced stage. The most commonly recognized signs are lethargy and weight loss. Other clinical signs are pneumonia, diarrhea, skin ulceration, and suppuration of lymph nodes. In some cases, no signs of illness are observed prior to sudden death of the animal.

Yellowish caseous nodules in the lungs and hilar lymph nodes are characteristic gross findings at necropsy. The liver, spleen, and lymph nodes of the abdominal, inguinal, and axillary areas are also frequently involved. Histologically, a classic presentation consists of tubercles with a central zone of caseous necrosis infiltrated with neutrophils and lymphocytes surrounded by a zone of epithelioid cells. Multinucleated giant cells of Langhans type may be seen. Mineralization and fibrosis of the tubercles are rare in nonhuman primates.

Diseases from which tuberculosis must be distinguished include lung mites, pulmonary nocardiosis, pseudotuberculosis, systemic mycoses, and neoplasms. No one premortem test is both sufficiently sensitive and specific to definitively diagnose tuberculosis infection (or

lack of infection). As a result, although a single test may be used in periodic colony screening, a combination of tests is recommended for more intensive screening or in the face of a potential disease outbreak. The intradermal tuberculin skin test (TST) remains one of the most commonly used screening tools. With this test, a known quantity of tuberculin is injected intradermally, usually in the upper palpebrae. The surrounding area is observed at 24, 48, and 72 hours postinjection for a delayed-type hypersensitivity response characterized by tissue edema, discoloration, ulceration, and necrosis. The eyelid is the preferred site as it facilitates reading of the test without recapturing the animal. Unfortunately, infected animals may not appropriately respond to this test (i.e., produce a false negative) due to anergy from repeated testing, recent measles vaccination, and immunosuppression such as may occur secondary to overwhelming tuberculosis infection or other concurrent disease. Alternatively, false-positive TST results may occur due to an animal's previous exposure to an organism antigenically similar to *Mycobacterium* spp., recent exposure to Freund's complete adjuvant, or traumatic injection technique. Other diagnostic methods developed for colony screening include *in vitro* assays to detect gamma interferon response or humoral immune response to tuberculin antigens.

Additional pre- and postmortem diagnostics are employed to confirm suspected *Mycobacterium* infections, including tissue, sputum, bronchoalveolar lavage, and gastric aspiration for acid-fast staining and cultures as well as polymerase chain reaction (PCR). Thoracic x-rays are occasionally employed to detect lung lesions in suspect animals. The lesions of nonhuman primate tuberculosis are often difficult to detect because calcium deposits within lymph nodes are rare and the heart blocks visualization of lesions around the hilar lymph nodes. Other factors that limit the value of thoracic radiographs are the small size of early lesions, lack of encapsulation, and the incidence of granulomas in many organs other than the lungs. Although treatment, such as long-term (approximately one year), systemic administration of isoniazid is possible for extremely valuable animals (e.g., great apes or select experimental animals), infected animals are most frequently culled due to the risk they present to animal colonies and humans. Combining isoniazid with streptomycin or other drugs may enhance its effectiveness. Disadvantages of using isoniazid include that it may (1) induce resistant strains, (2) mask the disease, (3) cause pyridoxine deficiency, and (4) alter experimental results. The most effective means of control is quarantine, testing, and elimination of reactors. Animals with a known or expected exposure to a tuberculosis-infected animal should be isolated and repeatedly tested for the disease or culled from the colony.

Another devastating pathogen of nonhuman primates is *Streptococcus pneumoniae*. *S. pneumoniae* is a common cause of fibrinopurulent pneumonia in OW primates that occasionally progresses to fibrinopurulent meningitis and arthritis with quick death. Transmission is by aerosol. Stress and waning passive immunity in neonates play a role in predisposing animals to infection and disease. *Bordetella bronchiseptica* has been reported to cause a high incidence of fibrinopurulent hemorrhagic bronchopneumonia in NW monkeys while it is less of a problem in OW primates. Other organisms often associated with pneumonia in primates include *Klebsiella pneumoniae*, *Pasteurella multocida*, *Haemophilus influenzae*, *Staphylococcus aureus*, and other *Streptococcus* spp.

Clinical signs of pneumonia are generally nonspecific and include fever, tachycardia, sneezing, coughing, mucopurulent nasal discharge, lethargy, anorexia, and dyspnea. In severe cases, cyanosis and prostration may be evident. With *S. pneumoniae*, meningitis with accom-

panying central nervous system signs is a frequent complication. The disease course may be several days to weeks.

Necropsy findings in pneumonia include lungs that are typically consolidated and red-to-gray. A fibrinous pleuritis, pericarditis, and occasionally empyema are characteristic of infections with *S. pneumoniae*. Meningitis appears grossly as a diffuse gray opacity of the meninges with an accumulation of yellow–white viscous material in the sulci.

Culture and determination of antibiotic sensitivities are important in selection of the most effective antibiotic. Penicillins, cephalosporins, enrofloxacin, tetracycline, and chloramphenicol are some antibiotics commonly used to treat bacterial pneumonia. Supportive treatment includes maintaining the ambient temperature at a comfortable level and providing nutritional supplements and fluids if animals refuse food and water. Bronchial dilators and decongestants are often useful.

Moraxella catarrhalis is the causative agent for "bloody nose syndrome" in macaques. *M. catarrhalis* is a common cause of sinusitis in people although it is debated if an identical bacterial strain infects both nonhuman primates and humans. The organism can be isolated from the nasopharynx of clinically normal macaques and thus does not always induce disease. Clinical signs include epistaxis and occasionally periorbital edema. Diagnosis is by isolation of the diplococcal organism and response to select antibiotics (e.g., amoxicillin–clavulanic acid, extended spectrum cephalosporins, trimethoprim–sulfa, tetracyclines, and fluoroquinolones).

Bacterial Gastroenteritis

Three of the most common types of bacterial gastroenteritis in primates are campylobacteriosis, shigellosis, and salmonellosis.

Campylobacter jejuni is the organism most frequently isolated from active cases of campylobacteriosis diarrheal disease and is primarily found in OW primates. Asymptomatic carriers are common, and transmission is fecal–oral. Clinical signs include watery diarrhea, most frequently without blood or mucous, and severe dehydration. Diagnosis requires fecal culture on special media incubated in a 5%–10% CO_2 environment, although serologic tests have been developed. Treatment includes supportive care including rehydration and correction of electrolyte abnormalities. The use of antibiotics has been debated.

Clinically apparent shigellosis is most frequently due to *Shigella flexneri* although other *Shigella* species may also induce disease. Transmission between nonhuman primates and from humans to nonhuman primates is by the fecal–oral route. Stress may precipitate clinical disease from a silent infection. Though adult humans seldom become clinically ill, the disease can be severe or even fatal in children. Infections in nonhuman primates and humans range from asymptomatic carriers to acute fulminant dysentery. Clinical signs in nonhuman primates include depression; blood-tinged, mucoid diarrhea; weakness; emaciation; and dehydration. Abdominal pain is often evident, and the affected primate may bend forward in a sitting position with its hands folded across its abdomen. Typically, as the disease progresses, the animal becomes semicomatose; death may occur from 24 hours to 2 weeks after onset of illness. Alternatively, nonenteric *Shigella* infections may occur and include gingivitis, abortion, and air sac infection. Culture is required for diagnosis although it should be noted that *Shigella* spp. may frequently be isolated from asymptomatic individuals. Treatment includes administration of antibiotics selected through antibiotic sensitivity screening and

supportive care and may be provided to both symptomatic and asymptomatic, but exposed, individuals. Strict environmental decontamination should be instituted.

Although less common than *Shigella* spp. and *Campylobacter* spp. infections, gastroenteritis from *Salmonella* spp. infections also occurs in nonhuman primates. Contaminated feed and contact with infected animals are the principal sources of infection with *Salmonella* spp. The clinical features of *Salmonella* spp. infection are similar to those of *Shigella* spp. infection except that vomiting is more common and the course of the disease is often less acute.

The gross lesions of shigellosis and salmonellosis are not readily distinguishable, and the two may exist as a mixed infection. In fatal cases of shigellosis, the colon is usually distended and contains mucus and occasionally blood. The mucosa is thickened, reddened, and may be ulcerated, and the surface covered by an exudate composed of fibrin and necrotic cells. Typical gross findings with salmonellosis are pasty-to-liquid intestinal contents, a swollen and reddened intestinal mucosa, and splenic congestion. *Salmonella* spp. infections frequently involve the ileum, whereas ileal involvement is infrequent with shigellosis.

It is often difficult to recover *Shigella* spp. organisms from rectal swabs; thus a negative culture is not conclusive. In culturing for intestinal pathogens, the swab should be inserted into the rectum and the surface of the mucosa scraped. The bacteria are very sensitive to drying, so swabs must be protected before presentation to the laboratory. Ideally, plates are streaked directly from rectal swabs immediately following specimen collection.

In general treatment of bacterial gastroenteritis includes provision of supportive care and antibiotic administration based on culture and sensitivity testing. Antisecretory drugs such as diphenoxylate hydrochloride, kaolin and pectin, or other intestinal absorbents may be indicated. Fluid and electrolyte replacement is essential in cases of severe diarrhea and are ideally administered intravenously although intraosseous or subcutaneous administration can be used if necessary. NW monkeys often require higher relative volumes of fluids compared with OW monkeys. Animals exposed to known infected animals should be evaluated for infection and considered for treatment.

Tetanus

Tetanus is caused by two neurotoxins produced by *Clostridium tetani*: tetanospasmin and tetanolysin. Both OW and NW monkeys, as well as apes, are susceptible. Clinical signs include tonic muscle spasms, trismus (lockjaw), dysphagia, opisthotonos, seizures, respiratory paralysis, and death. Vaccines (e.g., tetanus toxoid) are recommended for primates housed outdoors with soil contact. Tetanus antitoxin can be effective in treating cases of tetanus.

Other Zoonotic Bacterial Organisms

Other bacterial organisms, including *Helicobacter pylori*, *Lawsonia intracellularis*, *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, and *Burkholderia pseudomallei*, known to infect humans have been shown to cause disease in nonhuman primates. To date, the natural transmission of these organisms from nonhuman primates to humans has not been documented. However, their zoonotic potential should be understood and appropriate precautions instituted to guard against their transmission between species.

Several species of OW monkeys, especially rhesus, harbor *Helicobacter pylori* in their stomachs. *Helicobacter* spp. have been identified as causative agents for gastritis and gastric ulcers in humans and many other species. There are usually no clinical signs in nonhuman primates except inappetence and occasional vomiting. Diagnosis is based on gastric biopsy and culture results. Histologically, a lymphocytic plasmacytic gastritis may be present. Treatment is similar to that of humans, including a multiday, multidrug regimen; however, eradication of the organism may not be successful.

Lawsonia intracellularis, the cause of proliferative enteritis in several species, has been isolated from young rhesus, 6–16 months of age, with fatal proliferative enteritis. Epizootics of the disease are usually confined to younger animals. Infection occurs primarily in weanlings, when passive maternal immunity declines. Transmission is fecal–oral. Organisms localize to and multiply in intestinal proliferating crypt epithelial cells. Clinical signs included depression, mild diarrhea, and abdominal distension. Anemia and hypothermia are reported in severely affected individuals.

Pseudotuberculosis is caused by *Yersinia pseudotuberculosis* or *Yersinia enterocolitica* and has been reported in both OW and NW monkeys. Wild rodents and birds are reservoir hosts, and transmission is by ingestion of contaminated feed. Acute yersiniosis typically occurs as enteritis primarily affecting the jejunum and ileum. Clinical signs may include diarrhea, depression, and dehydration, or simply acute death. In its chronic form, septicemia results in necropurulent lesions in the liver, spleen, and other organs and may be confused grossly with tuberculosis. Microscopic liver or lung lesions consist of a central area of necrosis with neutrophils and clumps of bacteria surrounded by a zone of macrophages.

Melioidosis is caused by *Burkholderia pseudomallei*, which is a soil and water saprophyte typically found in tropical areas, including Southeast Asia and select regions of Australia, Central and South America, Africa, and the Middle East. It is currently designated as a Tier 1 select agent (www.selectagents.gov) by the Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture (USDA). The disease has been reported in OW monkeys and apes. The organism can remain clinically latent for years. Disease transmission typically results from contact with a contaminated environment via the integumentary, respiratory, or gastrointestinal system. The disease is not readily transmitted between individuals. The clinical course of melioidosis may be acute and fulminant or chronic. Clinical signs can be nonspecific or dependent on the affected organ system but may include bronchopneumonia, multiple subcutaneous abscesses, lymphadenitis, and swollen joints. Gross findings include suppurative or caseous nodules in tissues or organs—including the lungs, spleen, liver, and lymph nodes—and/or pneumonia. Infections are most frequently resistant to treatment.

Mycotic Diseases

Dermatophytosis

Ringworm in nonhuman primates is caused by several *Microsporum* spp. and *Trichophyton* spp. Long-term, systemic treatment with griseofulvin is suggested.

Systemic Mycoses

Candidiasis occurs in animals that are immunosuppressed, debilitated, or on long-term antibiotic therapy. Thrush is the most common presentation of *Candida albicans* and is seen as a white, velvety overlay in the mouth and throat.

