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Biology and Diseases of Dogs

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I. INTRODUCTION

A. Taxonomic Considerations

Dogs are mammals in the order Carnivora, suborder Caniformia (or superfamily Canoidea), and family Canidae. The domesticated dog has been designated as a subspecies of the gray wolf: *Canis lupus familiaris* (Wilson and Reeder, 2005). Other members of the genus *Canis* include four species of jackal and the coyote (*C. latrans*). *Canis lupus familiaris* is subdivided into approximately 400 breeds, ranging in size and shape from the teacup chihuahua to the large Irish wolfhound. The domesticated dog may have descended from prehistoric canids in Europe roughly 18,000–32,000 years ago (Thalmann *et al.*, 2013), although an East Asian origin is also possible (Savolainen *et al.*, 2002).

B. Use in Research

1. Historical Use of Dogs in Research

The dog played an important role as a laboratory animal in the early history of biomedical research, primarily because of its status as a cooperative companion animal of reasonable size. Dogs were used in the mid-1600s by William Harvey to study cardiac movement, by Marcello Malpighi to understand the basic lung anatomy and function, and by Sir Christopher Wren to demonstrate the feasibility of intravenous delivery of medications (Gay, 1984). The use of dogs continued as biomedical research advanced, and they were featured in many noteworthy studies, including those by Pavlov to observe and document the conditioned reflex response and by Banting and Best to identify the role of insulin in diabetes

mellitus. A comprehensive but concise review of the use of the dog as a research subject is available in [Gay \(1984\)](#).

2. Current Use of Dogs in Research

The breed of dog most commonly bred for use in biomedical research is the beagle. Some commercial facilities also breed foxhounds or other larger dog breeds for use in surgical research studies. Some specific breeds with congenital or spontaneous disorders have also been maintained by research institutions (see examples below). Random-source dogs used in research are most frequently mongrels or larger dog breeds (e.g., German shepherd, Doberman pinscher, Labrador and golden retrievers) that are used for surgical research and/or training.

According to a computerized literature search for “beagle” for the years 2012–2013, a significant portion of the biomedical scientific publications identified were in the fields of pharmacology or toxicology. Especially common were studies focusing on pharmacokinetics, alternative drug delivery systems, and cardiovascular pharmacology. Other common areas of research using beagles were dental and periodontal disease and surgery, orthopedic surgery, skeletal physiology, and imaging studies. Other research areas that utilized beagles included canine infectious disease, prostatic urology, and ophthalmology.

Most large-sized dogs (either purpose-bred or random-source) are used in biomedical research because of their suitability for surgical procedures. Anesthetic protocols and systems for dogs are well established and the organs of larger dog breeds are often an appropriate size for trials of potential pediatric surgical procedures. Surgical canine models have been used extensively in cardiovascular, orthopedic, and transplantation research.

There are also some unique spontaneous conditions for which dogs have proven to be valuable animal models. A colony of gray collies had been maintained at the University of Washington (Seattle) for the study of cyclic hematopoiesis. This condition is manifested by periodic fluctuations of the cellular components of the blood, most notably the neutrophil population. These dogs can be used to study the basic regulatory mechanisms involved with hematopoiesis, as well as possible treatments for both the human and the canine conditions ([Brabb et al., 1995](#)). Golden retrievers affected with muscular dystrophy have been used as models of Duchenne muscular dystrophy in human children. Duchenne muscular dystrophy is caused by an absence of the muscle protein dystrophin, inherited in an X-linked recessive manner. The dystrophy in golden retrievers is caused by the absence of the same protein and is inherited in the same way. The clinical signs (such as debilitating limb contracture) are also similar between the canine and human conditions ([Kornegay et al., 1994](#)). Other genetic disorders studied in dog colonies include hereditary canine spinal muscle atrophy ([Cork, 1991](#)) and narcoplepsy in

Doberman pinschers ([Ripley et al., 2001](#)). Bedlington terriers have been used to study copper storage diseases (such as Wilson’s disease) and the development of spontaneous diabetes mellitus and hypothyroidism has been studied in several breeds of dogs for comparisons with the human conditions.

3. Decline in Numbers Used

Although historically the dog has been a common laboratory animal, their use in research has waned over the past 30 years. According to the [U.S. Department of Agriculture \(USDA\), Animal and Plant Health Inspection Service \(1998, 2011\)](#), the number of dogs used in research has declined from 211,104 in 1979 to 75,429 in 1997 (prior to the previous edition of this text) and 64,930 in 2010. This decrease was caused by a variety of factors, including (but not limited to) decreased availability, local restrictive regulations, conversion to other animal models (such as livestock or rodents), increased cost, and shift in scientific interest from pathophysiology to molecular biology and genetics.

C. Availability and Sources

Dogs used for research are generally segregated into two classes: purpose-bred and random-source. Purpose-bred dogs are those produced specifically for use in biomedical research; they are intended for use in long-term research projects and/or pharmacologic studies in which illness or medication would require removal from the study. Usually these dogs are either beagles or mongrel foxhounds, although other breeds may be available. Purpose-bred dogs typically receive veterinary care throughout their stay at the breeding facility. They are usually vaccinated against rabies virus, canine distemper virus, parvovirus, adenovirus type 2, parainfluenza virus, *Leptospira* serovars Canicola, Icterohaemorrhagiae, Grippotyphosa, and Pomona, and *Bordetella bronchiseptica* (Jasmin, personal communication). Purpose-bred dogs are also usually treated prophylactically for intestinal helminths and ectoparasites, and possibly given a heartworm preventative.

Random-source dogs are not bred specifically for use in research. They may be dogs bred for another purpose (e.g., hunting and racing) or stray dogs collected at pounds or shelters. The health status of these dogs can be the same quality as purpose-bred dogs, or it can be an unknown entity. Random-source dogs that have been treated and vaccinated in preparation for use in research are termed *conditioned* dogs. These dogs are then suitable for long-term studies or terminal preparations that require unperturbed physiologic parameters. Conditioned dogs are often tested for heartworm antigen because of the implications that infestations can have on cardiovascular status and surgical risk. Nonconditioned

random-source dogs are useful only in a limited number of research studies, such as nonsurvival surgical training preparations and tissue/organ harvest.

Options for procurement of dogs for biomedical research typically include purchase from a USDA-designated Class A or Class B licensed dealer or directly from a municipal pound. The requirements for USDA licensure are detailed in Code of Federal Regulations (CFR), Title 9, Chapter 1 (1-1-92 edition), Subchapter A, Animal Welfare, 1.1 Definitions, and 2.1 Requirements and Application ([Office of the Federal Register, 2002](#)). Briefly, Class A licensees are breeders who raise all animals on their premises from a closed colony. Class B licensees purchase the dogs from other individuals (including unadopted animals from municipal pounds) and resell them to research facilities. There are additional regulations that apply to Class B dealers (such as holding periods and record-keeping documentation) because of the public concern that stolen pets could enter biomedical research facilities in this manner. In December 2013, the National Institutes of Health (NIH) issued notice NOT-OD-14-034 entitled *Notice regarding NIH plan to transition from use of USDA Class B dogs to other legal sources* ([National Institutes of Health, 2013](#)). This NIH policy begins in the fiscal year 2015 and prohibits the procurement of dogs from Class B dealers using NIH grant funds. From that point forward, dogs on NIH-funded studies will have to be obtained from Class A vendors, privately owned colonies (such as institutional breeding colonies), or client-owned animals (e.g., animals participating in veterinary clinical trials).

The best resource for identification of possible vendors are online 'Buyer's Guide' sites or 'Buyer's Guide' issues of trade periodicals. Online sites include the Buyer's Guide of the American Association of Laboratory Animal Science (<http://laboratoryanimalbuyersguide.com>), and the trade journals *Lab Animal* (<http://guide.labanimal.com>) and *Animal Lab News* (<http://www.alnmag.com/content/buyers-guide>). A 'Buyer's Guide' typically lists sources for both purpose-bred and random-source dogs, and denotes such features as pathogen-free status, health status, and availability of specific breeds and timed pregnant females. Some suppliers also have separate advertisements within issues of the journals.

D. Laboratory Management and Husbandry

Federal regulations promulgated by the Animal and Plant Health Inspection Service, USDA, in response to the Animal Welfare Act (7 CFR 2.17, 2.51, and 371.2[g]) are described in 9 CFR Chapter 1 (1-1-92 edition), Subchapter A, Animal Welfare ([Office of the Federal Register, 2002](#)). Regulations pertaining specifically to the care of dogs used in research are found in Subpart A, Specifications for the Humane Handling, Care,

Treatment, and Transportation of Dogs and Cats of Part 3 (Standards) of Subchapter A. Particular attention should be paid to Section 3.6c (*Primary Enclosures—Additional Requirements for Dogs*) because the space required for housing dogs is calculated using body length rather than weight (a parameter used for other species and also for dogs in the National Research Council (NRC) guidelines). Section 3.8 (Exercise for Dogs) describes the requirements that dealers, exhibitors, and facilities must follow in order to provide dogs with sufficient exercise.

The Institute for Laboratory Animal Research (ILAR) has written the *Guide for the Care and Use of Laboratory Animals* ([National Research Council, 2011](#)). The 'Guide' is the primary document used by institutional animal research units to develop their programs and by animal care evaluation groups, such as the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International), to facilitate site visits and inspections. The primary difference between the 7th and 8th editions of the 'Guide' ([National Research Council, 1996, 2011](#)) regarding the care of dogs is the notation that "Enclosures that allow greater freedom of movement and unrestricted height (i.e., pens, runs, or kennels) are preferable." The ILAR Committee on Dogs authored *Dogs: Laboratory Animal Management* ([National Research Council, 1994](#)). This publication describes "features of housing, management, and care that are related to the expanded use of dogs as models of human diseases" and includes "an interpretive summary of the Animal Welfare Regulations and the requirements of the Public Health Service Policy on Humane Care and Use of Laboratory Animals." The reader is encouraged to use these publications to obtain further information on care and husbandry of dogs in the biomedical research setting.