Pneumocystis carinii is another fungal organism primarily seen as an opportunistic infection in immunosuppressed primates. Clinical signs include fever, dyspnea, and coughing. Fungal cysts can be identified with special stains such as Gomori's methenamine silver.

Histoplasma capsulatum var. *duboisii* is the etiologic agent of African histoplasmosis. Histoplasmosis has been reported in baboons maintained in outdoor colonies within the United States. Transmission is from dermal contact, inhalation, or ingestion of spores from contaminated soil as well as direct contact with infected primates. Organs affected include the skin, lymph nodes, and bones. Treatment includes appropriate antibiotic administration with or without surgical excision of affected areas.

Nocardiosis in nonhuman primates is most commonly seen as a granulomatous disease of the lungs resembling tuberculosis. Nocardiosis can be differentiated from tuberculosis by the absence of involvement of hilar lymph nodes.

Moniliasis and aspergillosis rarely occur in animals not previously debilitated from other causes. Naturally occurring infection with *Coccidioides immitis* (coccidioidomycosis) is limited to arid portions of the southwestern United States. *Blastomyces dermatitidis* is occasionally seen in animals housed outdoors. Rare reports of systemic *Cryptococcus neoformans* infections in nonhuman primates exist in the literature.

Viral Diseases

Herpesviruses

Although a large number of herpesviruses have been isolated from different species of nonhuman primates, the pathogenic effects of many of these isolates have not been established. A number of specific alphaherpesviruses do produce highly fatal systemic diseases. In most alphaherpesvirus infections, there are two types of host: a reservoir or natural host, in which the virus exists as a subclinical or latent infection; and an aberrant or accidental host of a different species, in which the infection is usually fatal. These viruses and their natural and aberrant hosts are important to consider when selecting the physical placement and path of personnel and the movement of equipment between colonies of different primate species.

Herpes simplex virus (*Human herpesvirus 1*) is the cause of fever blisters in humans. Humans are the reservoir hosts, and certain species of nonhuman primates, primarily marmosets, tamarins, and owl monkeys, are aberrant hosts that suffer fatal infections. Lesions in affected nonhuman primates include ulceration of the lips, tongue, and gastrointestinal tract, and hepatic necrosis.

The natural host of *Saimiriine herpesvirus 1* (SaHV1), previously known as Herpesvirus tamarinus, is the squirrel monkey, and infection in this species is asymptomatic. It can exist as a latent infection in squirrel, cebus, and spider monkeys. Host species may show oral vesicles or ulcers, similar to those of herpes simplex virus in humans and herpes B virus in macaques. Fatal infections most commonly occur in owl monkeys, tamarins, and marmosets. Lesions in fatally infected monkeys include facial swelling with self-mutilation; ulceration of the lips, tongue, and gastrointestinal tract; and hepatic necrosis. The virus is not zoonotic.

Other select herpesviruses of note include *Cercopithecine herpesvirus 2* (formerly known as Simian agent 8), *Cercopithecine herpesvirus 9* (formerly Simian varicella virus), *Saimiriine herpesvirus 2* (formerly Herpesvirus saimiri), and *Ateline herpesvirus* (formerly Herpesvirus

ateles). While these viruses are not recognized zoonotic agents, they do induce serious, often fatal, disease in their aberrant nonhuman primate host species.

Macacine herpesvirus 1, previously known as Cercopithecine herpesvirus 1 and commonly referred to as herpes B virus, is an alphaherpesvirus that is an important zoonotic disease of macaques. Macaques are the natural hosts for herpes B in which infection is lifelong with intermittent reactivation and shedding of the virus in saliva or genital secretions, particularly during periods of stress or immunosuppression. Humans are one of the aberrant hosts of this virus. Transmission to humans is usually through bites and scratches from infected macaques although transmission may also occur through scratches or punctures from contaminated medical equipment, fomites, or caging or through contact of an animal's infectious secretions with a person's mucous membranes. Infected macaques may develop conjunctivitis, vesicles or ulcers on the oral or genital mucosa, or may be asymptomatic. Obtaining macaques from herpes B–negative colonies is recommended but does not eliminate the possibility of disease. Because B virus is intermittently shed, persons in close contact with macaques must treat all macaques as potentially infectious carriers and wear appropriate protective clothing (e.g., gown, gloves, mask, full eye protection or face shield) to prevent exposure (Box 12.5). Universal precautions should be followed in handling all equipment and specimens that may be contaminated with macaque blood, urine, saliva, or tissues.

Box 12.5

Because B virus is intermittently shed, persons in close contact with macaques must treat all macaques as potentially infectious carriers and wear appropriate protective clothing to prevent exposure.

Personnel working with or around macaques must be educated about the clinical manifestations of herpes B infection. In humans, a potentially fatal encephalomyelitis is often the result of exposure. Although the incidence of human disease is very low, the case fatality rate is approximately 80% with death occurring within 10–14 days. An expert panel of virologists and physicians have developed and published "Recommendations for prevention of and therapy for exposure to B virus" (Cohen et al., 2002). These recommendations state, "Materials including supplies used for first aid and specimen collection, copies of written instructional materials, and treatment protocols for exposures should be available in areas where exposure can occur. Signs that indicate the proper actions to take in the event of exposure should be posted in areas in which exposures to macaques may occur." Following an exposure to the herpes B virus, potentially exposed skin should be washed with a solution containing detergent soap for at least 15 minutes in an attempt to reduce or eliminate the number of viable viral organisms. Eyes or mucous membranes potentially exposed to B virus should be irrigated immediately with sterile saline solution or water for 15 minutes. Following the immediate washing, the exposed individuals must be evaluated by the designated occupation health physician knowledgeable about herpes B virus. The physician will then determine the need for additional wound cleaning and/or the initiation of antiviral treatment.

Paired sera should be obtained from the human and macaque on the day of exposure as well as approximately 3 weeks later. Viral cultures of the buccal mucosa, genital mucosa, and conjunctiva of each eye of the macaque and of the human exposure site should be collected to determine if virus is present. False negatives can occur. Viral cultures should be submitted to the B Virus Research and Reference Laboratory at Georgia State University. Clinical signs in humans may include vesicles, pain and itching at the exposure site, lymphadenopathy, fever, numbness, muscle weakness or paralysis in the exposed extremity, conjunctivitis, neck stiffness, sinusitis, headache, nausea, vomiting, altered mental state, and other central nervous system signs. Early treatment of humans with an antiviral agent, before they show central nervous system signs, has resulted in halting the disease progression but does not appear to eliminate the virus. Exposed individuals should be closely monitored by a physician until it can be safely assumed that the individual is free of infection. During the intervening time, the individual should be counseled to avoid activities that could result in disease dissemination to other humans.

Measles

Measles (rubeola), a human paramyxovirus, can infect both OW and NW monkeys and apes although it is not naturally found in wild populations removed from human contact. Humans serve as the disease reservoir for nonhuman primates. Measles is highly contagious with transmission by aerosol or direct contact with secretions. Measles commonly produces a mild upper respiratory infection in macaques and most other species of OW primates. Macaques may exhibit a more severe respiratory illness, including pneumonia. Clinical signs most frequently seen in OW monkeys include nasal and ocular discharge, conjunctivitis, facial edema, blepharitis, and a papular skin rash. Occasionally, the infection will progress to depression, anorexia, coughing, and dyspnea in conjunction with giant cell pneumonia. Abortions and neurologic signs have also been reported. Morbidity is high and mortality, caused by secondary bacterial infections, is low in OW monkeys. The disease is much more severe in marmosets, owl monkeys, and colobines, in which hemorrhagic gastroenteritis predominates and respiratory tract lesions and the skin rash are absent or less significant. Active infection with measles produces a general immunosuppression that mutes the antigenic response to the tuberculin skin test, possibly resulting in a false negative reading.

The most important preventive measure is avoiding exposure to infected humans, the primary source of nonhuman primate infections although the virus can be transmitted between nonhuman primates. Some facilities require measles vaccination or proof of serologic immunity of all humans who enter nonhuman primate facilities (e.g., animal caretakers, visitors, building support personnel). A monovalent human measles vaccine (Attenuvax) has been shown to be effective in protecting both OW and NW monkeys from the disease. It should be noted that this vaccination can cause immunosuppression that interferes with the intradermal tuberculin test, and animals may require up to 4 weeks to return to normal immunocompetency. Due to this complication as well as the high cost of the human vaccine, an alternative, less expensive but equally effective vaccine (e.g., canine distemper vaccine, Vanguard-2), had been used. Unfortunately, production of both vaccines has been suspended and an alternative monovalent measles vaccine has not yet been validated for use in nonhuman primates. Polyvalent vaccines may be shown to be efficacious in measles prevention; however, they may produce an immune response that interferes with select research (e.g.,

immune response to adenovirus from a human measles–mumps–rubella vaccine that interferes with adenovirus-based research).

Poxviruses

Five different poxviruses are known to naturally infect nonhuman primates: monkeypox, Yaba-like disease virus, Yaba monkey tumor virus, cowpox, and *Molluscum contagiosum*. All five viruses can infect humans. In most cases, only epidermal changes are seen in NHPs and humans, and infections are self-limiting. The incidence of each virus should be extremely low in a modern nonhuman primate research facility.

Monkeypox is closely related to smallpox (variola) and vaccinia viruses and affects NW and OW monkeys and apes (including humans). Characteristic lesions associated with the disease in nonhuman primates are 1- to 4-mm circular papules that develop predominantly on the skin of the hands, feet, and face and that progress in approximately 10 days to vesicles, pustules, and scabs. The respiratory system may also be affected. No specific treatment exists. Human disease is seen mainly in Africa with clinical signs similar to early smallpox disease, including fatigue, fever, muscular and back pain, and disseminated pox skin lesions. A small percentage of fatalities have occurred in infected children. In 2003, there was an outbreak of monkeypox in people associated with pet prairie dogs. Immunization with vaccinia protects both human and nonhuman primates against the disease.

Yaba-like disease virus (YLDV), was once believed to be identical to the closely related *Tanapox virus*, a benign cutaneous infection of humans. While NW primates are resistant, OW primates are susceptible to the disease and develop skin lesions that are circular, with umbilication and an adherent scab in the center. Papules initially appear similar to monkeypox lesions but do not progress to pustules. Histologically, there is hyperplasia of the epithelium of the skin and epidermal necrosis with little damage to the underlying dermis. A notable characteristic of this disease is that there are usually only a few epidermal lesions seen on each infected animal. The lesions usually regress spontaneously in 3–4 weeks. Human infections can occur and are characterized by pyrexia and skin papules and nodules that progress to pock lesions.

Yaba monkey tumor virus (YMTV) is an oncogenic virus originally isolated from rhesus monkeys at Yaba, Nigeria. Few natural outbreaks of disease have been reported in rhesus and baboons; NW primates are resistant to the disease. Transmission is unknown but is suspected to be through arthropod vectors, trauma, or nosocomial from the use of contaminated needles between animals. The virus can infect both NHPs and humans and is characterized by the presence of nodular lesions, primarily on the extremities, that progress up to multiple centimeters in diameter, may ulcerate, and then completely regress within approximately 6 weeks. Histologically, the tumors consist of proliferations of histiocytes, frequently with eosinophilic intracytoplasmic inclusions.

Cowpox virus has been identified in OW primates as well as in NW primates, in which the disease may be fatal. Transmission is through rodent vectors not present in the United States; therefore, while it is a zoonotic agent, the risk to personnel in U.S. facilities is exceedingly low.

Molluscum contagiosum is a poxvirus disease primarily of humans. Transmission is usually by direct contact. A similar, if not identical, viral infection has been reported in chimpanzees with skin lesions that appear as waxy papular cutaneous elevations, especially on the eyelid

and groin. Histologically, there are large, basophilic intracytoplasmic inclusion bodies called molluscum bodies. It is unknown if the viral agent of nonhuman primates can directly infect humans.

Treatment of poxvirus lesions, if indicated, should be directed at prevention of self-mutilation and secondary infection through the use of sedatives, antipruritic drugs, and antibiotics.

Hepatitis Viruses

Hepatitis A virus (HAV), a hepatovirus, causes a disease that was formerly known as infectious hepatitis. Naturally occurring infections have been reported in several nonhuman primate species, including rhesus, cynomolgus, African green monkeys, owl monkeys, chimpanzees, and marmosets. Transmission is fecal–oral. Humans and nonhuman primates are usually asymptomatic. Prevention involves attention to hand washing and personal hygiene as numerous cases of nonhuman primate-to-human transmission have been documented. Personnel working with experimentally infected HAV monkeys should be vaccinated with a commercially available HAV vaccine.

Hepatitis B virus (HBV), known as *Hepadnavirus B*, induces a significant viral hepatitis in humans in which approximately 8% of infected people become chronic carriers and may eventually develop hepatocellular carcinoma. Naturally occurring infections have been reported primarily in wild chimpanzees and gorillas which remain asymptomatic. Transmission within these species is by parenteral inoculation, droplet exposure of mucous membranes, or contact exposure of broken skin with contaminated blood, saliva, semen, cerebrospinal fluid, or urine. Although disease transmission has not been documented between nonhuman primates and humans, the possibility remains. As such, personnel working with chimpanzees and gorillas should be vaccinated with a commercially available HBV vaccine.