II. BIOLOGY

A. Normal Values

The information presented in the tables represents a range of normal values that can vary depending on the analytical method, as well as the age, breed, and sex of the animal. [Table 12.1](#) shows representative normal

TABLE 12.1 Normal Vital Signs

Physiologic parameters ^a	Reference ranges
Temperature	37.9–39.9°C, 100.2–103.8°F
Heart rate (beats/min)	70–120
Respiratory rate (breaths/min)	18–34
Capillary refill time (seconds)	<2

^aModified from Detweiler D.K. and Erickson H.H., *Regulation of the Heart, in Dukes' Physiology of Domestic Animals, 12th edn., Reece W.O., Ed. Copyright 2004 by Cornell University.*

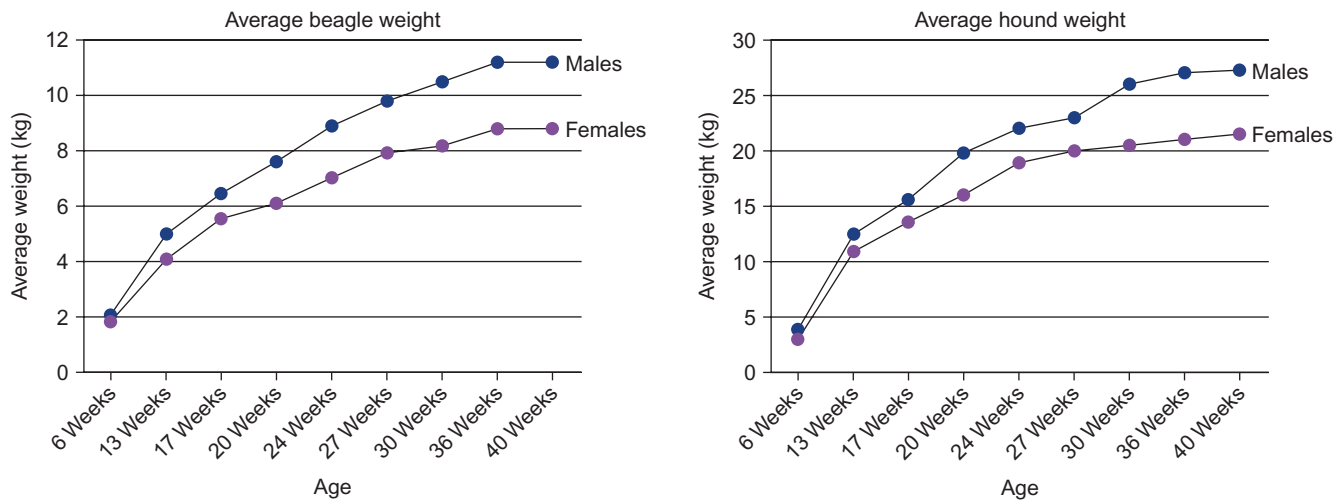


FIGURE 12.1 Average weight in kilograms by age in weeks for male and female beagle and hound dogs. Data courtesy of Dr. Asheley Wathen, Covance Laboratories, Inc., Madison, WI, and Dr. Kimberley Cohen, Covance Laboratories, Inc., Cumberland, VA (2013).

TABLE 12.2 Hematology Data from Purpose-Bred Beagles at Covance Laboratories, Inc.

Complete blood count ^a	Reference range	Units	4-Month male beagle	4-Month female beagle	6-Month male beagle	6-Month female beagle	8-Month male beagle	8-Month female beagle
HGB	12.1–20.3	g/dl	12.1	11.8	14.2	14.4	15.2	15.7
HCT	36–60	%	40.8	38.8	47.9	47.6	49.7	51.6
WBC	4.0–15.5	thousand/ μ l	18.6	20.3	14.4	11.5	11.6	11.8
RBC	4.8–9.3	million/ μ l	5.61	5.7	6.5	6.5	6.8	7
MCV	58–79	fl	73	68	74	73	73	74
MCH	19–28	pg	21.6	20.8	21.8	22.7	22.4	22.3
MCHC	30–38	g/dl	29.7	30.5	29.6	30.2	30.6	30.4
PLT	17–400	thousand/ μ l	512	438	436	345	329	344
DIFFERENTIAL								
Neutrophils	3000–13000	μ /l	12211	13172	9265	5202	8844	7074
Bands	0–300	μ /l	0	0	0	0	0	0
Lymphocytes	530–4800	μ /l	4648	5697	3916	3216	3036	3812
Monocytes	100–1800	μ /l	1325	1011	840	717	792	679
Eosinophils	0–1900	μ /l	446	369	310	253	528	187
Basophils	0–150	μ /l	0	0	19	20	0	71
OTHER TESTS								
T4	1.0–4.0	ng/dl	2.1	2.1	2	1.6	2.8	2.3

HGB, hemoglobin; HCT, hematocrit; WBC, white blood cells; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; T4, thyroxine.

^aBeagle baseline data averaged from blood testing performed in December 2011. From Dr. Asheley Wathen, Covance Laboratories, Inc., Madison, WI, and Dr. Kimberley Cohen, Covance Laboratories, Inc., Cumberland, VA (2013).

physiological data for a mixed population of dogs of both sexes. Fig. 12.1 demonstrates the normal weights and corresponding ages for both male and female beagle and hound dogs. Tables 12.2 and 12.3 feature

hematology data from beagles of both sexes from two commercial facilities. Tables 12.4 and 12.5 list serum chemical data for beagles of both sexes from two commercial facilities. Representative blood gas, coagulation

TABLE 12.3 Hematology Data from Purpose-Bred Beagles at Marshall BioResources

Complete blood count ^a	Units	6-Month male beagle (n =>1000)	6-Month female beagle (n =>1000)
HGB	g/dl	14.7 ± 1.3	15.2 ± 1.3
HCT	%	45.6 ± 3.6	47.0 ± 3.7
WBC	Thousand/ μ l	15.2 ± 3.9	14.7 ± 3.8
RBC	Million/ μ l	6.5 ± 0.5	6.7 ± 0.5
MCV	fl	70.3 ± 2.7	70.1 ± 3.0
MCH	pg	22.7 ± 0.9	22.7 ± 1.0
MCHC	g/dl	32.3 ± 1.2	32.4 ± 1.3
MPV	fl	11.2 ± 2.1	10.8 ± 1.9
RDW	%	13.6 ± 0.8	13.6 ± 0.9
HDW	g/dl	1.8 ± 0.2	1.8 ± 0.2
PLT	$\times 10^3/\mu$ l	410.1 ± 112.2	397.0 ± 115.7
DIFFERENTIAL			
Neutrophils	$\times 10^3/\mu$ l	8.6 ± 3.1	8.3 ± 2.8
Lymphocytes	$\times 10^3/\mu$ l	5.1 ± 1.1	5.1 ± 1.1
Monocytes	$\times 10^3/\mu$ l	1.0 ± 0.4	0.9 ± 0.4
Eosinophils	$\times 10^3/\mu$ l	0.3 ± 0.2	0.2 ± 0.1
Basophils	$\times 10^3/\mu$ l	0.2 ± 0.1	0.2 ± 0.1
PERCENTAGE			
Neutrophils	%	55.6 ± 6.6	55.7 ± 6.1
Lymphocytes	%	34.4 ± 6.4	35.2 ± 6.1
Monocytes	%	6.3 ± 1.7	5.8 ± 1.4
Eosinophils	%	2.0 ± 1.1	1.6 ± 0.9
Basophils	%	1.2 ± 0.5	1.3 ± 0.5

HGB, hemoglobin; HCT, hematocrit; WBC, white blood cells; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MPV, mean platelet volume; RDW, red cell distribution width; HDW, hemoglobin distribution width; PLT, platelets.

^aData generated by the Marshall Farms USA Bayer Advia 120 Hematology Analyzer. From Dr. Bambi Jasmin, Marshall BioResources, North Rose, NY (2013).

data, and normal urinalysis parameters can be found in Tables 12.6–12.8, respectively. Finally, the reviews in *Arterial and Venous Blood Gas Analyses* (Rieser, 2013) and the *Manual of Canine and Feline Cardiology* (Tilley *et al.*, 2007) are excellent resources.

B. Nutrition

Good nutrition and a balanced diet are essential to the health, performance, and well-being of the animal. The NRC of the United States National Academy of Sciences is the leading provider of nutrient recommendations for dogs and provides average requirements

needed to maintain growth and prevent deficiencies (Subcommittee on Dog and Cat Nutrition, 2006). The NRC publications form the basis for the Association of American Feed Control Officials (AAFCO) nutrient profiles, which are updated periodically (Baldwin *et al.*, 2010). The AAFCO is an advisory body comprising state representatives from across the United States. It provides a mechanism for developing and implementing uniform and equitable laws, regulations, standards, and enforcement policies, and establishes nutrient profiles for cat and dog foods (Dzanic, 1994; Thatcher *et al.*, 2010). Additional resources should be consulted for details on the nutritional requirements for dogs of all ages (Dzanic, 1994; Subcommittee on Dog and Cat Nutrition, 2006; Baldwin *et al.*, 2010; Thatcher *et al.*, 2010; Hand *et al.*, 2010).

Recommendations for feeding the appropriate amount of diet are determined by the dog's metabolic requirements. The maintenance energy requirement (MER) is the amount of energy used by a moderately active adult animal in a thermoneutral environment. The MER for most breeds may be calculated using the following equation: MER (metabolizable kcal/day) = BW \times 0.75 \times 550 kJ DE, where BW = body weight (kg), kJ = kilojoules, DE = digestible energy (Kienzle and Rainbird, 1991).

In-depth overviews of diets used in biomedical research are available in diet-specific literature. *Open-Formula Diets* have defined concentrations of all ingredients and the information is publicly available. This allows researchers to control for this important environmental variable and enables retrospective analysis of possible diet composition effects on research results (Barnard *et al.*, 2009). Open-formula diets occasionally may require changes in formulation to maintain nutrient composition or meet changing nutrient requirements. These changes in quantitative ingredient formulation are made public when open-formula diets are modified. In contrast, *closed-formula diets* are commercially available, balanced diets that meet and label the minimum requirements for protein and fat and the maximum values for ash and fiber; however, the exact composition of ingredients may vary from batch to batch. Ingredient composition varies as the manufacturer applies a least-cost strategy, referring to formulating diets to maximize profit by using the least-expensive ingredients. Although the ingredients are listed, the quantitative ingredient formulation is not publicly available and can vary without public disclosure, due to proprietary nature of commercial diets produced and marketed under vendor trade names. Closed-formula diets have also been referred to as 'fixed formula' or 'constant nutrition' (LabDiets, PMI Nutrition International, St. Louis, Missouri) by manufacturers (Barnard *et al.*, 2009). In *fixed-formula diets*, the quantitative ingredient formulation does not change; however, this information is proprietary and therefore

TABLE 12.4 Clinical Chemistry Data from Beagles at Covance Laboratories, Inc.^a

Blood chemistry	Reference range	Units	4-Month male beagle	4-Month female beagle	6-Month male beagle	6-Month female beagle	8-Month male beagle	8-Month female beagle
Total protein	5.0–7.4	g/dl	5.4	5.3	5.9	5.5	5.8	5.8
Albumin	2.7–4.4	g/dl	2.9	2.8	3.2	3.2	3.2	3.4
Globulin	1.6–3.6	g/dl	2.5	3.5	2.6	2.4	2.6	2.3
Albumin/globulin ratio	0.8–2.0	ratio	1.2	1.2	1.2	1.4	1.3	1.5
AST (SGOT)	15–66	U/l	51	48	50	36	40	63
ALT (SGPT)	12–118	U/l	41	39	42	47	44	57
ALP	5–131	U/l	124	122	97	98	99	72
GGTP	1–12	U/l	6	5	5	5	6	5
Total bilirubin	0.1–.03	mg/dl	0.1	0.1	0.2	0.2	0.1	0.2
BUN	6–25	mg/dl	20	22	25	24	18	21
Creatinine	0.5–1.6	mg/dl	0.4	0.4	0.4	0.4	0.5	0.5
Phosphorus	2.5–6.0	mg/dl	8.6	9.2	8.2	7.5	6.5	6.3
Glucose	70–138	mg/dl	87	76	65	90	82	80
Calcium	8.9–11.4	mg/dl	11	11	7.9	9.2	10.5	10.4
Magnesium	1.5–2.5	mEq/dl	1.7	1.7	1.8	1.7	1.6	1.9
Sodium (Na)	139–154	mEq/dl	144	147	147	145	145	148
Potassium (K)	3.6–5.5	mEq/dl	5.6	5.5	6.4	5.6	5	5.1
Na/K ratio		ratio	26	27	23	26	28	29
Chloride	102–120	mEq/dl	98	108	105	105	108	109
Cholesterol	92–324	mg/dl	159	152	136	127	151	141
Triglycerides	29–291	mg/dl	91	96	72	64	62	44
Amylase	290–1125	U/l	614	604	713	543	671	597
Lipase	77–695	U/l	160	189	200	224	147	280
CPK	59–895	U/l	945	829	606	318	624	741

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGTP, gamma-glutamyl transpeptidase; BUN, blood urea nitrogen; CPK, creatine phosphokinase.

^aBeagle baseline data averaged from blood testing performed in December 2011. From Dr. Asheley Wathen, Covance Laboratories, Inc., Madison, WI, and Dr. Kimberley Cohen, Covance Laboratories, Inc., Cumberland, VA (2013).

not disclosed publically (Barnard *et al.*, 2009). *Semi-purified and purified* diets provide the strictest control of ingredients and are formulated from purified components: amino acids, lipids, carbohydrates, vitamins, and minerals. Although purified and semipurified diets do differ in the types of ingredients used, the terms are generally used to mean the same thing. Purified-ingredient diets are generally ‘open’ formulas, meaning that they are published and available to the scientific community.

The animal care provider should be aware of the manufacture date of the diet, which should be clearly visible on the bag. As a general rule, diets are safe for consumption up to 6 months following the manufacture date when stored at room temperature. Refrigeration may prolong

the shelf-life, but the best strategy is to feed only fresh diets and use each lot based on the date of manufacture. Specifications for feeding and watering of dogs are provided in the regulations of the Animal Welfare Act.

C. Reproduction

Management of a breeding colony requires broad knowledge of the dog’s anatomy, reproductive physiology, and behavioral needs during breeding, gestation, and parturition. Although a comprehensive discussion of the biology of canine reproduction is beyond the scope of this chapter, essential features of the broad topics noted above are presented.

TABLE 12.5 Clinical Chemistry Data from Beagles at Marshall BioResources

Blood chemistry ^a	Units	6-Month male beagle (n = 50)	6-Month female beagle (n = 50)
Total protein	g/dl	5.1 ± 0.3	5.2 ± 0.4
Albumin	g/dl	2.7 ± 0.3	2.7 ± 0.3
Globulin	g/dl	2.5 ± 0.1	2.4 ± 0.2
AST (SGOT)	U/l	43.7 ± 7.1	46.3 ± 7.0
ALT (SGPT)	U/l	33.7 ± 5.6	37.5 ± 7.2
ALP	U/l	154.4 ± 36.4	113.3 ± 24.8
GGTP	U/l	6.8 ± 1.0	7.7 ± 2.9
Total bilirubin	mg/dl	0.2 ± 0.1	0.3 ± 0.1
BUN	mg/dl	19.0 ± 4.8	17.7 ± 3.5
Creatinine	mg/dl	0.9 ± 0.2	0.8 ± 0.2
Phosphorus	mg/dl	7.8 ± 0.6	7.8 ± 0.8
Glucose	mg/dl	87.8 ± 10.0	88.7 ± 8.8
Calcium	mg/dl	11.5 ± 0.4	11.1 ± 0.3
Magnesium	mEq/dl	1.9 ± 0.1	1.9 ± 0.1
Sodium (Na)	mEq/dl	146.6 ± 2.5	145.5 ± 1.6
Potassium (K)	mEq/dl	5.2 ± 0.4	5.1 ± 0.4
Na/K ratio	ratio	28.1 ± 2.0	28.7 ± 2.0
Chloride	mEq/dl	114.6 ± 1.8	111.9 ± 1.6
Cholesterol	mg/dl	202.2 ± 32.0	181.8 ± 26.0
Amylase	U/l	688.5 ± 99.2	687.9 ± 84.6
CPK	U/l	243.7 ± 64.1	230.2 ± 52.5

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGTP, gamma-glutamyl transpeptidase; BUN, blood urea nitrogen; CPK, creatine phosphokinase.

^aData generated by the Marshall BioResources Vitros 250. From Dr. Bambi Jasmin, Marshall BioResources, North Rose, NY (2013).

TABLE 12.6 Normal Physiologic Data for Dogs

Arterial blood gas ^a	Reference range
pH	7.3–7.5
PCO ₂ (mmHg)	30.8–42.8
PO ₂ (mmHg)	80.9–103.3
HCO ₃ (mEq/l)	18.8–25.6

^aFrom Tieser T.M., *Arterial and Venous Blood Gas Analysis, Topics in Companion Animal Medicine* 28 (2013) 86–90.

1. Anatomy and Reproductive Physiology of the Bitch

Overall health, body condition, nutrition, and age greatly influence reproductive efficiency (Gavrillovic *et al.*, 2008; Johnson, 2008). Therefore, only normal,

TABLE 12.7 Normal Coagulation Data in Dogs

Coagulation tests ^{a,b}	Time (seconds)
Prothrombin time (OSPT)	6–10
Activated partial thromboplastin time	11–19
Thrombin clotting time	3–8
Activated clotting time	55–80

^aModified from *The Laboratory Canine*, Taylor & Francis, New York, Garrett Field, Todd A. Jackson (2007).

^bValues from coagulation tests may have wide variability due to collection method, anticoagulant used, analyte (whole blood, serum, plasma, platelet poor plasma), temperature, and method of analysis.

TABLE 12.8 Normal Chemistry-Urinalysis in Dogs

Urine volume and specific gravity ^a	
Volume (ml/kg body weight/day)	24–41
Specific gravity (g/l)	1.016–1.060
pH	5.5–7.5
Glucose/ketones	0

^aModified from Reece W.O., *Kidney Function in Mammals, in Dukes' Physiology of Domestic Animals*, 12th edn., Reece W.O., Ed. Copyright 2004 by Cornell University.

healthy animals in excellent body condition should be used in breeding programs. Beagles between 2 and 3.5 years of age have the best conception rates and litter size with the lowest neonatal mortality. After 5 years of age, conception rates and litter size decline and neonatal mortality increases (Johnson, 2008).

The vagina is a long, musculomembranous canal that extends from the uterus to the vulva. During physical examination, the gloved finger or examination instrument should be introduced through the dorsal commissure of the vulva, avoiding the deep ventral clitoral fossa. Examination should proceed at an angle of approximately 60° until the instrument or fingertip has passed over the ischial arch, after which it can be directed further cranial toward the cervix. The uterus consists of the cervix, uterine body, and uterine horns. The cervix is an abdominal organ, located approximately halfway between the ovaries and the vulva. When the bitch is in proestrus and estrus, the cervix can be distinguished during abdominal palpation as an enlarged, turgid, walnut-shaped structure.

Female dogs are monoestrous, typically nonseasonal, spontaneous ovulators that have a spontaneous luteal phase approximately 5 days longer than the 65 ± 1 days of pregnancy followed by obligate anestrus. Puberty (beginning of the first estrus) occurs between 6 and 14 months in most breeds. The time of onset positively correlates with the body size (Concannon, 2011).

The canine cycle is divided into four phases: proestrus, estrus, diestrus, and anestrus. The duration of

proestrus is 5–20 days with an average of 9 days and reflects the follicular phase rise in estrogen. During this stage, the vulva is enlarged and turgid, and a serosanguinous vaginal discharge is present (Concannon, 2011). Estrus may be from 5 to 15 days in duration but generally lasts 9 days. The endocrine feature of estrus is the first abrupt increase in progesterone ($>5\text{ ng/ml}$), which occurs concomitantly with the luteinizing hormone (LH) surge 95% of the time, followed by ovulation within 24–72 h. The vulva is softer and smaller than in proestrus. The vaginal discharge persists and may remain serosanguinous or become straw colored.

Diestrus begins approximately 9 days after the onset of standing heat. The end of this stage is 60 days later, which would be coincident with whelping if the bitch had become pregnant. Defined behaviorally as starting when estrous behavior ceases (Concannon, 2011), diestrus represents the peak of serum progesterone. Anestrus may last from 80 to 240 days and is the stage of reproductive quiescence. It is characterized by an absence of ovarian activity and serum progesterone levels of less than 1 ng/ml .

2. Anatomy and Reproductive Physiology of the Male Dog

The onset of puberty in the male ranges from 5 to 12 months of age and is affected by breed, season, nutrition, and disease status. This process is initiated by the secretion of LH from the anterior pituitary, which stimulates the production of testosterone by the interstitial or Leydig's cells. At this time, the testicular growth is rapid, the seminiferous tubules begin to differentiate, and Sertoli cells form the blood–testis barrier. Secretion of follicle-stimulating hormone (FSH) by the anterior pituitary stimulates the production of other key hormones by the Sertoli cells, including, inhibin, androgen binding protein, and estrogen. FSH stimulates spermatogenesis in the presence of testosterone, whereas inhibin and estrogen provide negative feedback to the pituitary gland to decrease FSH production. Spermatogenesis in the dog is completed in 45 days, with subsequent maturation of sperm occurring in the epididymis for approximately 15 days. Thus, the entire process from the initiation of spermatogonial mitosis to the delivery of mature sperm to the ejaculate is 60 days.

A breeding soundness exam should be conducted to assess the probability of a male dog's successful production of offspring. Factors affecting male fertility include libido, ability to copulate, testicular size, and quality semen production. Suppression of sexual behavior and problems with libido may occur in dogs due to early weaning, isolation, or inherited abnormalities. Animals with poor hind limb conformation or trauma to the back may be unable to properly mount the female. There is a positive correlation between scrotal circumference and

the number of sperm produced. Finally, the quality of sperm is assessed by motility, morphology, volume, and concentration. An ejaculate (5 ml) that contains approximately 500 million progressively motile sperm without significant morphological abnormalities is a good indicator of normal male fertility. Complete anatomy of the bitch and dog can be found in *Miller's Anatomy of the Dog* (Evans and de Lahunta, 2012).

3. Detection of Estrus and Pregnancy

Cells of the vaginal epithelium mature to keratinized squamous epithelium under the influence of estrogen. Because of the rise in estrogen throughout proestrus, with peak levels occurring just prior to the onset of standing heat, the vaginal smear can be used as an indicator of the bitch's readiness for breeding. The smear will not confirm the presence of ovulation nor is it of prognostic value in normal bitches during anestrus.

The percentage of vaginal epithelial cell cornification is an index of estrogen secretion by the ovarian follicles. Cornification occurs approximately 2 days prior to the estrogen peak and 4 days prior to standing heat. As cornification of vaginal epithelial cells proceeds, the cells become larger, with more angular borders. The nuclear/cytoplasmic ratio decreases until the nuclei reach a point where they no longer take up stain (coincident with the onset of estrus). The cells appear 'anuclear' and are classified as 'cornified' or 'anuclear squames.' The vaginal cytology smear of the bitch changes from predominantly cornified to noncornified 6 days after ovulation. The day of this change is the first day of diestrus. Other epithelial cell types noted on vaginal cytology include superficial cells (large, angular cells with small nuclei); intermediate cells (round or oval cells with abundant cytoplasm and large, vesicular nuclei); and parabasal cells (small round or elongated cells with large, well-stained nuclei, and a high nuclear/cytoplasmic ratio). Based on vaginal cytology, the estrous cycle is classified as follows:

Proestrus, early: intermediate and superficial cells, red blood cells, and neutrophils

Proestrus, late: superficial cells, anuclear squames, and red blood cells

Estrus: more than 50% anuclear squames, superficial cells, \pm red blood cells

Diestrus: more than 50% intermediate cells, superficial cells, and squames early, but becoming completely noncornified with neutrophils present as diestrus proceeds

Anestrus: small numbers of parabasal cells and intermediate cells, \pm neutrophils

Although vaginal cytology is a useful tool, observation of behavioral estrus is the best criterion to use in breeding management. During proestrus, the male is attracted to the bitch and will investigate her hindquarters, but she

will not accept breeding. Estrus is characterized by proactive receptivity to mounting by males and increased male-seeking behavior (Concannon, 2011). During this stage, the bitch will exhibit 'flagging,' or elevation of her tail with muscular elevation of the vulva to facilitate penetration by the male. In order to maximize the conception rate and litter size, it is recommended to breed the bitch on days 1, 3, and 5 of the standing heat. Due to the long life span of canine sperm, fertilization occurs in the oviduct up to 8 days after coitus. The ovulated oocyte is a primary oocyte that must undergo two meiotic divisions before fertilization can occur. This overall maturation process takes approximately 2 days. After maturation, the oocyte remains viable for 4–5 days. Optimal conception rates tend to occur when the bitch is bred from 4 days before to 3 days after ovulation; best litter size is achieved when the bitch is bred 2 days after ovulation.