Hepatitis C virus (HCV), a *Hepacivirus*, causes a hepatic disease that was previously called non-A, non-B hepatitis (NANBH). Transmission is parenteral and is similar to that for HBV. It appears that there are no naturally occurring cases of this viral disease in nonhuman primates, and only experimentally infected chimpanzees pose a zoonotic hazard to personnel. There is no vaccine available to prevent this disease.

Viruses Causing Hemorrhagic Fevers

Multiple hemorrhagic fever viruses (e.g., Ebola virus, Marburg virus, and simian hemorrhagic fever virus) can infect nonhuman primates and may induce seroconversion or often fatal disease in humans. While these viruses are of specific concern when working with nonhuman primates (or their cells or tissues) imported from select foreign locations or with purposefully infected animals, the viruses are not expected in U.S. facilities as they are heavily screened for in all nonhuman primates imported into the country.

Encephalomyocarditis Viruses

Encephalomyocarditis viruses (EMCV) are a collection of cardioviruses that have been shown to cause myocarditis in nonhuman primates, elephants, pigs, and other species. Various nonhuman primate species, including orangutans, chimpanzees, baboons, squirrel monkeys, rhesus macaques, tamarins, and lemurs have been affected, most commonly in zoo

populations. Rodents, especially rats and mice, are believed to be the reservoir host. Sudden death and frothy exudate from the nose and mouth are the primary clinical signs seen in nonhuman primates. Gross findings include pericardial effusion and pale streaks in the myocardium. Histologically, there is necrosis of the myofibers with inflammation and edema. Controlling rodent populations around outdoor housing as well as protecting food and water sources from rodent contamination is important in preventing infections. EMCV detection by PCR is the most rapid, sensitive, and specific method for the diagnosis of this infection. PCR methodology can reduce the frequency of false negative diagnoses of this virus. Although this virus can infect humans, human infections are exceedingly rare and the means of disease transmission are not understood.

Retroviruses

The *Retroviridae* family consists of two subfamilies: *Orthoretrovirinae* and *Spumaretrovirinae*. To date, only viruses within *Orthoretrovirinae* have been shown to cause clinical disease in nonhuman primates. There are endogenous and exogenous viruses within the *Retroviridae* family. *Endogenous viruses* have become incorporated into the host's genomic material and are transmitted vertically. *Exogenous viruses* exist outside of the host and are transmitted horizontally.

Simian T-cell leukemia virus (STLV) belongs to the genus *Deltaretrovirus* and is an exogenous type C oncovirus. It is 90%–95% homologous to the human T-cell leukemia virus (HTLV) that induces adult T-cell leukemia/lymphoma and other disorders. There are numerous strains of STLV, just as there are numerous strains of HTLV. Naturally occurring infections have been reported in baboons, patas monkeys, African green monkeys, macaques, and chimpanzees. Transmission is by sexual contact or parenteral inoculation. Most animals remain asymptomatic; however, a leukemia/lymphoma syndrome has been reported in baboons, African green monkeys, and macaques. Diagnosis is by serology and identification of the virus in tumor cells by molecular biologic techniques. The STLV infection is an important model for studying HTLV infections in humans. PCR can be used to identify monkeys that are positive for STLV viruses.

Two groups of simian retroviruses are of particular importance to nonhuman primates: simian retrovirus (SRV; formerly known as simian retrovirus type D) and simian immunodeficiency virus (SIV), which is closely related to the etiologic agent of human AIDS. SRV and SIV differ in the species of nonhuman primates they infect, their prevalence in captive populations, their clinical course in symptomatic individuals (often variants of immunodeficiencies), and their associated diseases. However, susceptible animal populations should be screened for these viruses as they may be significant confounders of study results. The zoonotic potential of the simian retroviruses is not fully understood but is suspected to be low, except possibly in severely immunocompromised humans.

Rabies

Rabies occurs occasionally in nonhuman primates. Animals housed outdoors are particularly at risk for contracting the disease due to their possible interaction with rabid wildlife. Clinical signs include irritability, aggression, hypersalivation, self-mutilation, paralysis, and sudden death. Only killed vaccines should be administered to nonhuman primates as vaccination with a modified-live rabies vaccine has been suspected to induce the disease.

Miscellaneous Viral Infections

Other viral infections that have been reported less frequently in nonhuman primates include yellow fever, chicken pox, Epstein–Barr virus, adenovirus, papillomavirus, papovavirus, lymphocytic choriomeningitis, and cytomegalovirus.

Parasitic Diseases

Protozoa

Entamoeba histolytica is pathogenic for OW and NW monkeys and apes, including humans, in whom amebic dysentery is induced. *E. histolytica* must be differentiated from the nonpathogenic protozoan *E. dispar*, which is morphologically identical. Asymptomatic carriers are common although clinical diarrhea is occasionally seen. The most common lesions are gut ulceration and hepatic abscesses. Ulcers in the gut typically have a flask shape. Special techniques are needed to recover and identify the organism because it is not ordinarily detected by routine fecal flotation. Detection of specific *E. histolytica* antigens in stools is a fast, sensitive technique that should be considered the method of choice. Stool PCR is a highly sensitive and specific technique, but it is more costly. The utility of serologic tests in distinguishing *E. dispar* from *E. histolytica* is controversial. However, serology is still considered the method of choice for diagnosis of extraintestinal amebiasis. Recommended treatments include metronidazole, tetracycline, chloramphenicol, paramomycin, and diiodohydroxyquin.

Balantidium coli is frequently found in fecal specimens of various nonhuman primate species, especially the anthropoid apes. Asymptomatic infections are common. Transmission is fecal–oral through ingestion of contaminated food or water. The pathogenicity of the organism for nonhuman primates has not been clearly established, but ulcerative lesions in the large intestine have been reported and set the stage for systemic spread. Infections in humans may result in severe diarrhea and dysentery. Recommended treatments for elimination of *B. coli* include metronidazole, tetracycline, and diiodohydroxyquin.

Both OW and NW monkeys as well as apes can contract malaria through infection with various *Plasmodium* spp. Females of various species of anopheline mosquitoes transmit malaria. The life cycle in nonhuman primates occurs in the hepatocytes and red blood cells. Some *Plasmodium* spp. cause cyclic bouts of fever and anemia due to lysis of red blood cells caused by multiplication of the protozoan organisms. Infected animals may be a source of infection for humans if the required species of mosquito vectors are present or via parenteral inoculation. Gross findings include gray lungs, liver, and spleen due to malarial pigment in the macrophages as well as hepatosplenomegaly. Common treatment regimens include use of chloroquine and primaquine; however, chloroquine-resistant strains of malarial organisms are increasingly common.

Hepatocystis spp. are protozoans related to *Plasmodium* spp. These organisms infect African and Asian OW monkeys, primarily baboons, African green monkeys, and macaques. Transmission is through the bite of a midge, the intermediate host. Infected monkeys remain asymptomatic because replication of the organism occurs only in the hepatocytes. Gross findings include 2- to 4-mm opaque cysts or scars in the liver, and adhesions to the liver may be present. Neither treatment nor prophylaxis is commonly necessary as the intermediate host is not present in the United States. However, infections of imported nonhuman primates are common.

Nematodes

Oesophagostomum spp., or nodular nematodes, are the most common helminth parasites affecting OW monkeys and apes; they also infect multiple other species of primates, but rarely NW monkeys. The infection is usually asymptomatic, but heavy burdens can cause diarrhea, unthriftiness, weight loss, and intra-abdominal adhesions with parasite death or migration. The parasite produces firm, smooth, black or white nodules in the wall of the large intestine and cecum and mesentery that initially contained the viable adults. Premortem diagnosis is by observation of the eggs or adults in the feces, although this can be difficult as the eggs are indistinguishable from hookworm eggs. Postmortem diagnosis is by histologic observation of the characteristic nodules. Thiabendazole, levamisole phosphate, and mebendazole can be effective treatments. The parasite may infect humans, thus appropriate sanitation and animal handling procedures should be followed.

Strongyloides fulleborni and *Strongyloides cebus* are very common zoonotic parasites of nonhuman primates. Infections are frequently asymptomatic, but heavy infections can cause severe diarrhea and coughing from migration of the larvae through the lungs. Apes are more prone to develop severe clinical disease. Thiabendazole and ivermectin are effective in treating strongyloidiasis. Organisms are capable of penetrating human skin if appropriate personal protective equipment is not used when contacting infected animals and their feces.

Adult *Dipetalonema* spp. are commonly seen in the peritoneal cavity of NW monkeys and occasionally are found in OW monkeys. Midges serve as parasite vectors. Microfilaria appear in the blood of infected animals. Although often found in large numbers, the parasite appears to cause only mild proliferative lesions in the peritoneal cavity. Treatment is not indicated. Zoonotic transmission is possible.

Enterobius vermicularis, the common pinworm of humans, is commonly found in captive chimpanzees. Cross-infection between chimpanzees and humans occurs readily. Pinworm infections in simian primates are usually not serious but can cause perianal pruritus and restlessness. Heavy infections, however, are occasionally fatal. The ova are more readily detected by application of cellophane tape to the perianal area or by perianal swabs than by fecal flotation. It is best to perform diagnostic exams on consecutive days to increase the chance of detecting ova. Effective treatments include thiabendazole, mebendazole, and pyrantel pamoate.

Tapeworms

Larval cestodes, including cysticercus larvae (*Taenia* spp.), coenurus larvae (*Multiceps* spp.), hydatid larvae (*Echinococcus* spp.), and *Hymenolepis nana* larvae, are seen with some frequency. Transmission is by ingestion of infective eggs or intermediate hosts, such as arthropods. These are usually incidental findings at necropsy or surgery, as there are rarely clinical signs of infection. Larvae may be found in subcutaneous tissues, muscles, and in the abdominal, peritoneal, or cranial cavities. Select cestodes of nonhuman primates (e.g., *Hymenolepis nana* and *Echinococcus granulosus*) are infectious to humans.

Acanthocephalans

Prosthenorchis elegans and *P. spirula* are the thorny-headed helminths of NW primates. The cockroach and beetle serve as intermediate hosts. The adults burrow deeply into the mucosa of the terminal ileum, cecum, or colon, causing abscesses, granulomas, and occasionally

peritonitis. A large population of adults can cause intestinal blockage. The use of praziquantel reduces the population of adults but will not eliminate all the parasites. Zoonotic infections have not been reported and are unlikely.

Arthropods

The lung mite, *Pneumonyssus simicola*, is an arthropod capable of infecting OW primates. Although previously a common finding in wild macaques, the use of anthelmintics in captive breeding programs has almost eliminated this mite. Affected animals are usually asymptomatic. At necropsy, pale yellow foci containing the mites are found throughout the lungs, and in some cases, cavitation of the lungs is seen. The lesions of lung mites may grossly resemble those of tuberculosis. Histologically, the presence of brown to black pigment and a focal bronchiolitis are characteristic of lung mite infestation. Radiographically, diffuse interstitial densities or discrete opaque densities may be seen throughout the lung lobes. Secondary bacterial bronchopneumonia may develop. Ivermectin appears to be an effective treatment. The parasite is not zoonotic.

Ectoparasites of nonhuman primates include lice and mites. Several of the more common external parasites reported are *Sarcoptes scabiei*, *Demodex* spp., *Pedicinus* spp., and *Psorergates simplex*. Topical organophosphate insecticides and pyrethrins, as well as parenteral ivermectin, are effective in treating external parasites.

Miscellaneous Conditions

Acute Gastric Dilation

Acute gastric dilation with or without torsion occurs with some frequency in macaques and baboons. The exact etiology is unknown, but there is evidence that clostridial organisms are involved. *Clostridium perfringens* is usually isolated from the colon contents of animals dying of bloat; however, the condition has not been reproduced by administration of the organism. Chronic broad-spectrum antibiotic administration, anesthesia, excessive food intake, and excessive water intake are considered predisposing causes. Large amounts of raw vegetables or fruits may be particularly problematic. The condition is most often seen in animals that hoard food from cagemates or animals in which full feeding was resumed after a period of restricted feeding. Most deaths occur at night following the evening feeding. Gaseous distention of the stomach develops rapidly. The abdominal distention is extreme and may be accompanied by an accumulation of subcutaneous gas. Ingesta may be seen in the oral cavity, but neither frank vomiting nor bloody diarrhea is a feature of acute gastric dilation.

Mortality is high even when the condition is detected early and vigorous treatment is instituted. Recommended treatment consists of passing a gastric tube to relieve the gas and excess fluid contents until peristalsis is resumed. Supportive therapy in the form of intravenous fluids and analgesics should be administered. The use of antibiotics and steroids is also thought to increase the chances of survival. Bloat in primates is prevented by multiple feedings of small quantities of food plus full access to water.

Trauma

Bite wounds are the most frequent problem seen in group-housed primates. Wounds may be very extensive and often involve the extremities and face. Fresh lacerations should be

thoroughly cleaned and, depending on the degree of tissue trauma, gross contamination, and time until treatment, sutured using a subcuticular pattern to decrease the chance of the animal disturbing the sutures. Broad-spectrum antibiotics, such as enrofloxacin, are indicated, since the oral cavity carries a heavy microbial burden. Bandages are difficult, if not impossible, to keep in place, and self-mutilation following injury often poses a problem. Crushing injuries of the muscles may release large amounts of methemoglobin, which can lead to acute renal failure if left untreated. Intravenous fluid therapy must be aggressive to help diurese the kidneys; otherwise, treatment is essentially the same as for other species. Primates should receive tetanus antitoxin after penetrating injuries if they have not been previously immunized.