4. Pregnancy

Implantation is evident by areas of local endometrial edema 17–18 days after breeding. There is no correlation between the number of corpora lutea and the number of fetuses in the corresponding uterine horn, suggesting transuterine migration of embryos.

The dog has endotheliochorial placentation. The endothelium of uterine vessels lies adjacent to the fetal chorion, mesenchymal, and endothelial tissues, so that maternal and fetal blood are separated by four layers. The canine placenta is also classified as zonary, indicating the placental villi are arranged in a belt, and deciduate, reflecting that maternal decidual cells are shed with fetal placentas at parturition.

The length of gestation is 59–63 days. Luteal progesterone is responsible for maintaining pregnancy and canine corpora lutea retain their structural development throughout gestation. Serum progesterone rises from less than 1 ng/ml in late proestrus to a peak of 30–60 ng/ml during gestation, and then declines to 4–5 ng/ml just prior to parturition. Progesterone is essential for endometrial gland growth, secretion of uterine milk, attachment of the placentas, and inhibition of uterine motility (Johnson, 2008; Verstegen-Onclin and Verstegen, 2008).

Pregnancy detection can be performed by several methods. Abdominal palpation of the uterus may be most informative at approximately 28 days after breeding. The embryos and chorioallantoic vesicles form a series of ovoid swellings and are approximately 2 inches in length at 28–30 days. By day 35, the uterus begins to enlarge diffusely and the vesicles become difficult to identify by palpation. Radiology can be used to confirm pregnancy and facilitate determination of gestational age, beginning 45 days after the LH surge (Lopate, 2008). Bitches in which a difficult whelping is anticipated should be radiographed in late pregnancy to determine

the litter size and to evaluate the size of the fetal skulls in relation to the bony maternal birth canal.

Ultrasonography can be used to confirm pregnancy beginning on days 18–22, at which point the gestational sacs will be approximately 1 cm in diameter, and until parturition (Shille and Gontarek, 1985; Lopate, 2008). Ultrasonography can assess fetal viability by visualizing fetal heartbeats and fetal movement beginning on gestational days 23–25 and 35, respectively (Lopate, 2008). It can also predict gestational age using the inner diameter of the chorionic cavity in early pregnancy and the biparietal diameter in late pregnancy (Beccaglia and Luvoni, 2006; Luvoni and Beccaglia, 2006). However, ultrasonography for determination of gestational age is most accurate at day 30 of pregnancy when using correction factors for small (<9 kg) and large (>40 kg) body weight dogs (Kutzler *et al.*, 2003).

5. Parturition and the Neonate

Thermal support should be provided prior to parturition. Dogs housed on grated flooring should be provided with mats and those on solid floors would benefit from blankets placed in a corner of the primary enclosure. Shavings are discouraged because they may adhere to the umbilical cord and predispose to ascending infections. Heat lamps may be placed 24 h prior to parturition and remain until all neonates demonstrate vigorous suckling behavior. However, the use of heat lamps necessitates strict supervision in order to prevent thermal burns. If possible, whelping bitches should be housed in a quiet corridor in order to decrease periparturient stress, especially in primiparous or young mothers. Monitoring of parturition is important, but human intervention should be minimal in order to prevent stress-induced cannibalism.

An abrupt drop in body temperature to less than 100°F indicates impending parturition within 18–24 h. The process of parturition has been divided into three stages. Stage 1 of labor lasts 6–12 h and is characterized by uterine contractions and cervical dilation. During this stage, the bitch may appear restless, nervous, and anorexic. Other common clinical signs include panting and increased pulse rate (Johnson, 2008).

Fetal expulsion occurs during stage 2, which lasts approximately 3–6 h. As the fetus engages the cervix, there is release of oxytocin, referred to as the Ferguson reflex, which strengthens the uterine contractions and may elicit abdominal contractions as well. The bitch is able to inhibit this stage of labor if disturbed. The chorioallantois ruptures either during passage of each neonate through the birth canal or by the bitch's teeth at birth. Interestingly, posterior presentation is common in dogs but does not predispose to dystocia. The time interval between deliveries of each pup is irregular, but the average is less than 1 h between pups. Veterinary assistance

is necessary if the bitch remains in stage 2 for more than 5 h without delivering the first pup, or for more than 2 h before delivering subsequent pups.

During stage 3 of labor, the placentas are expelled either immediately or within 15 min of delivery of each pup. If two pups are delivered from alternate uterine horns, then the birth of both puppies may precede expulsion of the respective placentas. The bitch will lick the newborn vigorously to remove the membranes from its head and to promote respiration. She will also sever the umbilical cord. The bitch may ingest the placentas, although they confer no known nutritional benefit and may induce a transient diarrhea.

The peripartum use of oxytocin is required only in the event of uterine inertia, stillbirths, or agalactia. Oxytocin should not be used in the event of systemic illness or abnormalities precluding vaginal delivery. Indications for its use include lack of delivery 24 h after onset of stage 1 labor, greater than 1 h of unproductive stage 2 labor, inadequate contractions, or abnormal vaginal discharge. In these cases, radiographs are recommended to assess fetal size in relation to the birth canal and any possible obstructions, followed by 0.25–2.00 IU of oxytocin intramuscularly or subcutaneously. The oxytocin can be repeated 30–60 min after the first dose for a total of two doses (Plunkett, 1993). In some cases, treatment with 0.5–1.5 ml/kg of 10% calcium gluconate, delivered slowly IV while monitoring closely for bradycardia, and 25% dextrose IV may be indicated.

Uterine involution occurs during anestrus within 4–5 weeks of parturition. During this time, a greenish to red–brown vaginal discharge, or lochia, is considered normal. The presence of an odiferous, purulent discharge, accompanied by systemic signs of illness, indicates metritis or pyometra. Desquamation of the endometrium begins by the 6th postpartum week, with complete repair by 3 months.

Newborn puppies are easily sexed by examination of the anogenital distance. In female puppies, the vulva is evident a short distance from the anus, whereas the prepuce of male puppies is nearly adjacent to the umbilicus. Eyes are open at approximately 12 days, and ears are patent at approximately 12–20 days. Solid food can be introduced between 4.5 and 6 weeks of age, and puppies can be weaned at 6–8 weeks.

6. Artificial Insemination

Artificial insemination (AI) is indicated when the male is physically incapable of mounting or penetrating the bitch, when there are vaginal abnormalities such as a vaginal–vestibular stricture, narrow vagina, vaginal septum, and vaginal hyperplasia, or if there is a behavioral incompatibility between the male and female dogs (Kutzler, 2005).

Semen is collected using a plastic centrifuge tube and rubber latex artificial vagina. The male is introduced to the scent of an estrous bitch and manually stimulated. The first two fractions are collected followed by a sufficient amount of the third fraction (predominantly of prostatic fluid) to bring the total semen volume to 4–6 ml.

The semen can be introduced into the cranial vagina or directly into the uterus either through trans-cervical catheterization with a Norwegian AI catheter or utilizing fiberoptic endoscopy. Use of the Norwegian AI catheter for intrauterine insemination of frozen-thawed, fresh, and chilled-extended semen results in significantly higher whelping rates than intravaginal insemination (Linde-Forsberg *et al.*, 1999; Thomassen and Farstad, 2009). For trans-cervical insemination, the bitch is either standing on all four legs or standing with hindquarters raised. The AI catheter and guiding tube are inserted into the vestibulum as far as the pseudocervix. Firm abdominal palpation is then used to locate and fix the cervix in the other hand, at which point the catheter is further inserted along the dorsal vaginal fold until the cervical opening is located and semen is deposited into the uterus lumen (Thomassen and Farstad, 2009).

Surgical and laparoscopic AI has been used successfully for intrauterine and intratubal insemination; however, these techniques are invasive and require anesthesia. Therefore, the nonsurgical techniques mentioned above are recommended, as these approaches are less invasive and can be completed without anesthesia in nonsedated or sedated dogs depending on the experience of the personnel and personality of the dogs.

AI with freshly collected sperm can be done on days 1, 3, and 5 of standing heat or on days of maximal vaginal cornification. The viability of frozen-thawed sperm is significantly reduced compared to fresh or chilled sperm that may live up to 5 or 6 days in the reproductive tract of the bitch; frozen-thawed sperm live only a few hours. Therefore, the ova must be mature and insemination with frozen-thawed semen must be done 2–3 days after ovulation in the bitch as determined by serum progesterone concentrations (Thomassen *et al.*, 2006).

7. False Pregnancy

False pregnancy (pseudocyesis), a stage of mammary gland development and lactation associated with nesting or mothering behavior, is common in the bitch. The condition occurs after the decline in serum progesterone toward the end of diestrus. There is no age or breed predisposition. Pseudopregnancy does not predispose the bitch to reproductive disease or infertility. A comprehensive review of canine pseudocyesis exploring its cause, clinical features, and treatments is covered by C. Gobello (Gobello *et al.*, 2001).

8. Reproductive Life Span

Reproductive performance in the bitch is optimal prior to 4 years of age. Cycling does not completely cease; however, after 5–8 years of age, bitches demonstrate significant decreases in conception rate and the number of live pups whelped. By 8–9 years of age, pathologic conditions of the uterus, such as cysts, hyperplasia, atrophy, and neoplasia, are extremely common.

D. Behavior

Dogs prefer living in a social environment. Dogs have well developed olfactory glands, vision, and auditory and tactile senses that allow them to gain environmental cues and information from other dogs and humans (Field and Jackson, 2006; Joint Working Group on Refinement, 2004). Much of their instinctive behavior is dependent on learning to interact with other members of their species. Beagles have been a popular animal model because of their docile nature. They are easily handled and, for the most part, respond favorably to repetitive manipulations such as body weight measurements, physical examination, electrocardiograms (ECGs), oral gavage, and venipuncture.

Although sexually mature by 6–9 months of age, dogs are not socially mature until 18–36 months of age. The socialization process should begin early during development, when puppies are receptive to conspecific and human contact. For example, from 3 to 8 weeks of age, puppies are most capable of learning about how to interact with other dogs. Between weeks 5 and 12, puppies are most capable of learning how to interact with people. By 10–12 weeks of age, dogs voluntarily wander and explore new environments. Thus, early handling and mild stress (such as vaccination) appear to be extremely beneficial components of a dog's social exposure.

Canid social systems use signals and displays that minimize the probability of outright aggression. These behavior patterns are most likely elicited during distressful situations, such as strange environments, being handled by strange people, or encountering new animals. An excellent, illustrated discussion of normal canine behavior patterns can be found in the canine behavior section of the *Manual of Clinical Behavioral Medicine for Dogs and Cats* (Overall, 2013).

III. DISEASES

By virtue of the dog's status as a companion animal, there are many veterinary publications and reference texts on the diagnosis, medical management, pathology, and epidemiology of its disorders. The authors of this chapter have chosen to emphasize those diseases that

are more frequently encountered in the research setting, especially infectious diseases associated with the use of random-source dogs and conditions seen frequently in the beagle. For more thorough and detailed discussion of these diseases, as well as those not discussed in this chapter, the reader should consult standard veterinary textbooks.

A. Infectious Diseases

1. Bacterial and Mycoplasmal Diseases

a. Canine Infectious Respiratory Disease (Kennel Cough Complex and Infectious Tracheobronchitis)

Etiology Canine infectious respiratory disease (CIRD) is a highly contagious illness and several organisms have been incriminated including *Bordetella bronchiseptica*; *Streptococcus equi* subsp. *zoepidemicus*; canine parainfluenza virus (CPIV); canine influenza virus (CIV); canine respiratory coronavirus; canine adenovirus type 2 (CAV-2); canine herpesvirus; canine reovirus types 1, 2, and 3; and mycoplasma and ureaplasma. Naturally occurring infection can result in coinfection by two or more organisms (Garnett *et al.*, 1982; Ford, 2012).

Clinical Signs CIRD can be subdivided into mild or severe forms. The mild form is more common and is characterized by an acute onset of a loud, dry, hacking cough. Increased formation of mucus sometimes results in a productive cough, followed by gagging or retching motions. Cough may be elicited by tracheal palpation and may be more frequent with excitement or exercise. Otherwise, dogs are typically asymptomatic. Mild tracheobronchitis usually lasts 7–14 days, even when untreated.

The severe form results from poor general health, immunosuppression, or lack of vaccination. Secondary bronchopneumonia can occur and can be the determinant of severity (Sherding, 1994). Animals are clinically ill and may be febrile, anorexic, and depressed. Productive cough and mucopurulent naso-ocular discharge are more common than in the mild form.

Epizootiology and Transmission The natural reservoir for *B. bronchiseptica* is the respiratory tract (Bemis, 1992), and it is very easily spread by aerosol and direct contact. Transmission is heightened by confined housing of multiple animals. *Bordetella bronchiseptica* is highly infectious with an incubation period of 3–10 days.

Pathogenesis The most common clinical isolates are CPIV and *B. bronchiseptica* (Mochizuki *et al.*, 2008). However, *B. bronchiseptica* is often recovered from clinically healthy animals (Chalker *et al.*, 2003). During clinical infection, *B. bronchiseptica* attaches to the cilia of the upper airway epithelium, causing suppurative tracheobronchitis and bronchiolitis. Infections with CPIV

or CAV-2 alone are usually subclinical but can cause necrotizing tracheobronchiolitis (Dungworth, 1985).

Diagnosis and Differential Diagnosis Diagnosis is often based on clinical signs and known history; however, cough elicited by tracheal palpation may be inconsistent and should not be used for definitive diagnosis. Presumptive diagnosis can be made by isolation of *B. bronchiseptica* or mycoplasma by nasal swabs. Viral isolation or paired serology is often impractical and expensive. If cough persists for more than 14 days, other disease conditions should be considered. Differential diagnoses include CIV, canine distemper virus, pneumonia, heartworm disease, tracheal collapse, mycotic infections, and diseases resulting in tracheal compression (Johnson, 2000).

Prevention Prevention is best achieved by avoiding exposure to infected animals. Dogs should be vaccinated prior to or upon admission to the animal facility. Intranasal vaccines protect against infection and disease and can be given to dogs as young as 3 weeks of age (Greene and Levy, 2012). Combination vaccines for *B. bronchiseptica*, CAV-2, and CPIV are preferred. Vaccinations should be boosted every 6 months when multiple animals are housed in a confined area.

Control Staff must practice proper hygiene to prevent transmission by fomites. Sanitation, proper ventilation, and proper humidity are critical for control. Symptomatic animals should be isolated and kennels should be disinfected with agents such as bleach, chlorhexidine, or quaternary ammonium chloride.

Treatment *Bordetella bronchiseptica* is sensitive to potentiated sulfas, chloramphenicol, quinolones, tetracyclines, gentamicin, and kanamycin. Use of antibiotics is indicated when severe or persistent clinical signs occur and should be continued for 14 days. For severe or unresponsive infection, treatment should be based on bacterial culture/sensitivity patterns. Nebulized gentamicin or kanamycin may be helpful in severe cases. Antitussives should be avoided if the cough is productive; however, their use is indicated if coughing is causing discomfort or interfering with sleep. Bronchodilators such as aminophylline, theophylline, or terbutaline can be helpful in reducing reflex bronchoconstriction.

Research Complications Due to the altered respiratory tract physiology, infected animals should not be used for pulmonary studies.

b. Group C *Streptococcus* Infections

Etiology β -Hemolytic Lancefield's group C streptococcus (*S. equi* ssp. *zooepidemicus*) is a gram-positive, non-spore-forming coccus that causes pneumonia and sepsis in dogs.

Clinical Signs Clinical signs vary based on the organ system affected. Pneumonic disease is typically associated with sudden onset of clinical signs including



FIGURE 12.2 Open view within the thoracic cavity of a dog after acute death from *Streptococcus equi* subsp. *zooepidemicus*. Petechial and ecchymotic hemorrhages evident on the parietal pleura and hemorrhagic fluid within the thoracic cavity. Reprinted from *The Veterinary Journal*, vol. 188, Priestnall, S. and Erles, K., *Streptococcus zooepidemicus: an emerging canine pathogen*, pp. 142–148, 2011, with permission from Elsevier.

coughing, weakness, fever, dyspnea, and hematemesis. The rapid progression of disease is similar to that seen in humans with toxic shock syndrome (TSS) caused by *Streptococcus pyogenes*. Peracute death has been reported in research and shelter dogs (Bergdall *et al.*, 1996; Pesavento *et al.*, 2008).

Epizootiology and Transmission *Streptococcus equi* ssp. *zooepidemicus* is not considered a commensal of healthy dogs as most of the β -hemolytic commensal organisms belong to group G, specifically *Streptococcus canis*. Asymptomatic carriers are suspected to be the route by which infection enters populations. *Streptococcus equi* ssp. *zooepidemicus* is considered an opportunistic pathogen and stressful factors such as transport can predispose to disease (Priestnall *et al.*, 2010).

Pathologic Findings In peracute cases, hemorrhage from the mouth and nose and within the pleural cavity can be the most striking lesion. Ecchymotic and petechial hemorrhages can be noted on several organs (Fig. 12.2). 'Bull's-eye' lesions may be observed on the pleural surface of affected lung lobes. Histologic lesions can include fibrino-suppurative, necrotizing, and hemorrhagic pneumonia. Gram-positive cocci can be found in intracellular clusters throughout the lung (Fig. 12.3), tonsils, and spleen of affected animals (Bergdall *et al.*, 1996; Priestnall and Erles, 2011).

Pathogenesis Predisposing factors such as transport stress and viral coinfection have been shown to contribute to the virulence of *S. zooepidemicus* (Priestnall and Erles, 2011). Due to the similarities with the clinical

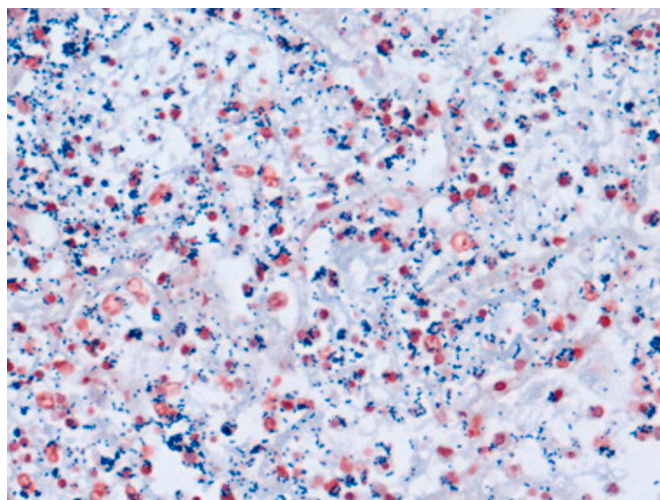


FIGURE 12.3 Gram stain of *Streptococcus zooepidemicus*-positive canine lungs. Numerous gram-positive coccoid bacteria within the alveolar spaces. Some bacteria evident within neutrophil cytoplasm. Magnification $\times 400$. Reprinted from *The Veterinary Journal*, vol. 188, Priestnall, S. and Erles, K., *Streptococcus zooepidemicus: an emerging canine pathogen*, pp. 142–148, 2011, with permission from Elsevier.

signs seen in human cases of TSS, superantigens are thought to contribute to the virulence of *S. zooepidemicus* in cases of acute hemorrhagic pneumonia. These superantigens work by bypassing the conventional mechanisms of antigen presentation and binding to major histocompatibility complex class II receptors. As a result, there is a hyperactive proinflammatory response and an ‘avalanche’ of cytokines including interleukin 1 β (IL-1 β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF- α). Three novel superantigen-encoding genes have been identified from a case of acute fatal hemorrhagic pneumonia, *szeF*, *szeN*, and *szeP*. However, it is currently unclear what effect these superantigens have *in vivo* (Paillot *et al.*, 2010; Priestnall *et al.*, 2010). While superantigens have been detected in some isolates, there is not enough data to determine if superantigens play a role in the pathogenesis (Byun *et al.*, 2009; Kim *et al.*, 2007).

Diagnosis and Differential Diagnosis Definitive diagnosis is based on bacterial culture of nasal swabs or transtracheal lavage. Polymerase chain reaction (PCR) can be done on post-mortem lung tissue. Bacterial pneumonias or bacteremias can be caused by other pathogenic *Streptococcus* spp., *Staphylococcus* spp., *Escherichia coli*, *Pasteurella multocida*, *Pseudomonas* spp., *Klebsiella pneumoniae*, and *B. bronchiseptica*. Nonbacterial causes of respiratory disease include rodenticide intoxication, coagulopathies, heartworm disease, pulmonary thromboembolism, ruptured aneurysm, and left-sided congestive heart failure.

Prevention and Control There is no vaccine for prevention of *S. zooepidemicus*. The organism has been

isolated from the environment during active outbreaks (Pesavento *et al.*, 2008), so dogs diagnosed with *S. zooepidemicus* should be quarantined and any potential fomites (e.g. food bowls, enrichment) should be properly disinfected.

Treatment Antibiotic therapy should be based on culture and sensitivity. Resistance to doxycycline and tetracycline has been demonstrated (Garnett *et al.*, 1982; Pesavento *et al.*, 2008).

Research Complications Dogs with severe hemorrhagic pneumonia or systemic disease are not appropriate for research study. The association between epizootics of this disease and transportation supports operational policies that require adequate acclimation periods for animals upon arrival.

c. Leptospirosis

Etiology Serovars Canicola, Bratislava, and Grippotyphosa result in renal or hepatic disease, whereas serovars Icterohaemorrhagiae and Pomona predominantly result in hepatic disease (Greene *et al.*, 2012).