Metabolic Bone Disease and Vitamin D Deficiency

Metabolic bone disease, referred to as simian bone disease, cage paralysis, or osteodystrophia fibrosa (secondary hyperparathyroidism), results from a deficiency of vitamin D and an imbalance in the calcium-to-phosphorus ratio (a much higher content of phosphorus than calcium), combined with inadequate exposure to direct sunlight. This disease syndrome is due to a nutritional secondary hyperparathyroidism, which results in bone resorption and fibrous replacement.

Vitamin D deficiencies, most frequently seen in NW primates, cause rickets in young animals and osteomalacia in adult animals. In rickets, calcification of the cartilage never occurs. In osteomalacia, calcium resorption causes decalcified bones. NW primates cannot utilize vitamin D_2 and thus require a diet containing vitamin D_3. Clinical signs include decreased activity, pain, paresis, osteomalacia, kyphosis, deformities of long bones, multiple fractures, and high levels of serum alkaline phosphatase. Radiographs show thinning of cortical bone. Treatment should be aimed at correcting the diet by providing vitamin D_3, calcium, and a proper calcium-to-phosphorus ratio. A full-spectrum light source will also aid in the production of adequate vitamin D_3 levels. Appropriate treatment with 5000 IU of vitamin D_3 per kilogram per week plus a diet high in calcium and protein will prevent further progression of metabolic bone disease. This disease should not occur in modern nonhuman primate research facilities as commercial, nutritionally complete diets are readily available for all primate species.

Scurvy

Without an adequate dietary supply of vitamin C, scurvy develops in nonhuman primates. The usual clinical signs are swelling of the epiphyses of long bones and hemorrhage of the gums, eyes, and periosteum. Cephalohematoma is a unique clinical manifestation seen in squirrel monkeys. Treatment of hypovitaminosis C consists of administration of 25 mg/kg per day of vitamin C until clinical signs disappear. Response to therapy is usually rapid, with clinical signs resolving in several weeks. Prevention of scurvy is dependent upon feeding diets formulated with appropriate vitamin C levels that have been stored at an appropriate temperature and for no longer than recommended by the feed manufacturer. As with metabolic bone disease, scurvy is largely a historical disease as nutritionally complete diets are readily available for all nonhuman primate species. There have been, however, recalls of primate diets by manufacturers due to errors in vitamin C. If suspicious clinical signs are present, diet recall information should be checked and the animal treated.

Endometriosis

Endometriosis is a condition in which endometrial glands and stroma are found in abnormal locations, most commonly on the serosal surface of pelvic organs. Endometriosis is very commonly seen in middle-aged to older rhesus monkeys. Clinical signs may be absent or may consist of irregular menstrual cycles, infertility, periodic inactivity, or decreased food consumption. Abdominal masses may occasionally be palpable, especially when adhesions have developed. The pathogenesis of this disease in humans and nonhuman primates is unresolved. Treatment usually consists of surgical removal of the abnormally placed endometrial tissue, ovariectomy, hysterectomy, and possibly hormonal therapies such as medroxyprogesterone and leuprolide. Analgesic use should be strongly considered. Extremely advanced cases may warrant euthanasia.

Fatal Fasting Syndrome of Obese Macaques (Fatal Fatty Liver Syndrome)

This syndrome is seen predominantly in obese female macaques, older animals, and in periods of anorexia. Clinical signs include anorexia, lethargy, weight loss, and high mortality. Histologically, there is fatty change in both the liver and kidney. The mechanism of action is unknown but may be similar to hepatic lipidosis of cats. Treatment using a percutaneous endoscopic gastrotomy (PEG) tube and enteral feeding has been described as an effective method to provide nutritional support until normal eating behaviors resume.

Amyloidosis

Amyloidosis, a condition in which extracellular accumulations of amyloid protein fibrils are deposited in various organs or tissues, is common. While amyloidosis can develop as a result of multiple underlying causes, the most frequently associated inciting cause is a chronic infectious or inflammatory process resulting in amyloid disposition primarily in the liver and intestines with subsequent weight loss with or without diarrhea. Clinical signs are dictated by the organ(s) or tissue(s) affected. Affected organs may be notably large on palpation. The condition is diagnosed through observation of the birefringent amyloid deposits seen with Congo Red stain of affected tissues collected through biopsy or at necropsy. Some characteristic blood chemistry abnormalities may be noted. Most severely affected animals are euthanized without treatment.

Arthritis

Arthritis is a common disease of many species of older nonhuman primates and is commonly seen in macaques. Clinical signs include enlarged joints, limited joint mobility, muscle contracture, and wasting. Interphalangeal and knee joints are most commonly affected. Treatment with polysulfated glycosaminoglycans has been proposed by some to be beneficial in alleviating clinical signs. Analgesics and an increased opportunity for regular movement and exercise are recommended; human arthritis medications may be indicated for severe cases.

Marmoset Wasting Syndrome

Marmoset wasting syndrome is poorly understood but is characterized by marked and progressive weight loss, muscle atrophy, and hair loss concurrent with chronic diarrhea, colitis, and hemolytic anemia in both captive marmosets and tamarins. The syndrome's etiology is unknown but has been postulated to involve nutritional vitamin E deficiency and

possibly protein deficiency as a sequela to chronic diarrhea. Treatment of affected animals has not been successful, but nutritional supplementation of vitamin E, selenium, zinc, and copper has been successful for disease prevention.

Dental Disease

Dental disease is a common condition of captive nonhuman primates, frequently involving excess tartar accumulation, gingivitis, tooth abscesses, dental caries, and dental trauma. Primary dental disease can progress to severe systemic disease (e.g., tooth root abscess progressing to cardiac valve disease). As such, the oral cavity and associated structures should be assessed at the time of each physical exam and routine dental cleaning. Abscesses of the upper canine teeth are common in squirrel monkeys. Diagnosis and treatment of severe dental disease in all species should be performed in collaboration with dental professionals.

Although once common, the routine extraction or blunting of canine teeth ("disarming") is no longer acceptable due to animal welfare concerns and the high incidence of severe, long-term complications (e.g., tooth root abscesses, unintended tooth migration resulting in malocclusion) induced.

Extreme caution should be taken when performing dental work on nonhuman primates, especially macaques, because the possibility of aerosols, splatter of blood, and accidental self-injury (such as during tooth extraction) is significant. Personnel involved in the dental work must wear personal protective equipment (PPE) appropriate for all work with macaques. Additional PPE may be indicated based on the procedures to be performed (e.g., respirator use during ultrasonic dental scaling).

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CHAPTER 12 REVIEW

Fill in the Blank

Fill in the blank with one of the following:

NW = New World monkeys
OW = Old World monkeys

1. _____ Are narrow-nosed.
2. _____ Do not require dietary vitamin D_3.
3. _____ Some have prehensile tails.
4. _____ Do not have opposable thumbs.
5. _____ Some have cheek pouches.
6. _____ Have three premolar teeth per quadrant.
7. _____ Ischial callosities are present in some.
8. _____ Are from Central and South America.
9. _____ Are broad-nosed.
10. _____ Are from Asia and Africa.

(*Continued*)

Multiple Choice

11. Scurvy is the result of a vitamin _____ deficiency.
    A. E
    B. B
    C. C
    D. D
12. Nodular nematodes
    A. *Enterobius*
    B. *Strongyloides*
    C. *Prosthenorchis*
    D. *Oesophagostomum*
13. Retrovirus that is closely related to the etiologic agent of human AIDS
    A. SIV
    B. EMCV
    C. Ebola
    D. HAV
14. Nonhuman primate extensively used to study *Plasmodium* spp.
    A. squirrel monkey
    B. rhesus
    C. owl monkey
    D. baboon
15. The common marmoset
    A. *Macaca mulatta*
    B. *Callithrix jacchus*
    C. *Sapajus apella*
    D. *Papio anubis*
16. Zoonotic virus that is commonly referred to as herpes B
    A. *Macacine herpesvirus 1*
    B. hepatitis B virus
    C. Yaba monkey tumor virus
    D. *Saimiriine herpesvirus 1*
17. *Mycobacterium* strain that is the most frequent cause of simian tuberculosis
    A. *M. tuberculosis*
    B. *M. avium*
    C. *M. bovis*
    D. *M. leprae*
18. Metabolic bone disease is caused by a deficiency of vitamin D and an imbalance of
    A. sodium and chloride
    B. selenium and vitamin E
    C. calcium and phosphorus
    D. potassium and sodium

19. Type of caging system that facilitates examination and immobilization of nonhuman primates
    A. metabolic
    B. gang
    C. exercise
    D. squeeze
20. Twinning is normal in this monkey as they are biovulatory.
    A. marmoset
    B. rhesus
    C. squirrel
    D. owl

Critical Thinking

21. There are 10 cynomolgus monkeys singly housed in your research facility. Your new supervisor wants you to develop a plan for environmental enrichment and social housing. Make suggestions, considering the welfare, health, and safety of the primates and the human caretakers.

Suggested Activities

Read the book *The Hot Zone* by Richard Preston. This best seller is a dramatic story about an Ebola virus outbreak in a suburban Washington, DC, laboratory and demonstrates the important role the laboratory animal medicine community plays in preventing zoonotic diseases.

13 Research Variables and Quality Control

RESEARCH VARIABLES: INTRINSIC AND EXTRINSIC FACTORS

In a research setting, it is important to keep nonexperimental variables to a minimum. A variety of factors can confound experimental results and lead to erroneous conclusions from the data collected. Complicating factors can be divided into two primary areas of interest: intrinsic factors inherent to the animal, such as genotype, age, and sex; and extrinsic factors that are external to the animal, such as environment, diet, and infectious agents. All may affect an animal's physiology and behavior. Figure 13.1 depicts intrinsic and extrinsic factors that can act as nonexperimental variables and affect the biologic responses of laboratory animals.

Intrinsic Factors

When designing a research project, the genotype of the animal used is an important intrinsic consideration, as genetic composition often determines the biologic responses of individual animals within a species. Different strains of mice and rats will have different responses to select pharmaceuticals and different immune responses to pathogenic organisms. In toxicological testing, drug responses may vary between strains. For example, administration of chloramphenicol succinate induces leukopenia in BALB/c mice, but not in CD-1 mice. DBA/1J mice are highly susceptible to Sendai virus, whereas SJL mice are highly resistant to developing clinical disease. Likewise, LEW rats are more susceptible to infection with sialodacryoadenitis virus than are F344 rats.

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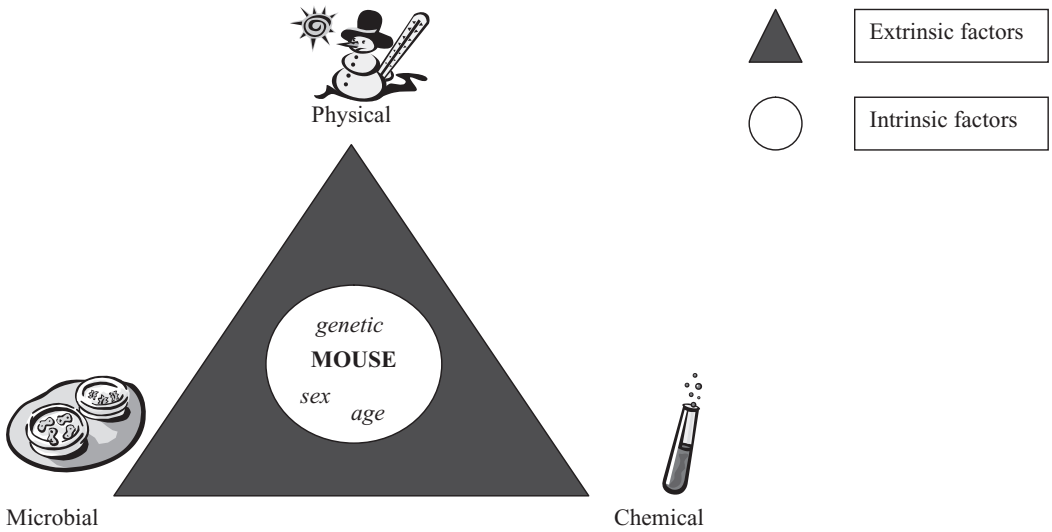

Fig 13.1. Intrinsic and extrinsic factors that can act as nonexperimental variables.

It is critical that the genetic integrity of the rodent is maintained to the extent possible so as not to impact or alter experimental data. Inbred animals are used to collect reproducible and comparable research data. They are produced using a pyramid breeding scheme involving foundation stock, pedigree expansion stock, and production stock to maintain animal homogeneity. However, genetic differences, even from animals of the same inbred strain, can occur. The most common cause of this variation is human error involving mismatings and inadvertent outcrossings with other strains. For this reason, it is advisable to house different strains of breeding mice with the same coat color in separate rooms. Spontaneous mutations and chromosomal aberrations can also be involved. Conversely, outbred animals are produced from large breeding colonies to maintain heterogeneity. Management techniques must be employed to ensure the genetic variability of the outbred stock.

Transgenic animals, produced by gene targeting to create knockout or knock-in animals, present additional genetic integrity concerns. The original mouse strain selected to develop the transgenic animal may influence phenotypic expression due to the transgene's interaction with innate genes, ultimately affecting normal physiology and behavior. The transgene may also produce unwanted or unexpected research results.

Whether using inbred, outbred, or transgenic animals, genetic drift cannot be entirely prevented as it is a natural occurrence in all populations; divergence is unavoidable due to residual heterozygosity and spontaneous mutations. The goal is to minimize the effect of genetic drift by slowing its progression and by making the changes due to drift available to all individuals in the strain population, regardless of geographic separation. Commercial vendors have international genetic standards programs to manage the health and genetics of their outbred stocks and inbred strains. To maintain heterozygosity and manage genetic

drift in outbred stock, inbreeding is minimized. These management programs also help minimize divergence of inbred strains due to drift and prevent contamination by mismatings with other strains. Vendors are diligent, consistently monitoring the genetic integrity of animals produced for research. Methods include monitoring physical characteristics or behavior of colony animals and the use of molecular-based testing. Knowledge of the individual strain is of utmost importance to distinguish normal from deviant characteristics. Expected coat color, frequency of inherited medical conditions such as microophthalmia or hydrocephalus, and behavior patterns such as aggression can all be used to monitor the colony.

DNA-based assays that can distinguish extremely subtle genetic alteration, such as polymerase chain reaction (PCR), single nucleotide polymorphism (SNP), and microsatellite panels, form the cornerstone of genetic monitoring and quality control programs. Typically, PCR is used to determine the presence or absence of the gene of interest in a transgenic animal. Quantitative PCR, also called real-time PCR, enables both detection and quantification of a targeted DNA molecule. It is used to determine the genetic condition of the zygote (such as a homozygote or a heterozygote) and copy number of the gene. Point or single nucleotide mutations may occur spontaneously or be induced as part of a research study. Microsatellites are short segments of DNA that have repeat sequences. SNP and microsatellite marker panels are available to validate the genetic status of an animal and identify specific genes. Samples for genetic testing can be taken from a variety of sources, including buccal swabs, tail or ear tissue, saliva, fecal pellets, and hair follicles. A tail biopsy of approximately 0.5 cm is a preferred sample for submission. Due to welfare considerations, the use of less invasive methods for sample collection is advocated.

Other intrinsic factors include the age, sex, and immune status of the animal. Age reported variables include respiratory rate, blood pressure, demand for oxygen, hepatic microsomal enzyme system development, renal concentration and dilution ability, and the ability to thermoregulate. Compared with adults, neonates have immature body systems that are more sensitive to various carcinogenic compounds. Older animals frequently have decreased cardiovascular, hepatic, and kidney functions that can confound experimental results. It is not surprising to see divergent responses to a variety of research manipulations, such as marked differences in pharmacologic and toxicologic responses to compounds between male and female rats due to sex-linked hormonal variations. As an example, male rats are significantly more sensitive to the analgesic properties of morphine than are females. Immune function is another important intrinsic factor that plays a role in response to experimental manipulations. Nude, severe combined immunodeficient (SCID), and many transgenic strains of mice lack specific components of a complete immune system, thereby eliciting a different response to antigens than immunocompetent animals.

Extrinsic Factors

Extrinsic factors are generally divided into three categories: physical, chemical, and microbial. Physical factors include macroenvironmental (the housing room) or microenvironmental (the cage) conditions, as well as stress. The macro- and microenvironmental conditions may be much different depending upon the caging or housing system used. Rodents housed in static microisolation or standard open-top shoebox caging in an animal room will be

exposed to much different environmental conditions than those housed in microisolation cages on ventilated racks. Temperature, humidity, ammonia, and carbon dioxide levels can be substantially higher in static microisolation cages. The macroenvironment, the caging or housing system used, and the animals all influence the microenvironment. An increase in animal density within a cage will also clearly affect the ammonia and temperature levels within the microenvironment.

Cage design, temperature, relative humidity, ventilation, lighting, and noise are several of the more important environmental variables. The *Guide for the Care and Use of Laboratory Animals* (the *Guide*; ILAR, 2011) has established guidelines for the macroenvironment to assure the well-being of animals. Physical factors can affect animals directly and indirectly. According to the *Guide*, regulation of body temperature within normal variation is necessary for the well-being of homeotherms. Exposure of unadapted animals to temperature extremes, above 85°F or below 45°F, may produce life-threatening physiologic effects. Elevation of the macroenvironmental temperature of breeding rodents can cause sterility or reduced milk production. Temperature affects studies of drug-induced toxicity, which may increase or decrease depending upon the temperature at which the study is conducted. Low environmental humidity may cause ringtail in rat pups and epistaxis in some species of nonhuman primates. High environmental humidity causes gerbils to have greasy, ruffled appearing coats. Humidity levels affect the animal's ability to thermoregulate, the amount of food eaten, and the transmission of infectious agents and can impact skin absorption studies. The relative humidity has a vast impact on intracage ammonia levels, especially in static microisolation cages. According to the *Guide*, ventilation of 10–15 air changes per hour is an acceptable general standard for the macroenvironment of a conventional facility but does not take into account the many variables that can affect air quality, such as room dimensions and heat load. Mechanical engineers can assist in making a more accurate determination of ventilation requirements to accommodate the needs of animals being housed. Noise generation, although inherent in an animal facility, has different effects on each species of animal. Intraspecies communication at the ultrasound level is of particular importance to rodents and can easily be affected by environmental noise. Cage-changing activities, cleaning and movement of equipment, fire alarm activation, and construction noise are of particular concern. Even the animals themselves are a source of noise generation. Use of ventilated racks, laminar flow, and biological safety cabinets can generate noise that may impact research studies. Noise is often outside the range audible to humans, but may be clearly perceived by some animal species. Studies have demonstrated ventilated caging systems can influence body weight, food and water consumption, and intracage location of animals. Low-frequency sounds can alter blood pressure and excess noise levels can cause audiogenic seizures in a number of strains of mice, such as DBA. Noise can even induce effects postexposure, as demonstrated by the reduced learning ability of mouse pups exposed to noise during pregnancy.

Light intensity and duration can impact behavior and physiology. Excessive light intensity for a prolonged period can cause retinal degeneration in albino rodents. These effects can be confounding in a 2-year carcinogenicity study; thus light intensity and cage position become important variables for consideration. Light cycles are also extremely important for maintenance of circadian rhythms and breeding production in most species.

Stressors such as housing method, cage population, and transportation can directly affect animals. Cage designs should take into account an animal's space, health, and social needs in order to create a less stressful environment. Gerbils, for example, have a semierect posture, so their cages should be tall enough to allow for this species-typical behavior. The cage height required for rabbits has been recently increased from 14 inches to 16 inches to also allow for normal postural adjustments. Studies have demonstrated effects on the welfare of breeding mice housed in individually ventilated caging systems. Mice have a preference for 70 to 75 air changes per hour versus 50 and for air injected at the rear top of the cage rather than directly at the animal level. Species and strain differences should be taken into account when determining the number of animals to house in a cage. Some strains of mice prefer living with a more dense population, yet others become aggressive with an increased density. Providing greater floor space to male mice can alter their dominance rank. Solid-bottom cages are preferred by rodents. Mice and other rodents can be provided with nesting material and other devices to hide in to decrease stress. Enrichment items, however, may induce unintended effects, including physical trauma, as some strains of mice may become more aggressive when given huts or other items to "guard." Providing complex cage environments as enrichment to mitigate stress has also been shown to change research parameters such as the development of learning and memory in behavioral testing. Rodents should be housed in groups whenever the research allows as studies have shown that close interaction between group-housed rats appears to be a more important parameter in stress reduction than is providing increased floor space per animal. Transportation of animals between institutions causes alterations in humoral immunity, serum biochemical indices, food consumption, and weight. Rodents should be allowed a minimum of 48 hours to acclimate prior to starting an experiment.

Xenobiotics, chemicals that are foreign to a biological system, can impact the animal through its macro- and/or microenvironment. Air, diet, bedding material, caging materials, and cleaning agents are potential sources of chemical exposure. The effect or toxicity is based on the dose and disposition of the chemical. Chemicals may cause direct damage to animal tissues and organs or may have indirect effects through biotransformation and the production of metabolites. The chemical or its metabolites may cause physiologic alterations in the animal, affecting the immune response or acting as a mutagen and/or as a teratogen.

Food is usually the principal source of chemical or compound exposure. According to the *Guide*, laboratory animals should be fed palatable, uncontaminated, and nutritionally adequate food unless the protocol in which they are being used requires otherwise. Diets may contain either natural or synthetic compounds that can affect the experimental outcome by altering the animal's physiologic processes. Heavy metals, nitrosamines, aflatoxins, estrogenic compounds, organophosphates, and polychlorinated biphenyls are examples of dietary contaminants. Exposure to heavy metals such as lead and cadmium lowers disease resistance in rodents. Nitrosamine contamination can have a carcinogenic effect. Diets can also be contaminated with estrogenic compounds during formulation of the product or by aflatoxin during storage of various cereal grains. Organophosphate and polychlorinated biphenyls are dietary contaminants from agriculture residues. The source and amount of protein and fat in a diet can have profound effects on physiologic processes such as breeding efficiency, body weight, longevity, and mortality. Caloric restriction has been shown to be beneficial to animals, retarding disease processes and delaying death due to some forms of cancer.

According to the *Guide*, animals should have access to potable, uncontaminated drinking water. There is substantial variation in water quality depending upon locality, source, and treatment prior to consumption. Drinking water can be contaminated by a variety of agents, including pesticides, heavy metals, volatile organics, asbestos fibers, polychlorinated biphenyls, and radionuclides that can alter experimental outcomes. Water is frequently treated using chlorine or acids to reduce microbial contamination. The treatment, however, can also produce physiologic changes in the animal and indirectly alter experimental outcomes. Chlorine may cause changes in the immune response, and acidification can cause decreased water intake and weight gain. The delivery system used can also impact studies. Autoclaving glass bottles can cause formation of silicon crystals, and rubber stoppers can leach minerals into the water. Bedding materials can directly cause biologic changes or can become contaminated with chemicals and indirectly affect studies. Softwood bedding materials such as cedar are not recommended because they emit aromatic hydrocarbons that induce hepatic microsomal enzymes and cytotoxicity. Mice housed on cotton bedding have been shown to have lower intestinal humoral immune responses than those on hardwood bedding.

Another area of chemical exposure that is often overlooked is exposure to pharmaceutical agents. Drugs are administered to anesthetize animals, relieve pain, prevent or treat disease, or as part of a protocol to activate promoters that turn on or off specific genes. Administration of the pharmaceutical agents is usually a necessary protocol component; however, these agents may produce different or confounding physiologic effects that alter the experiment. Cardiovascular and respiratory depression are two common side effects of a variety of anesthetic agents; these biologic effects could clearly impact an animal's response to an experimental manipulation. Chemical variables must be monitored and eliminated when possible.

Microbes are one of the most obvious factors that can affect research integrity. Pathogenic organisms frequently cause clinical disease that directly impacts the biologic responses of affected animals. Many organisms cause latent or subclinical infections in animals. Although there are no overt clinical signs of illness, latent microbial infections often cause subtle changes in the physiologic responses of infected animals. Microbes can be transmitted through direct contact between animals or through food, bedding, water, fomites, vectors, or caretakers. Direct contact transmission usually occurs by the aerosolized or fecal–oral route. Indirect contact occurs by exposure through the microenvironment. Green vegetables or hay contaminated with *Salmonella* spp. fed to guinea pigs can cause high mortality. Cleaning equipment and supplies transported from one animal housing room to another can transfer microbes. Caretakers can be the source of *Staphylococcus aureus* infections, especially to immunodeficient rodents. Limiting animal–human contact by use of microisolation caging, biological safety cabinets for cage changing, disinfected forceps for animal handling, and personnel protective equipment can greatly reduce the chances for introduction of infectious agents.

Biologic materials such as tissue cultures and tumor lines that are injected into animals are yet another source of infectious agents. Lactate dehydrogenase elevating virus is a common contaminant of tumors maintained by passage in mice. Contaminated biologic materials can serve as a nidus of infection. Each research facility should employ a program to ensure that biologic materials are tested prior to using them in animals. Table 13.1 summarizes major rodent infectious agents and their adverse effects.