Clinical Signs Canine leptospirosis can present as subclinical, acute, or chronic disease. Clinical signs in acute infection can be nonspecific and include lethargy, depression, abdominal discomfort, stiffness, anorexia, vomiting, muscle tenderness, and pyrexia. Clinical signs can be related to renal failure including polyuria and polydipsia, with or without azotemia, oliguria, or anuria. Leptospirosis can also lead to hepatic failure with signs such as icterus or bleeding abnormalities. Peracute leptospirosis is characterized by shock, vascular collapse, and rapid death. Uveitis, abortions, stillbirths, and pulmonary hemorrhage have also been associated with leptospirosis (Klopfleisch *et al.*, 2010; van de Maele *et al.*, 2008).

Epizootiology and Transmission Bivalent vaccines against the most common canine serovars, Icterohaemorrhagiae and Canicola, have resulted in the increased prevalence of other serovars including Grippotyphosa, Pomona, Bratislava, and Autumnalis. Increased movement of wild animal reservoirs (rats, raccoons, skunks, opossums) into urban/suburban areas have also contributed to the greater prevalence of previously uncommon serovars (Sykes *et al.*, 2011). Transmission occurs primarily through environmental contact, although direct transmission between hosts may also occur. Leptospire passing from urine into water is the most common route of contamination (Goldstein, 2010). Leptospirosis is a zoonotic disease.

Pathologic Findings The kidneys consistently have gross and microscopic lesions. In the acute phase, the kidneys are swollen with subcapsular and cortical ecchymotic hemorrhages. Petechial or ecchymotic hemorrhages and swelling of the lungs may also be noted. Hepatic lesions during the acute phase consist of diffuse

hemorrhage and necrotic foci (Searcy, 1995). In chronic stages of leptospirosis, the kidneys become small and fibrotic. Endothelial cell degeneration and focal to diffuse lymphocytic–plasmacytic interstitial nephritis are the characteristic histopathological findings.

Pathogenesis The severity and course of leptospirosis depend on the causative serovar as well as the age and immune status of the dog. Infection occurs after the leptospires penetrate a mucous membrane or abraded skin. The organisms then invade the vascular space and multiply rapidly, reaching the renal tubular epithelium several days postinfection. Acute or progressive renal failure leading to oliguria or anuria may occur. Nephritis may or may not be accompanied by hepatitis, uveitis, pulmonary hemorrhage, and meningitis. Disseminated intravascular coagulation is often a secondary complication.

Diagnosis and Differential Diagnosis Paired serology for the microscopic agglutination test is the most reliable means of definitive diagnosis, and successive serum sampling should be done 7–14 days after the first sample. PCR can be used to identify active infection early in the disease when serologic testing is negative or in previously vaccinated animals (Sykes *et al.*, 2011). Differential diagnoses include other causes of acute renal failure and hepatitis.

Prevention and Control According to the American Animal Hospital Association's 2011 vaccination guidelines, vaccination for leptospirosis is recommended based on geographic location and exposure risk (Welborn *et al.*, 2011). Both quadrivalent and bivalent inactivated bacterins are available. Quadrivalent bacterins protect against Canicola, Icterohaemorrhagiae, Grippotyphosa, and Pomona serovars, whereas bivalent bacterins cover only Canicola and Icterohaemorrhagiae. Immunization does not prevent the development of the carrier state or protect against other serovars. Control requires preventing contact with wildlife reservoirs as well as identification of carrier animals.

Treatment Doxycycline is the drug of choice as it can eliminate renal colonization. If vomiting or allergic reactions prohibit treatment with doxycycline, ampicillin or other penicillins should be utilized. Aggressive fluid therapy and supportive care may also be needed.

Research Complications Due to the zoonotic potential, dogs with clinical leptospirosis should not be used in research studies.

d. *Campylobacteriosis*

Etiology *Campylobacter* spp. are thin, curved or spiral, microaerophilic, thermophilic motile gram-negative rods. Many species of *Campylobacter* have been isolated from normal and diarrheic animals; however, the most common pathogenic species include *Campylobacter jejuni* ssp. *jejuni* and *C. coli* (Marks *et al.*, 2011).

Clinical Signs Most adult animals infected with *C. jejuni* are asymptomatic carriers; clinical signs are most commonly noted in dogs that are less than 6 months of age (Greene, 2000; Burnens *et al.*, 1992). In cases of clinical illness, mild and intermittent mucoid or watery diarrhea, with or without frank blood, is most commonly noted. Signs typically last 5–21 days but can persist for several months. Tenesmus, inappetance, vomiting, and a mild fever may accompany the diarrhea (Marks *et al.*, 2011). Bacteremia and cholecystitis secondary to *C. jejuni* have also been documented in dogs (Fox, 2012).

Epizootiology and Transmission The role of *Campylobacter* spp. as a primary pathogen has been questioned; it may require a coenteropathy to produce disease (Sherding and Johnson, 1994). Stress or immunosuppression may make animals more susceptible. Transmission is via the fecal–oral route, mostly through contaminated food or water. *Campylobacter jejuni* can be zoonotic with immunocompromised individuals at greatest risk.

Pathologic Findings Lesions depend on the mechanism of the enteropathy (Van Kruiningen, 1995). Enterotoxin production results in dilated, fluid-filled bowel loops, with little or no histopathologic alteration. Cytotoxin-mediated disease results in a friable, hemorrhagic mucosal surface. Histologically, the mucosa is ulcerated with lymphoplasmacytic infiltration. Translocation can result in edema and congestion of the lamina propria with focal accumulation of granulocytes. Epithelial hyperplasia and decreased goblet cell numbers are also noted. *Campylobacter jejuni* may be visualized between enterocytes with Warthin–Starry silver-stained sections.

Pathogenesis Clinical disease may be produced by several different mechanisms as *Campylobacter* spp. have a variety of virulence factors including enterotoxins, cytotoxins, and adherence or invasion properties. *Campylobacter jejuni* can cause an erosive enterocolitis by invasion of epithelium and production of the cytolethal distending toxin (cdt) (Fox, 2012; Van Kruiningen, 1995). In addition, *C. jejuni* can produce illness via translocation to regional lymph nodes causing a mesenteric lymphadenitis.

Diagnosis and Differential Diagnosis Fresh feces (per rectum) can be used for presumptive diagnosis by demonstration of highly motile, curved or spiral organisms with dark-field or phase-contrast microscopy. Gram-stained *C. jejuni* appear as gull-winged rods. Definitive diagnosis requires isolation of the organism (Sherding and Johnson, 1994). Culture requires selective isolation media, and growth is favored by reduced oxygen tension and a temperature of 37°C. A PCR multiplex assay for differentiation of *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* ssp. *fetus* has been developed (Wang *et al.*, 2002). Any disorder that can cause diarrhea in dogs should be considered as a differential diagnosis.

Prevention and Control Proper environmental sanitation, waste disposal, and food storage can prevent campylobacteriosis. In enzootic situations, group housing should be avoided. Outbreaks are controlled by isolation and treatment of affected individuals.

Treatment Antibacterial treatment should be considered in severely ill dogs. Erythromycin, neomycin, enrofloxacin, clindamycin, and doxycycline are all effective. Resistance to quinolones and ciprofloxacin has been documented (Acke *et al.*, 2009). Treatment should be a minimum of 10–14 days with bacterial cultures repeating 1 and 4 weeks after treatment.

Research Complications Dogs with clinical campylobacteriosis have temporary derangements to digestive and absorptive functions.

e. Helicobacteriosis

Etiology Helicobacters are gram-negative, microaerophilic, spiral bacteria that infect the gastrointestinal tract. *Helicobacter* spp. can be separated into gastric and enterohepatic groups. The gastric helicobacters commonly identified in dogs are referred to as non-*Helicobacter pylori* helicobacters or *H. heilmannii sensu lato* and include *H. felis*, *H. bizzozeronii*, *H. salomonis*, *H. cynogastricus*, and *H. heilmannii sensu stricto*, formerly *Candidatus H. heilmanni* (Haesebrouck *et al.*, 2011; Joosten *et al.*, 2013). The most common enterohepatic species found in dogs include *H. bilis*, *H. canis*, and *H. cinaedi* (Castiglioni *et al.*, 2012; Dewhurst *et al.*, 2005; Fox, 2012). Canine *Helicobacter* spp. isolated from human tissues, suggesting zoonotic transmission, include *H. bizzozeronii*, *H. salomonis*, *H. bilis*, *H. canis*, and *H. heilmannii s.s.* (Haesebrouck *et al.*, 2009; Fox, 2012).

Clinical Signs Most infections are subclinical in the dog. Gastric infections may present with vomiting, diarrhea, and fever, accompanied by anorexia, pica, or polyphagia. Enterohepatic helicobacters have been linked with inflammatory bowel disease in experimental animal models. Heavy infections in dogs have been associated with inflammatory lesions of the large intestine (Castiglioni *et al.*, 2012; Nguyen *et al.*, 2013).

Epizootiology and Transmission The epizootiology and transmission of *Helicobacter* spp. in the dog remain unknown. Both oral–oral and fecal–oral routes for transmission have been suggested in humans, but transmission via canine saliva is a less reliable source of infection (Craven *et al.*, 2011). Enterohepatic infections of pet dogs are as high as 52% (Castiglioni *et al.*, 2012). Prevalence of gastric *Helicobacter* infections in colony or shelter dogs can be as high as 82–100% (Fox, 1995; Hermanns *et al.*, 1995).

Pathologic Findings Gastritis is usually mild and characterized by reduced mucus content of the surface epithelium with vacuolation, swelling, karyolysis, and karyorrhexis of parietal cells. Multifocal infiltrates of

plasma cells and neutrophils occur around blood vessels and between gastric pits (Hermanns *et al.*, 1995). Intestinal lesions include mild to moderate lymphoplasmacytic infiltration as well as crypt dilation and crypt hyperplasia (Castiglioni *et al.*, 2012).

Pathogenesis Gastric helicobacters are urease positive, which assists with survival in the acidic environment of the stomach (Kusters *et al.*, 2006; Uberti *et al.*, 2013). Enterohepatic helicobacters are urease negative and typically reside in the lower intestine. The mechanism by which enterohepatic helicobacters colonize the liver is thought to be through portal circulation after uptake by enterocytes or through retrograde movement from the intestine into the bile duct (Fox, 2012).

Diagnosis and Differential Diagnosis Organisms may be demonstrated with histopathology on endoscopic or surgical biopsy tissue samples. Warthin–Starry silver stain may increase the sensitivity for histopathologic diagnosis. Culture may be difficult depending on the *Helicobacter* spp. For species that produce urease, a positive urease test on a gastric biopsy specimen may give a presumptive diagnosis. The urea breath test has been successfully used to diagnose *Helicobacter* spp. in laboratory beagles with a sensitivity and specificity of 89% (Kubota *et al.*, 2013). Western blot has been used to detect serum antibodies to enterohepatic species and PCR can be used to detect *Helicobacter* spp. in fecal samples (Oyama *et al.*, 2012; Wadström *et al.*, 2009). Any causes of acute or chronic vomiting and diarrhea in the dog are differential diagnoses.

Prevention and Control Until more is known about the epizootiology and transmission of *Helicobacter* spp., specific recommendations cannot be made for prevention and control.