Table 13.1. Major rodent infectious agents

Organism	Host	Adverse effects
Cilia-associated respiratory bacillus (CAR bacillus)	Mouse, rat, rabbit	Respiratory disease resulting in morbidity and mortality; interferes with rabbit research involving the respiratory tract; enhances severity of other concurrent infections
Corynebacterium bovis	Mice and rats: nude, hairless, and SCID	Weight loss; decreased transplantable tumor take
Ectromelia virus	Mouse	High mortality of animals; can alter phagocytic response; tumor contaminant
Encephalitozoon cuniculi (ECUN)	Rabbit, mouse, rat, hamster, guinea pig	Histologic changes in brain and kidney complicates interpretation of toxicology and other studies; tumor contaminant
Hantaviruses	Wild and laboratory rodents	Infectious to humans and can cause fatal hemorrhagic disease
Helicobacter spp. *H. bilis* *H. hepaticus*	Mice, all mammals	Inflammatory response in liver and gut in susceptible strains; confounds investigations with digestive diseases; linked to hepatocellular carcinoma in A/J strain; may alter mammary carcinogenesis
Lactate dehydrogenase–elevating virus (LDV)	Mouse	Immunosuppression; decreased cellular immunity; increased cytokine activity; alters incidence and behavior of spontaneous neoplasms; tumor contaminant
Lymphocytic choriomeningitis virus (LCMV)	Mouse, rat, hamster	Infectious to humans and can cause serious disease; immunosuppression; inhibits tumor induction; decreased cellular immunity
Mouse adenoviruses (MAd-1 and Mad-2)	Mouse	Lesions in kidneys; increased susceptibility to *Escherichia coli* induced pyelonephritis
Mouse cytomegalovirus (MCMV)	Mouse	Immune, respiratory, reproductive, hematopoietic systems affected
Mouse hepatitis virus (MHV)	Mouse	Focal necrotizing hepatitis; alters hepatic enzymes; prolonged immune system effects; modulates course of infections; alters phagocytic and tumoricidal activities
Mouse parvoviruses (MPV-1, MPV-2, MPV-3, MVM)	Mouse	Modification of responses in cells that depend on cell multiplication; immune function changes
Mycoplasma pulmonis	Mouse, rat	Potential to interfere with a wide variety of research; affects immune response and may predispose animal to other infections; increased total lymphocyte and neutrophil counts
Murine norovirus (MNV)	Mouse	Only in mice with severe deficiencies in innate immunity; wasting and death
Pasteurella multocida	Rabbit	Interferes with a wide variety of research; affects numerous organ systems; organism can infect humans

Table 13.1. (*Continued*)

Organism	Host	Adverse effects
Pinworms (*Aspiculuris tetraptera*, *Syphacia obvelata*, *S. muris*)	Mouse, rat, hamster, gerbil	Subtle effects on immune response
Polyoma viruses (polyoma virus, K virus)	Mouse	Tumor induction in newborns from susceptible colony; shortened life span; wasting disease in immunodeficient mice
Rat parvoviruses (RPV-1, RPV-2, RV, H-1)	Rat	Alteration of immune response; lower tumor take; induction of cytokine production
Reovirus (REO-3)	Mouse, rat, hamster, guinea pig	May interfere with research involving transplantable tumors and cell lines; tumor contaminant
Rotaviruses EDIM IDIR	Mouse Rat	Interfere with research in young animals; modify intestinal absorption and enzymes; increased neonatal mortality
Sendai virus	Mouse, rat, hamster, guinea pig	Immunosuppression; lung changes; predisposes to bacterial infections; infertility; alters host response to transplantable tumors
Sialodacryoadenitis virus/rat corona virus (SDAV/RCV)	Rat	Interferes with studies involving eyes, salivary glands, respiratory tract; reduced reproductive rate; slow growth rate of young
Theiloviruses (TMEV, GDIII)	Mouse, rat	Interferes with research on nervous, immune, and musculoskeletal systems

Source: Adapted from Rodent Health Surveillance Program, University of Miami. Information compiled from *Infectious Diseases of Mice and Rats* by the National Research Council (1991, Washington, DC: National Academy Press) and Charles River Infectious Agent Technical Sheets (www.criver.com/en-US/ProdServ).
EDIM, epizootic diarrhea of infant mice; IDIR, infectious diarrhea of infant rats; SCID, severe combined immunodeficient.

Chain of Infection

Adventitious agents originate from an external source or occur in an unusual place or manner. They are not inherited or innate but occur accidentally or spontaneously. Central to the success of preventing, containing, and potentially eradicating adventitious agents is an understanding of the chain or pathway of infection. The reservoir, sources, and modes of transmission all play a crucial role. Figure 13.2 depicts these pathways. The reservoir of an infectious agent can be a particular animal species or may include the environment. Wild and domestic rodents are the principal reservoir for laboratory rodents; secondary sources include food or bedding contaminated by another animal or the environment.

An infection can be transmitted to a susceptible host directly through vertical or horizontal transmission or indirectly through fomites or vectors. Vertical transmission is the transfer of a pathogen from a parent, usually the dam, to the offspring and occurs *in*

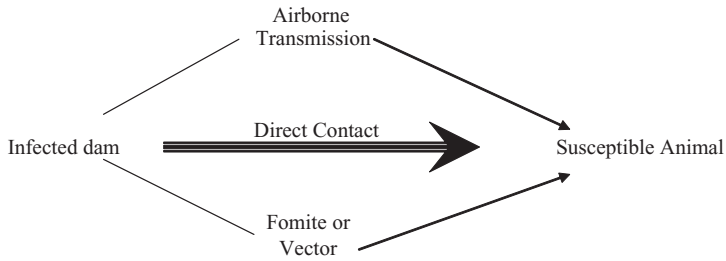

Fig 13.2. Chain of infection.

utero—or at birth (Figure 13.3). Horizontal transmission is the transfer of a pathogen from an infected animal to a naive animal and occurs through multiple routes, including respiratory aerosols and by direct contact. Fomites (inanimate objects that may transmit infectious organisms) transfer contaminated material via food, water, bedding, and equipment that then contacts the animal. Vectors such as mites, lice, and flies may be a part of the life cycle of the organism, or they can mechanically transmit the organism from one animal to another. The mode of transmission varies with the infectious agent; for example, mouse hepatitis virus is transmitted primarily via feces, whereas lymphocytic choriomeningitis virus is transmitted via urine, saliva, and milk.

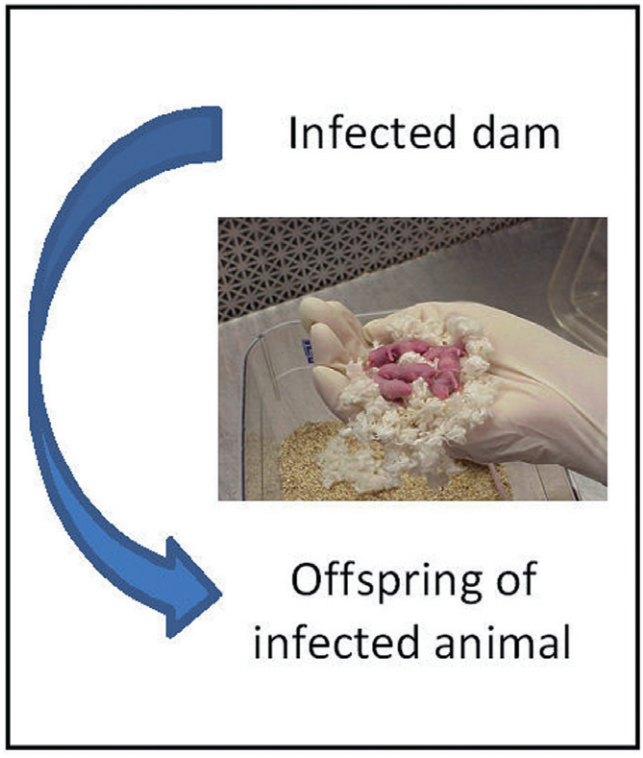

Fig 13.3. Vertical transmission of infection.

Incoming rodent transport containers should be inspected to ensure they are intact and the outside surface sprayed with dilute bleach or similar solution prior to uncrating animals. Rodents transferred into the animal facility from conventional source colonies (e.g., universities and institutions) should be quarantined and monitored for health status prior to release into the facility. Microisolation or individually ventilated caging use is appropriate as it provides a higher level of biosecurity. Food and bedding supplies should be obtained from quality vendors and stored away from chemicals and off the floor to prevent contamination from vermin. In some instances, food and bedding materials may require sterilization via autoclave or gamma irradiation. The use of autoclaved water bottles or a reverse osmosis water purification system may be indicated. Personnel manipulating animals should wear appropriate protective clothing such as gloves, gowns, and masks to minimize contact with animals, and change this personal protective equipment (PPE) at appropriate intervals throughout the day. Supplies and equipment should be appropriately disinfected and transport limited in the facility. A pest control system should be in place to address escaped and/or wild rodents, insects, and other vermin.

Prevention is the key! Animals to be used in research should be of the healthiest status possible and obtained from vendors that monitor and can ensure their microbial and genetic status. Attention should be given to potential sources and means of transmission of adventitious infectious agents. Minimizing risk factors will protect the health status of colony animals.

QUALITY CONTROL

A quality-control program safeguards the health of animals and personnel. A well-trained staff capable of recognizing signs of illness is an important component of a quality-control program. Adventitious infections, especially viral, are common in conventionally housed rodents and are recognized as important complicating factors for biomedical research. A standardized method for health surveillance to identify pathogens and environmental monitoring should be in place.

Health Surveillance Methods

Health surveillance methods include pathology, histology, parasitology, serology, molecular biology, and microbiology. Gross and microscopic examination of tissues and organs from asymptomatic and clinically ill animals can be useful. Analysis can reveal disease in an early phase of infection, can allow for etiological identification, or may uncover emerging infectious agents. A variety of age groups should be sampled because the prevalence of infection can be age specific. Multiple systems can be evaluated quickly with histologic examination of selected target tissues. Histopathology played a major role in recognizing *Helicobacter hepaticus* as a mouse pathogen. Histopathological monitoring can be used in immunodeficient rodents, since serologic tests are of little use. Parasitology exams include pelage and skin examination for mites and lice, using a low-power microscope. Microscopic examination of adhesive tape applied to the dorsal fur has been reported to be of greater value than skin scrapings to identify the presence of select mites. Direct examination of gastrointestinal tract, fecal flotation, and perianal adhesive tape tests can be used to check for the presence

of internal parasites. Polymerase chain reaction (PCR) methodology can be used to detect endoparasites from a fecal sample and fur mites from a fur swab.

Serologic testing using either serum or plasma is the primary method of monitoring rodent colonies. It provides an indirect measure of past exposure to an infectious agent and is most useful for viral and mycoplasmal infections. Serologic tests are sensitive, are specific, and allow for screening of multiple infectious agents using one serum sample. Both antibody and antigen assays are available for serologic testing. As antibody assays determine whether the serum sample contains antibodies to a particular infectious agent, they are considered an indirect test. Animals used for serologic monitoring must be immunocompetent, allowed adequate time to seroconvert, and of an appropriate age to be capable of an acceptable immune response. Antibody assay methods include the microbead-based assay, enzyme-linked immunosorbent assay (ELISA), and indirect immunofluoresence assay (IFA). Microbead assays are the most commonly used primary tests because they are extremely sensitive, require a minute sample size, and allow for high throughput volume. Beads are coated with a purified antigen, such as from a virus or bacteria, and will react with its specific antibody if present in the sample. This testing involves a multiplex format, where beads are used to detect multiple agents and/or strains of a single agent, and use of multiple controls for accuracy. ELISAs are used if no microbead assay is available for the agent or as a confirmatory test, while IFAs are employed for parasitology, some bacteria, and also as confirmation of other test results. Serologic antibody testing, however, can fail to identify an animal with an acute illness and does not provide information about the current infection status of an animal.

Polymerase chain reaction assays directly test for the presence of nucleic acids from an infectious agent. PCR detects DNA, and reverse transcriptase PCR (PCR-RT) detects RNA. Fecal pellets, tissue, skin, fur and lung swabs, saliva, buccal swabs, and serum can be used as the test sample. PCR assays are extremely sensitive and are available for more prevalent rodent viruses. These assays are excellent tests to detect current infection in immunodeficient animals that are incapable of mounting an immune response and incoming (quarantine) animals that might not have had time to produce antibodies. Animals can be screened cost-effectively; fecal pellets can be pooled and used to rapidly determine whether or not animals in quarantine pose a risk for common viral agents or *Helicobacter*. PCR can be used to identify a particular serotype of virus, such as parvovirus, and is also useful for agents that persist in tissues such as mesenteric lymph nodes and biological materials.

Microbiologic monitoring involves inoculating agar and/or broth with animal or environmental samples. The nasopharynx and cecum are routinely used in monitoring rodents for bacterial pathogens. PCR testing is used to detect *Corynebacterium bovis*, using a skin swab, and cilia-associated respiratory (CAR) bacillus and *Pneumocystis* spp. using oral/lung swabs.

Animals Used to Monitor Health Status of a Colony

Sentinel or resident animals or a combination of both can be used to monitor the health status of a colony. *Sentinel* animals are externally sourced animals, most commonly vendor purchased. They are introduced into the colony and exposed to soiled bedding or colony animals and then sampled. *Resident* animals include colony and study animals. Resident animals are more problematic to use. It is often difficult to select appropriately aged animals,

and it may be difficult to determine if animals that are genetically modified have a complete immune response.

Sentinel animals should be free of all infectious agents that are of concern in the area they are used to monitor. Females are preferred to decrease fighting incidence and lessen the chance of genetic contamination. They should be introduced into the colony at 3–5 weeks of age. Outbred stocks are recommended over inbred strains as they are good serologic responders and are less costly. Sentinels should be housed in a manner that maximizes their exposure to the adventitious agents of the animals being monitored. Infections are transmitted most efficiently through animal-to-animal contact; however, the transfer of soiled bedding material (5–15 mL) to sentinel cages is usually effective. Sentinels should be exposed to new soiled bedding at each cage change. New nesting material is provided to the sentinel cage rather than clean bedding; clean bedding placed in a sentinel cage dilutes infectious agents. As sentinels require 7–14 days to develop an antibody response, serology sampling should occur 2–3 weeks after the last exposure to soiled bedding. Seroconversion to some agents, such as mouse norovirus, can take 8 weeks or longer. Sentinels should not be kept longer than 3 months; the sensitivity to agents such as mouse parvovirus decreases with age. Airborne spread of infection is less reliable and is impeded when microisolation or ventilated caging systems are used.

The increased use of individually ventilated caging presents a new challenge in health monitoring. The air exhausted from the caging rack can be monitored using sentinel animals that are exposed to rack exhaust air or via filters that are placed on the air exhaust port. Filters are tested using PCR methodology. Studies have shown effective monitoring can occur whether the ventilated rack was operated under positive or negative air pressure. When using ventilated caging systems, it is best to utilize a multifaceted approach to monitoring, using soiled bedding and exhausted air.

An established protocol should be followed when collecting and processing samples for testing if the results are to be meaningful. As testing can be costly, it is best to prioritize a list of agents for monitoring in terms of prevalence and potential animal or human health impact. This list will vary depending upon the rodent population, sources of incoming animals, and housing method. The method of testing is also important as one may be more effective than another in monitoring for the presence of a pathogenic agent. Serology is preferred for routine colony screening to detect mouse parvovirus–infected mice, while PCR assay on fecal pellets is the primary test for murine helicobacter infection. In determining the frequency of testing, the impact of specific pathogens to ongoing research must be considered; in conventional animal facilities, monitoring is usually done on a quarterly or semiannual basis.

The number of animals needed for testing is based on the predicted prevalence of the disease in a population. A commonly used formula to determine the number of sentinels is based on the number of cages per rack; one sentinel cage of 2–3 animals is used to monitor a 50- to 80-cage rack. In reality, the budget plays the greatest role in determining the number of animals to sample. Disease prevention comes through proper management of data and of personnel, including animal care staff and researchers. One consideration for animal handlers at any research institution is personnel exposure to potentially infectious rodents away from the work site. There have been several reported incidents of employees handling infectious rodents at home and unwittingly carrying the infection to animals at work. The cost of an

outbreak can be quite significant when the potential effects on data, time, containment, and replacement of infected animals is considered. Test results should be interpreted using scientific and professional judgment. Any action taken should be based on sound quality-control principles.

Diagnostic Laboratories

A number of commercial laboratories and universities have diagnostic facilities available to process screening panels for the laboratory animal science community. Serology panels that test for a number of viral agents are routinely used to monitor the health status of rodent colonies. In the past, mouse antibody production (MAP) and rat antibody production (RAP) tests were used to check biologic materials such as transplantable tumors, cell lines, ascites, viral stocks, and purified antibodies for pathogens. PCR molecular-based testing has replaced MAP and RAP tests as it offers the advantage of rapid results and does not require the euthanasia of valuable animals. As no diagnostic test is 100% reliable, unexpected or unusual test results should always be confirmed by an alternative test. Consulting with an infectious disease expert at one of the diagnostic laboratories is another good method to obtain guidance on interpretation of and response to test results, based on a particular program's needs.

Food, water, and bedding samples can also be tested for chemical and microbial contamination. PCR assays are used to test for environmental contamination and to assess effectiveness of decontamination efforts after an outbreak. Replicate organism detection and counting (RODAC) plates and adenosine triphosphate (ATP) bioluminescent technology can be used to assess the effectiveness of a facility's sanitation program for inanimate objects or surfaces. Table 13.2 provides a partial listing of commercial and university comprehensive animal diagnostic laboratories. Table 13.3 presents a partial listing of laboratories that perform nonhuman primate diagnostic testing. The information provided is strictly a point of reference and in no way indicates author preferences. Other diagnostic laboratories may be available in your area.

Table 13.2. Comprehensive diagnostic laboratories

Company Contact Information	Comments
BioReliance Corporation Animal Health Services (AHS) 14920 Broschart Road Rockville, MD 20850-3349 Phone: 800-804-3586 Fax: 301-610-2587 www.bioreliance.com	Animal health screening, disease diagnosis, histopathology, environmental monitoring, biotechnical services
Charles River Laboratories 251 Ballardvale St. Wilmington, MA 01887 Phone: 877-274-8371 www.criver.com	Comprehensive health monitoring, serology, genetic testing, pathology services, environmental testing, serology reagents and test kits

Table 13.2. (*Continued*)

Company Contact Information	Comments
Harlan Laboratories Research Model Support Services 8520 Allison Pointe Blvd., Suite 400 Indianapolis, IN 46250 Phone: 800-793-7287 www.harlan.com	Comprehensive health monitoring, serology, genetic testing, pathology services
Taconic One Hudson City Centre Hudson, NY 12534 Phone: 888-822-6642 Fax: 518-697-3910 www.taconic.com	Comprehensive health monitoring, serology, genetic testing, pathology services, toxicology services
IDEXX RADIL Lab Animal Diagnostics & Biological Materials Testing 4011 Discovery Drive Columbia, MO 65201 Phone: 800-554-5205 Fax: 573-499-5701 www.idexxradil.com	Comprehensive health monitoring, serology, genetic testing, pathology services

Table 13.3. Nonhuman primate diagnostic testing laboratories

Company	Comments
National B Virus Resource Center Viral Immunology Center Georgia State University 161 Jesse Hill Drive Atlanta, GA 30303 Phone: 404-413-6550 Fax: 404-413-6556 bvirus@gsu.edu	B-virus screens for nonhumans and humans
VRL Laboratories P.O. Box 40100 7540 Louis Pasteur, Suite 200 San Antonio, TX 78229 Phone: 877-615-7275 Fax: 210-615-7771 www.vrl.net	Antibody assays, virus isolation, microbiology, polymerase chain reaction

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CHAPTER 13 REVIEW

Fill in the Blank

Fill in the blank with one of the following:

E = Extrinsic factor that may induce research variables
I = Intrinsic factor that may induce research variables

1. ____ Mouse hepatitis virus
2. ____ 18 months of age
3. ____ Pelleted rodent diet
4. ____ 72°F room temperature
5. ____ IVC housing system
6. ____ Female rat
7. ____ C57BL/6J
8. ____ *Staphylococcus*
9. ____ Transgenic mouse
10. ____ Lighting cycle

Multiple Choice

11. Chemicals that are foreign to a biological system are
    A. probiotics
    B. xenobiotics
    C. antibiotics
    D. radiobiotics
12. A common murine tumor contaminant is
    A. mouse hepatitis virus
    B. lactate dehydrogenase elevating virus
    C. Sendai virus
    D. lymphocytic choriomeningitis virus

(*Continued*)

13. Vertical transmission of a pathogen occurs
    A. by a fomite
    B. from animal to animal through sneezing
    C. from mother to offspring *in utero*
    D. by direct contact with another animal
14. A RODAC plate is used to
    A. grow *Helicobacter* species organisms
    B. count pinworm larvae
    C. check if autoclave is reaching temperature
    D. assess effectiveness of sanitation
15. False statement regarding environmental enrichment
    A. It is consistently associated with positive effects.
    B. Animals may become more aggressive.
    C. It may affect research results.
    D. Compatible group housing reduces stress.
16. Fomite example
    A. mouse parvovirus
    B. SCID mouse
    C. mop bucket
    D. personnel
17. Produced to maintain heterogeneity
    A. transgenic
    B. outbred stocks
    C. nude
    D. inbred strains
18. Commonly used sample for rodent genetic testing
    A. tail biopsy
    B. saliva
    C. fecal pellet
    D. hair follicle
19. Rodents should be allowed a minimum of _____ hours to acclimate after shipping and prior to initiation of a research protocol.
    A. 12
    B. 24
    C. 26
    D. 48
20. All are DNA-based assays *except*
    A. PCR
    B. SNP
    C. ELISA
    D. microsatellite panel

Appendix 1: Normal Values

The following tables provide "normal" hematologic and biochemical data for each species covered in the text. The values should be used as guidelines, for there are many inherent variables in reporting such data. Sample collection method; the breed, strain, or stock; health status; age; sex; environment; and analytic techniques are but a few of the many variables that can affect specific clinical laboratory values.

REFERENCES FOR NORMAL VALUES

Table A.1. Hematologic data: erythrocytes and platelets

Species	RBC (10^6/mL)	PCV (%)	Hb (g/dL)	Platelets (10^3/mL)
Mouse[a]	7.0–12.5	39–49	10.2–16.6	800–1100
Rat[a]	7–10	36–48	11–18	500–1300
Gerbil[a]	8–9	43–49	12.6–16.2	400–600
Hamster[a]	6–10	36–55	10–16	200–500
Guinea pig[a]	4.5–7	37–48	11–15	250–850
Chinchilla[b]	6.6–10.7	40	11.7–13.5	254–298
Rabbit[a]	4–7.2	36–48	10–15.5	200–1000
Ferret[c]	6.8–12.2	42–61	15–18	297–910
Rhesus monkey[d]	4.5–6	39–43	12.7	130–144
Squirrel monkey[d]	7.1–10.9	43–56	12.9–17.0	112

[a]From Harkness and Wagner (1995).

[b]Adapted from Newberne, Casella, Kraft, and Strike in Merry (1990).

[c]From Bernard et al. (1984).

[d]From Johnson-Delaney (1994).

RBC = red blood cells; PCV = packed cell volume; Hb = hemoglobin.

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Table A.2. Hematologic data: leukocytes

Species	WBC ($10^3/\mu L$)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
Mouse[a]	6–15	10–40	55–95	0.1–3.5	0–4	0–0.3
Rat[a]	6–17	9–34	65–85	0–5	0–6	0–1.5
Gerbil[a]	7–15	5–34	60–95	0–3	0–4	0–1
Hamster[a]	3–11	10–42	50–95	0–3	0–4.5	0–1
Guinea pig[a]	7–18	28–44	39–72	3–12	1–5	0–3
Chinchilla[b]	7.6–11.5	23–45	51–73	1–2	0.5–2.6	0–1
Rabbit[a]	7.5–13.5	20–35	55–80	1–4	0–4	2–10
Ferret[c]	4–19	11–84	12–54	0–9	0–7	0–2
Rhesus monkey[d]	11.5–12.4	20–56	40–76	0–2	1–3	0–1
Squirrel monkey[d]	5.1–10.9	36–66	27–55	0–6	0–11	<1

[a]From Harkness and Wagner (1995).

[b]Adapted from Newberne, Casella, Kraft, and Strike in Merry (1990).

[c]From Bernard et al. (1984).

[d]From Johnson-Delaney (1994).

WBC = white blood cells.

Table A.3. Serum biochemical data

Species	BUN (mg/dL)	Creatinine (mg/dL)	Glucose (mg/dL)	ALT (IU/L)	AST (IU/L)	AP (IU/L)	Total bilirubin (mg/dL)	Cholesterol (mg/dL)
Mouse[a,b]	12–28	0.3–1	62–175	26–77	54–269	45–222	0.1–0.9	26–82
Rat[a,b,c]	15–21	0.2–0.8	50–135	16–89	192–262	16–125	0.2–0.55	40–130
Gerbil[a,d]	17–27	0.6–1.4	50–135	—	—	12–37	0.2–0.6	90–150
Hamster[a,b]	12–25	0.91–0.99	60–150	22–128	28–122	45–187	0.25–0.6	25–135
Guinea pig[a,b,c]	9–31.5	0.6–2.2	60–125	10–25	45.5–48.2	18–28	0.3–0.9	20–43
Chinchilla[b,e]	10–25	0.4–1.3	60–120	10–35	96	3–47	0.6–1.28	40–100
Rabbit[a,e]	15–23.5	0.8–1.8	75–150	14–80	14–113	4–16	0.25–0.74	35–60
Ferret[f]	12–43	0.2–0.6	62.5–134	78–149	57–165	30–120	0–0.1	119–209
Rhesus monkey[e,g]	14.2–19.6	0.1–2.8	53–87	145–171	20–34	—	0.1–0.66	94–162
Squirrel monkey[e]	23–39	—	52–108	59–99	56–118	—	0.1–0.53	127–207

[a]From Harkness and Wagner (1995).

[b]From Anderson (1994).

[c]Adapted from Loeb and Quimby (1989).

[d]From Canadian Council on Animal Care (1980).

[e]From Carpenter et al. (1996).

[f]Adapted from Fox et al. (1986) and Lee et al. (1982).

[g]From McClure (1975).

BUN = blood urea nitrogen; ALT = alanine transferase; AST = aspartate transferase; AP = alkaline phosphate.