Treatment For gastric species, combination therapy of amoxicillin (10 mg/kg q12h), metronidazole (30 mg/kg q24h), and sucralfate (0.25–0.5 mg/kg q8h) has proven to be most effective (Hall and Simpson, 2000). Replacing sucralfate with famotidine, omeprazole, or bismuth subsalicylate may also be effective (Marks, 1997; Jenkins and Bassett, 1997; DeNovo and Magne, 1995). Recurrence rates within 60 days of treatment can be as high as 80% (Anacleto *et al.*, 2011). Treatment of enterohepatic helicobacters may depend on species susceptibility. Aminoglycosides have been successful in treating *H. cinaedi*, but resistance to fluoroquinolones has been documented (Tomida *et al.*, 2013). Combination therapy of amoxicillin, clarithromycin, metronidazole, and omeprazole in medicated chow has been successful in eliminating various enterohepatic helicobacters from mice (del Carmen Martino-Cardona *et al.*, 2010). Long-term antibiotic treatment at a minimum of 21 days is suggested for enterohepatic and gastric helicobacters.

Research Complication Dogs used in gastrointestinal physiology or oral pharmacology studies should be free from helicobacteriosis.

2. Viral and Chlamydial Diseases

a. Canine Parvovirus Enteritis

Etiology Parvoviral enteritis in dogs is caused by canine parvovirus strain 2 (CPV-2) of the family Parvoviridae, genus *Protoparvovirus*, species *Carnivore protoparvovirus 1*. Currently, there are three antigenic variants, 2a, 2b, and 2c. Parvoviruses are nonenveloped, single-stranded DNA viruses.

Clinical Signs While parvoviral infection can affect the gastrointestinal tract, bone marrow, myocardium, and nervous tissues, the most common manifestation of disease is acute enteritis. Clinical signs usually appear 5 days after fecal-oral inoculation and include anorexia, fever, depression, vomiting, and profuse intractable diarrhea which may become hemorrhagic. Excessive fluid and protein losses through the gastrointestinal tract result in rapid and severe dehydration. Dogs can develop severe leukopenia with a total leukocyte count of 1000 cells/ μ l or less. Repeated hemograms may provide prognostic value, as rebounds in leukocyte counts are indicative of impending recovery. Terminally ill dogs may develop hypothermia, icterus, or disseminated intravascular coagulation due to endotoxemia.

Epizootiology and Transmission Parvovirus can infect dogs of any age, but puppies between 6 and 20 weeks of age are particularly susceptible. Puppies less than 6 weeks of age are protected by passive maternal antibody. Strain CPV-2c has been associated with severe disease in adult vaccinated dogs (Calderon *et al.*, 2009).

Pathogenesis Canine parvovirus has an affinity for rapidly dividing cells of the intestine and causes acute enteritis with intestinal crypt necrosis and villus atrophy. The virus also has tropism for the bone marrow and lymphoid tissues; thus, leukopenia and lymphoid depletion accompany the intestinal destruction.

Diagnosis and Differential Diagnosis Parvovirus can be detected with a commercially available fecal enzyme-linked immunosorbent assay (ELISA). Due to intermittent and brief shedding of the virus, fecal ELISAs can have false-negative results. PCR can be used to confirm an ELISA result and to differentiate the viral strain. At necropsy, diagnosis is based on gross and histopathologic evidence of necrosis and dilatation of intestinal crypt cells with secondary villous collapse.

Prevention and Control Parvoviral-positive animals should be quarantined for at least 10 days as the infectious virus is shed for several days after onset of clinical signs. Although PCR has been used to detect viral DNA in feces for up to 6 weeks (Decaro *et al.*, 2005), it is currently unknown if the material being shed at this

time is still infectious. Disinfection of exposed areas with dilute bleach (1:30) or a commercial disinfectant is essential for elimination of the virus. Six-week-old puppies should be vaccinated every 2–4 weeks with a modified live vaccine until at least 16 weeks of age.

Treatment Treatment is largely supportive and aimed at restoring fluid and electrolyte balance. Antimicrobial therapy is recommended due to intestinal compromise and risk of sepsis. Early nutritional support continued throughout the disease has been shown to decrease recovery times (Mohr *et al.*, 2003).

Research Complications Infection with parvovirus precludes the use of a particular dog in an experimental protocol. Due to the significant discomfort of the animal, as well as the intensive therapy required, humane euthanasia is usually chosen in a research setting.

b. Rabies

Etiology Rabies virus is a *Lyssavirus* belonging to the family Rhabdoviridae.

Clinical Signs Clinical progression of neurologic disease occurs in three stages. The first, prodromal, stage is characterized by a change in species-typical behavior. The loss of the instinctive fear of humans by a wild animal is a classic sign of impending rabies. In the second, furious, stage, animals are easily excited or hyperreactive to external stimuli and will readily bite at inanimate objects. The third, paralytic, stage is characterized by incoordination and ascending ataxia of the hindlimbs due to viral-induced damage of motor neurons. Death due to respiratory failure usually occurs after onset of the third stage.

Epizootiology and Transmission Wild animals such as raccoons, skunks, and bats are common reservoirs of infection for domestic animals, which in turn are the principal source of infection for humans. Transmission occurs primarily by contact with infected saliva, usually via bite wounds.

Pathogenesis The incubation period for rabies is 3–8 weeks to the onset of clinical signs but can range from 1 week to 1 year. Bites to the head and neck result in shorter incubation periods due to the close proximity to the brain. Following infection, the virus migrates centripetally via peripheral nerve fibers to neurons within the brain, resulting in neurologic dysfunction. On reaching the brain, the virus migrates centrifugally to the salivary glands, thus enabling shedding and subsequent transmission.

Diagnosis and Differential Diagnosis Definitive diagnosis is based on immunofluorescence of the virus in Negri bodies of hippocampal cells. Submission of the whole, unfixed brain, including the cerebellum and proximal brain stem, should be done within 48 h of collection. The tissue should be kept refrigerated as freezing can cause delays in testing. Differential diagnoses

include pseudorabies, canine distemper, bacterial meningitis, and toxicants that affect neurologic function.

Prevention and Treatment Puppies should be vaccinated by 16 weeks of age, again at 1 year, and then annually or triennially, depending on state and local laws.

Research Complications Immuno-prophylaxis is recommended for animal care and research personnel who may have work-related risks of exposure. Due to risk of human exposure, animals with suspected infection should be humanely euthanized and brain tissue should be submitted for confirmation.

3. Parasitic Diseases

a. Protozoa

Giardiasis *Giardia lamblia*, also known as *G. duodenalis* and *G. intestinalis*, is a binucleate flagellate protozoan that usually causes subclinical infestation of the small intestine. Clinical disease is usually seen in young dogs and the characteristic sign is voluminous, light-colored, foul-smelling, soft to watery diarrhea, which is the result of malabsorption and hypersecretion. *Giardia* has a direct life cycle with infection resulting after consuming cyst-contaminated food or water. The change in pH between the stomach and duodenum activates excystation and trophozoites then attach to the enterocytes. For diagnosis, direct fecal smears are considered best for observing trophozoites and zinc sulfate centrifugation is preferred for detection of cysts. A commercial ELISA kit is licensed for use in dogs, but the positive predictive value is poor and zinc sulfate centrifugation techniques should be used in conjunction with ELISA (Rishniw *et al.*, 2010). PCR assays are also available for diagnosing giardiasis. Differential diagnoses for giardiasis include bacterial and protozoal enteritis, coccidiosis, and whipworm infestation. Metronidazole at 25–30 mg/kg PO q12h for 5–10 days is effective at treating giardiasis as well as other enteric protozoans, which may be potential differential diagnoses or coinfections. Albendazole, fenbendazole, pyrantel, and praziquantel are also effective.

Coccidiosis Intestinal coccidia associated with enteropathy in dogs include *Isospora canis*, *I. ohioensis*, *I. neorivolta*, *I. burrowsi*, and *Hammondia heydorni* (Dubey and Greene, 2012). Coccidian oocysts can be found in feces of clinically healthy dogs, as well as animals with diarrhea. Clinically affected animals are young or immunosuppressed and develop diarrhea, which can vary from soft to watery and may contain blood or mucus. Vomiting, dehydration, lethargy, and weight loss can also be seen. Coccidia oocysts are typically spread by fecal–oral transmission, but dogs can ingest mono-zoic cysts in intermediate host tissues. The coccidian life cycle is both sexual and asexual, and results in the

release of unsporulated eggs, which sporulate under appropriate environmental conditions. Other causes for diarrhea should be excluded before a coccidial etiology is implicated. Treatment may not be necessary, as infections are typically self-limiting and clinically insignificant. Treatment may help to limit the number of oocysts shed in a kennel-housing situation and may be necessary in cases of protracted clinical illness. Possible choices for treatment include daily administration of sulfadimethoxine (50–60 mg/kg PO q24h for 10–20 days) or trimethoprim sulfa (30 mg/kg PO q8h for 10 days).

b. Nematodes

Ascarids Roundworms of dogs are most often *Toxocara canis*; however, *Toxascaris leonina* can also affect dogs. Clinical illness is usually only seen in young animals with large worm burdens. Diarrhea, vomiting, dehydration, and abdominal discomfort with vocalization can be seen. Puppies may have a classical ‘potbellied’ appearance. Heavy infestations can cause intussusception and/or intestinal obstruction. Puppies that experience lung migrations of larval worms can develop fatal pneumonia. *Toxascaris canis* can infect dogs by transplacental migration, transmammary migration, or ingestion of infective eggs. The infective stage of *T. canis* is the third-stage larva (L3). In transplacental infections, puppies may be born with L3 larvae in their lungs (Sherding, 1989). For diagnosis, large (70–85 µm in diameter) and relatively round ascarid eggs can be seen by standard fecal flotation methods. Monthly administration of milbemycin or ivermectin plus pyrantel pamoate is recommended for prevention (Hall and Simpson, 2000). Most anthelmintics are effective for treatment. Puppies should be treated early and often (every other week until 16 weeks of age) because of the possibility of prenatal or neonatal infection. Pregnant bitches can be treated with extended fenbendazole therapy (50 mg/kg PO once a day from day 40 of gestation through day 14 of lactation).

Hookworms The most common and most pathogenic hookworm of dogs is *Ancylostoma caninum*. *Ancylostoma braziliense* can also be found in dogs, but only *A. caninum* infestation typically results in clinical illness. Puppies with hookworm infections can present as anemic with bloody diarrhea or melena. Other clinical signs include lethargy, anorexia, dehydration, vomiting, and poor weight gain. These signs are a direct result of the worms’ consumption of blood and body fluids. Infective larvae (L3) are ingested from the environment and develop directly in the intestinal tract. Infestation can also be transmammary, from ingestion of a paratenic host, and, less often, by transplacental migration. On histological sections, embedded worms with mouthparts may be identified. Diagnosis is made by identification of eggs or larvae by either fecal flotation or direct smear.

A differential diagnosis of parvovirus should be considered for puppies with bloody diarrhea, and autoimmune hemolytic anemia should be considered in young dogs with anemia. Pyrantel pamoate is the anthelmintic of choice because it is safest in young ill animals. Monthly administration of milbemycin or ivermectin plus pyrantel pamoate is recommended for prevention and control (Hall and Simpson, 2000). Due to transplacental or milk-borne infection, puppies should be treated q2 weeks from 2 to 16 weeks of age.