Table A.4. Serum biochemical data

Species	Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)
Mouse[a,b]	3.5–7.2	2.5–4.8	0.6	3.2–8.5	2.3–9.2	112–193	5.1–10.4	82–114
Rat[a–c]	5.6–7.6	3.8–4.8	1.8–3	5.3–13	5.3–8.3	135–155	4–8	94–116.3
Gerbil[a,b,k]	4.3–12.5	1.8–5.5	1.2–6	3.7–6.2	3.7–7	144–158	3.8–5.2	93–118
Hamster[a,b,d]	4.5–7.5	2.6–4.1	2.7–4.2	5–12	3.4–8.2	128–144	3.9–5.5	93–98
Guinea pig[a,b]	4.6–6.2	2.1–3.9	1.7–2.6	5.3–12	3–12	132–156	4.5–8.9	98–115
Chinchilla[e,f]	3.8–5.6	2.3–4.1	0.9–2.2	5.6–12.1	4–8	130–155	5–6.5	105–115
Rabbit[a,g]	2.8–10	2.7–4.6	1.5–2.8	5.6–12.5	2.7–7.3	131–155	3.6–6.9	92–112
Ferret[h]	5.3–7.2	3.3–4.1	1.8–3.1	8.6–10.5	5.6–8.7	146–160	4.3–5.3	102–121
Rhesus monkey[i]	4.9–9.3	2.8–5.2	1.2–5.8	6.9–13	3.1–7.1	102–166	2.3–6.7	84–126
Squirrel monkey[j]	6.6–7.8	3.5–4.5	2.6–3.6	9.5–10.5	3.2–6.6	143.1–152.9	3.6–5.4	107.6–118.4

[a]From Harkness and Wagner (1995).
[b]From Anderson (1994).
[c]Ringler and Dabich (1979).
[d]Mitruka and Rawnsley (1977).
[e]Adapted from Merry (1990).
[f]From Carpenter et al. (1996).
[g]From Quesenberry (1994).
[h]Adapted from Fox et al. (1986) and Lee et al. (1982).
[i]McClure (1975).
[j]Adapted from Manning et al. (1969).
[k]From Canadian Council on Animal Care (1980).

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Appendix 2: Comparative Biologic and Reproductive Values by Species

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Table B.1. Comparative biologic and reproductive values by species

	Mouse	Rat	Gerbil	Syrian Hamster	Guinea Pig	Chinchilla	Rabbit	Ferret	Rhesus Monkey	Cynomolgus Monkey	Baboon	Squirrel Monkey
Adult body weight: Male	20–40 g	450–520 g	80–130 g	85–130 g	900–1200 g	400–500 g	2–5 kg	1–2 kg	6–11 kg	6 kg	22–30 kg	700–1100 g
Adult body weight: Female	25–40 g	250–300 g	55–85 g	95–150 g	700–900 g	400–600 g	2–6 kg	600–950 g	4–9 kg	4 kg	11–15 kg	500–1000 g
Life span	1.5–3 y	2.5–3.5 y	3–4 y	18–24 mo	5–7 y	9–18 y	5–6 y or more	5–11 y	30+ y	37 y	40–45 y	20 y
Body temperature	36.5°–38°C (97.7°–100.4°F)	35.9°–37.5°C (96.6°–99.5°F)	37°–38.5°C (98.6°–101.3°F)	37°–38°C (98.6°–100.4°F)	37.2°–39.5°C (99°–103.1°F)	37°–38°C (98.6°–100.4°F)	38.5°–40°C (101.3°–104°F)	37.8°–40°C (100°–104°F)	36°–40°C (96.8°–104°F)	36°–38°C (96.8°–100.4°F)	36°–39°C (96.8°–102.2°F)	33.5°–38.8°C (92.3°–101.8°F)
Heart rate (beats per minute)	325–780	250–450	360	250–500	230–380	100–150	130–325	200–400	150–333	107–215	80–200	225–350
Respiratory rate (breaths per minute)	60–220	70–115	90	35–135	40–130	40–80	30–60	33–36	10–25	32–44	29	20–50
Tidal volume	0.09–0.23 mL	0.6–2 mL		0.6–1.4 mL	2.3–5.3 mL/kg		4–6 mL/kg					
Food consumption (per day)	12–18 g/100 g	5–6 g/100 g	5–8 g/100 g	8–12 g/100 g	6 g/100 g	30–40 g/d; 5.5 g/100 g	5 g/100 g	140–190 g/d (semi-moist diet)				
Water consumption (per day)	15 mL/100 g	10–12 mL/100 g	4–7 mL/100 g	Male, 4.5–5 mL/100 g; female, 13.6–14 mL/100 g	10 mL/100 g	10–20 mL/d; 8–9 mL/100 g	5–12 mL/100 g	75–100 mL				

(*Continued*)

Table B.1. (*Continued*)

	Mouse	Rat	Gerbil	Syrian Hamster	Guinea Pig	Chinchilla	Rabbit	Ferret	Rhesus Monkey	Cynomolgus Monkey	Baboon	Squirrel Monkey
Breeding onset: Male	50 d	65–110 d	70–85 d	10–14 wk	3–4 mo (600–700 g)	7–9 mo	6–10 mo	8–12 mo	38 mo	42–60 mo	73 mo	60 mo
Breeding onset: Female	50–60 d	65–110 d	65–85 d	6–10 wk	2–3 mo (350–450 g)	4–5 mo	4–9 mo	7–10 mo	34–43 mo	46 mo	51–73 mo	36–46 mo
Estrous cycle length	4–5 d	4–5 d	4–6 d	4 d	15–17 d	30–50 d	Induced ovulator	Continuous until intromission	28 d	28 d	31–36 d	18 d
Gestation period	19–21 d	21–23 d	24–26 d	15–16 d	59–72 d	105–120 d	29–35 d	42+2 d	167 d	162 d	175–180 d	170 d
Postpartum estrus	Fertile	Fertile	Fertile	Infertile	Fertile	Fertile	48 h	Occasional				
Litter size	6–12	6–12	3–7	5–9	1–6	1–6	4–10	8, avg. (1–18 range)				
Weaning age	21–28 d	21 d	20–26 d	20–25 d	14–21 d	6–8 wk	4–6 wk	6–8 wk	210–425 d	365–547 d	180–456 d	182 d
Breeding duration	7–9 mo	350–440 d	12–17 mo	10–12 mo	18–48 mo	3 y	1–3 y	2–5 y				
Chromosome number (diploid)	40	42	44	44	64	64	44	40	42	42	42	44

Sources: Adapted from Bennett et al. (1995), Fox (1998), Harkness et al. (2010), Harkness and Wagner (1995), Johnson-Delaney (1994), Mulder (2012), and Quesenberry and Carpenter (2004).

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Appendix 3: Supportive and Critical Care Products for Laboratory Animal Use

Rodents

- Bio-Serv, www.bio-serv.com; phone, 800-966-9908
  Nutra-Gel Diet: nutritionally complete, flavored gel diet; available as purified, grain based, or sterile
  Transgenic Dough Diet: nutritionally complete, soft sterile diet with higher protein and fat
  Bacon Softies: nutritionally complete, soft, pelleted diet, sterile and certified
  Rodent MD's 2mg Rimadyl Tablets
- PharmaServ, www.pharmaserv.net; phone, 877-LAB-DIET
  DietGel Recovery: purified ingredients, aids in support of postsurgical or impaired rodents
  DietGel Boost: high-calorie supplement that aids in support of postsurgical or impaired rodents
  DietGel 76A: nutritionally complete soft diet, purified ingredients, sterile product in sterile packaging
  MediGel CPF: nutritionally complete soft gel with carprofen, sterile product in sterile packaging

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Herbivores

- PharmaServ, www.pharmaserv.net; phone, 877-LAB-DIET
  DietGel Critical Care: dietary supplement with timothy hay

Ferrets

- Bio-Serv, www.bio-serv.com; phone, 800-966-9908
  Bacon Yummies Certified: certified supplement

Guinea Pigs

- Bio-Serv, www.bio-serv.com; phone, 800-966-9908
  Guinea Pig Stix: certified, high-fiber supplement

Nonhuman Primates

- Bio-Serv, www.bio-serv.com; phone, 800-966-9908
  Monkey Dough Diet: nutritionally complete, soft diet
  Prang Oral Rehydrator: certified, flavored rehydration solution with electrolytes

The partial list provided here is for general informational purposes and not intended as a complete accounting of available supplements; no endorsement is expressed or implied by the authors.

Answers to Review Questions

Chapter 1

1. G 2. I 3. D 4. E 5. B 6. H 7. A 8. F 9. C
10. J
11. REP 12. REF 13. REP 14. REP 15. RED 16. REF
17. REP 18. RED 19. REP 20. REF 21. RED 22. REF
23. REP 24. REP

Chapter 2

1. C 2. O 3. K 4. B 5. N 6. I 7. E 8. A 9. Q
10. F 11. S 12. M 13. H 14. G 15. J 16. P
17. NC 18. C 19. NC 20. C 21. C 22. C 23. NC
24. C 25. C 26. C 27. NC 28. NC

Chapter 3

1. C 2. D 3. A 4. B 5. D 6. C 7. B 8. B 9. D
10. C 11. A 12. C 13. A 14. D 15. B 16. A
17. C 18. B 19. D 20. D
21. 6 rats [(8.5 × 17) = 144.5 $in.^2$; 144 ÷ 23 = 6.2]

Chapter 4

1. B 2. A 3. D 4. D 5. C 6. B 7. A 8. D 9. B
10. C 11. N 12. K 13. M 14. E 15. A 16. C
17. B 18. G 19. I 20. F

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Chapter 5

1. D 2. A 3. B 4. B 5. C 6. A 7. D 8. C
9. A 10. B 11. B 12. J 13. A 14. L 15. F 16. M
17. E 18. N 19. G 20. K

Chapter 6

1. A 2. D 3. B 4. B 5. C 6. A 7. D 8. C 9. B
10. D 11. B 12. B 13. A 14. A 15. C 16. B 17. B
18. D 19. C 20. A

Chapter 7

1. A 2. C 3. C 4. D 5. B 6. B 7. D 8. A
9. A 10. C
11. Cytogenetics 12. Cannibalism 13. Chinese 14. Polychromasia
15. Enterocolitis 16. Hip or flank 17. *Mesocricetus auratus*
18. Lordosis 19. Wet tail 20. Amyloidosis
21. Suggestions include:
    a. Change light timer to provide 14 hours of light per day.
    b. Increase depth of bedding material.
    c. Provide nesting material.
    d. Change cages to tinted (amber) or opaque variety.
    e. Provide tunnels or huts.
    f. Replace breeders with younger animals (should be replaced by 14 months of age).
    g. Make sure animal caretaker does not disturb hamsters several days prior to delivery and a week postdelivery of young (schedule cage change day to avoid anticipated perinatal period).

Chapter 8

1. Cavy 2. Abyssinian 3. Peruvian 4. Palatal ostium 5. C
6. Kurloff 7. Farrowing 8. Trachea 9. Sow, boar 10. 4, 3
11. K 12. O 13. M 14. G 15. D 16. J 17. A
18. C 19. N 20. P
21. The guinea pigs are most likely in pain as they have not been given any preemptive or postoperative analgesics.

Chapter 9

1. A 2. D 3. C 4. A 5. B 6. D 7. B 8. C
9. A 10. D
11. Add compatible members of the same sex; use multilevel caging; add toys such as dumbbells and stainless steel chains; provide a hut with a flat-surface top or PVC tubes; feed twice daily; provide a dust bath several times weekly; add a pumice gnawing stone to cage; provide a fresh branch from a willow, beech, hazelnut, or unsprayed fruit tree in the cage.

Chapter 10

1. B 2. D 3. A 4. C 5. A 6. B 7. B 8. D 9. A
10. C 11. B 12. D 13. A 14. A 15. C 16. B
17. B 18. D 19. C 20. A
21. 21″ × 18″ = 378 in.^{2}; 378 ÷ 144 = 2.63 ft^2. Adequate floor space is provided; however, the cage height dimension is 2″ short of the required 16″ minimum. These cages do not meet the minimum housing requirements.

Chapter 11

1. E 2. A 3. F 4. B 5. D 6. H 7. D 8. B 9. C
10. A 11. C 12. D 13. B 14. C 15. A 16. B 17. D
18. C 19. A 20. A
21. The ferret is used as a replacement for higher carnivore species (dogs, cats) and has a strong emesis response.

Chapter 12

1. OW 2. OW 3. NW 4. NW 5. OW 6. NW 7. OW
8. NW 9. NW 10. OW 11. C 12. D 13. A 14. C
15. B 16. A 17. A 18. C 19. D 20. A
21. Select a number of different enrichment items produced for laboratory primate use to rotate every few days (e.g., Cyclone chews and manzanita sticks for chewing; foraging boards, grooming boards, rattles, puzzles, Linkables, Busy Buddy Footballs, and Flexi- Keys for manipulanda and mental stimulation; mirror for visual stimulation).

    Develop a list of appropriate dietary enrichment items such as fresh fruits, vegetables, and commercial primate treats; include novel delivery mechanisms such as paper bags or items frozen in fruit juice to increase desirability, variety, and time spent with each item.

    Add permanent or removable perches to cages to encourage species-typical behavior.

    Purchase a play cage with exercise wheel mounted inside.

    Purchase cages with adaptations for primate to make selections regarding heat, light, audio, and/or video use.

    Purchase cages designed to allow incremental socialization (social access wheel).

    Work with the facility veterinarian and principal investigator to develop the social housing plan and schedule for implementation.

Chapter 13

1. E 2. I 3. E 4. E 5. E 6. I 7. I 8. E 9. I
10. E 11. B 12. B 13. C 14. D 15. A 16. C
17. B 18. A 19. D 20. C

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