Whipworms *Trichuris vulpis*, the canine whipworm, can cause acute or chronic large intestinal diarrhea. The adult worm resides in the cecum or ascending colon. Most infections are subclinical, but in symptomatic cases, the typical clinical sign is diarrhea with blood and/or mucus. Abdominal pain, anorexia, and weight loss may also be seen. Dogs may have eosinophilia, anemia, and/or hypoproteinemia on clinical hematology. *Trichuris vulpis* has a direct life cycle with eggs passed in the feces. The penetration of the adult worm into the enteric mucosa, and the associated inflammation, can lead to diarrhea. Factors that influence development of clinical symptoms are the number and location of adult whipworms; the severity of inflammation, anemia, or hypoproteinemia in the host; and the overall condition of the host. Whipworm infestation is diagnosed by the presence of barrel-shaped, thick-walled eggs with bipolar plugs on fecal flotation. Adult worms intermittently release eggs; therefore, negative results do not exclude infection. Differential diagnoses for whipworm infestation include giardiasis, coccidiosis, and bacterial enteritis. Fenbendazole, oxbendazole, and milbemycin have all been recommended for treatment of whipworms. Treatment for whipworm infestation should be at monthly intervals for 3 months (Jergens and Willard, 2000).

c. Cestodes (Tapeworms)

Several species of cestodes parasitize the small intestine of dogs. The most common is *Dipylidium caninum*. Other species include *Taenia pisiformis* and, more rarely, *Echinococcus granulosus*, *Multiceps* spp., *Mesocestoides* spp., and *Spirometra* spp. Most cestode infestations are subclinical, but severe infestations with *Dipylidium* can cause diarrhea, weight loss, and poor growth. The cestode requires an intermediate host, which for *D. caninum* are fleas and lice. Ingestion of these arthropods results in transmission of the tapeworm. Definitive diagnosis is usually made by the identification of egg capsules or proglottids (tapeworm segments) on the surface of the feces or around the anus. The most significant means to limit cestode infestation is to control flea and/or louse exposure. Praziquantel at 5–12.5mg/kg orally or subcutaneously is the standard treatment for cestodiasis, especially *Taenia* or *Echinococcus* species. Fenbendazole,

mebendazole, or oxfendazole may also be effective against *D. caninum* (Hall and Simpson, 2000).

d. Mites

Demodicosis Canine demodicosis is caused by *Demodex canis*, a commensal mite that lives in the hair follicles and is passed from dams to nursing pups. Localized demodicosis is typically asymptomatic, but disease can present with variable and nonspecific clinical signs, such as alopecia, erythema, pruritus, crusts, and hyperpigmentation. It can occur anywhere on the body but is often seen on the feet and face, and around the ears (DeManuelle, 2000a). Generalized demodicosis can develop in juvenile or adult populations and is indicative of an underlying immunosuppressive disorder. *Demodex* has a characteristic 'cigar shape' and can be identified from deep skin scrapings mounted on mineral oil (Campbell, 2000; Noli, 2000). Differential diagnoses include dermatophytosis, allergic contact dermatitis, and seborrheic dermatitis. The primary differential diagnosis for generalized demodicosis is primary bacterial pyoderma, which is also a common secondary complication of generalized demodicosis. Ivermectin at 200–600 µg/kg and oral milbemycin at 1–2mg/kg/day are effective treatments. Treatment duration can be extensive and must be accompanied by repeated skin scrapings.

Sarcoptic Mange Canine sarcoptic mange is caused by *Sarcoptes scabiei* var. *canis*, which is zoonotic. The most common clinical sign is an intense pruritus, usually beginning at sparsely furred areas of the ear pinnae, elbows, ventral thorax, and abdomen. Lesions are characterized by alopecia and yellowish dry crusts with a macular papular eruption. These lesions may be exacerbated by excoriation due to the pruritic nature of the condition. Adult mites, mite eggs, or mite feces may be observed on superficial skin scrapings, but diagnosis may be difficult because multiple skin scrapings may yield negative results. Even if scrapings are negative, a therapeutic trial should be initiated if the clinical signs and history suggest a *Sarcoptes* etiology. Demonstration of anti-mite IgE either in the serum or via an intradermal antigen test can be used as a diagnostic aid (Campbell, 2000). Histologic examination is nondiagnostic; however, suggestive lesions include small foci of edema, exocytosis, degeneration, and necrosis (Scott et al., 1995). An important differential diagnosis is flea allergy dermatitis (FAD). Unless antiparasitic therapy would interfere with research objectives, all dogs with sarcoptic mange should be treated. In addition, their kennel mates should also be treated due to the contagious nature of the disease and its zoonotic potential. The usual means of treatment is either ivermectin at 200–400 µg/kg q14 days or milbemycin at 2mg/kg q7 days for three oral doses (Scott et al., 1995).

e. Ticks and Fleas

Ticks Ticks are obligate arachnid parasites that require vertebrate blood as their sole food source. Genera that more commonly infest dogs in the United States include species of *Rhipicephalus*, *Dermacentor*, *Amblyomma*, and *Ixodes*. The primary significance of tick infestation is vector-borne infectious diseases, including Rocky Mountain spotted fever (*Rickettsia rickettsii*), Lyme disease (*Borrelia burgdorferi sensu stricto*), thrombocytic anaplasmosis (*Anaplasma platys*), and canine monocytic ehrlichiosis (*Ehrlichia canis*). Ticks alone cause minimal signs unless the dog develops a hypersensitivity reaction leading to a more granulomatous response at the bite location (Merchant and Taboada, 1991). Some species (primarily *Dermacentor andersoni* and *D. variabilis*) produce a salivary neurotoxin that causes an ascending flaccid paralysis (Malik and Farrow, 1991). Uncomplicated tick bites and tick-bite paralysis are diagnosed by identification of the tick and clinical signs of paralysis. Dogs with tick-bite paralysis usually show improvement within 24h of tick removal, with complete recovery within 72h (Malik and Farrow, 1991). Formamidines (amitraz), pyrethroids, and phenylpyrazoles (fipronil) are available as spot-ons, collars, sprays, and foggers to treat tick infestations in both the animal and the environment (Halos *et al.*, 2014; Beugnet and Franc, 2012). Differential diagnoses for tick-bite paralysis include botulism, snakebite, polyradiculoneuritis, and idiopathic polyneuropathy (Malik and Farrow, 1991).

Fleas The most common flea to infest dogs is *Ctenocephalides felis felis*, the cat flea (Sousa, 2010). Flea infestations usually cause foci of alopecia and pruritus. Dogs that are hypersensitive to antigenic proteins in flea saliva develop severe FAD, which features papules, crusting, and excoriations over the lumbosacral region, flanks, thighs and abdomen. These animals may require oral corticosteroids to relieve clinical signs (Muller *et al.*, 1983). Secondary bacterial and fungal infections can also develop. Fleas can also transmit other parasitic diseases, such as *Dipylidium* tapeworms. Flea infestations and FAD are definitively diagnosed by observing the fleas on the host's skin; however, the presence of flea excrement can support a presumptive diagnosis (DeManuelle, 2000b). Treatment of flea infestations should use an integrated pest management (IPM) approach that targets adult fleas, immature stages, and environmental contamination in order to limit the risk of chemoresistance. Combining ovicidal treatments, such as lufenuron and selamectin, with adulticidal treatments, such as fipronil, spinosad, selamectin, and imidacloprid, is recommended (Halos *et al.*, 2014; Beugnet and Franc, 2012; Dryden *et al.*, 2012). Certain chemicals (i.e., imidacloprid and selamectin) have both adulticidal and larvicidal abilities, but the principles of IPM preclude the use of

one product solely for both adulticidal and larvicidal properties (Schwassman and Logas, 2009). Differential diagnoses include mite and louse infestations, bacterial folliculitis, and allergic or atopic conditions that present with skin lesions in dogs.

4. Fungal Diseases

a. Superficial Dermatophytoses (Ringworm)

Canine dermatophytoses are commonly caused by *Microsporum* spp., *Trichophyton* spp., and *Epidermophyton* spp. (Moriello and DeBoer, 2012). Uncomplicated infections are characterized by circular areas of alopecia and crusting with or without follicular papules, usually around the face, neck, and forelimbs. Dermatophytes infect the hair shaft and follicle, as well as the surrounding skin. Infected hairs become brittle and broken shafts remain infective in the environment for months. Dermatophytoses are zoonotic and easily transmitted to other animals through the environment or by direct contact. Definitive diagnosis is made using dermatophyte test medium for culture. Hair and crust material from infected sites can be plucked and placed on culture; however, the 'toothbrush' method is more effective for sampling multiple sites. The brush is used to comb hairs and scales from several infected sites and then pressed into the culture media. Media plates should be visually inspected daily for 14 days. Positive cultures will become red at the same time as growth of a fluffy white colony. Microscopic examination of hairs and scales to visualize fungal elements can be done using skin scrapings in 20% KOH or mineral oil; however, this method is not very sensitive. Topical and systemic therapy should be initiated together after all suspected areas are clipped to reduce spreading of contaminated fragile hairs. Whole-body topical therapies with antifungal shampoos, rinses, and creams are recommended rather than spot treatment. Systemic therapy can be achieved with griseofulvin, ketoconazole, itraconazole, or fluconazole. Due to the highly infective nature of this disease, animals should be isolated and the environment thoroughly disinfected. Chlorhexidine and Virkon[®] S are ineffective at clearing environmental spores, but lime sulfur (1:33), enilconazole (0.2%), and bleach (1:10) are effective across many strains of *Microsporum canis* (Moriello and DeBoer, 2002).

B. Metabolic and Nutritional Diseases

1. Endocrine Disorders

a. Hypothyroidism

Although the incidence of hypothyroidism in the canine population is not high (Kemppainen and Clark, 1994), deficiency in thyroid hormone can significantly

affect basal metabolism and immune function. Because these factors are important in many biomedical research studies, it is imperative that laboratory animal veterinarians be able to recognize, diagnose, and treat this problem.

Etiology Primary hypothyroidism affects the thyroid gland directly, whereas secondary hypothyroidism has indirect effects through dysfunction of the pituitary gland (Seguin and Brownlee 2012). Both of these causes result in a gradual loss of functional thyroid tissue (Avgeris *et al.*, 1990; Kempainen and Clark, 1994). The majority of cases of canine hypothyroidism are due to lymphocytic thyroiditis, an autoimmune disorder, or idiopathic atrophy of the thyroid gland. Lymphocytic thyroiditis is the major cause of hypothyroidism in laboratory beagles and appears to be familial in that breed (Tucker, 1962; Beierwaltes and Nishiyama, 1968; Manning 1979). Rarely, congenital defects or nonfunctional tumors may cause hypothyroidism (Peterson and Ferguson, 1989; Kempainen and Clark, 1994).

Clinical Signs Because it affects metabolism in general, hypothyroidism can produce a large number of clinical signs referable to many organ systems. An individual dog with hypothyroidism may have one or any combination of clinical signs. Hypothyroidism reduces the dog's metabolic rate, which then produces such signs as obesity, lethargy, cold intolerance, and constipation. Additionally, hypothyroidism can produce several dermatologic abnormalities, including nonpruritic, bilaterally symmetrical alopecia, hyperpigmentation, seborrhea, and pyoderma (Avgeris *et al.*, 1990; Peterson and Ferguson, 1989; Panciera, 1994). Several clinicopathologic abnormalities have also been reported in a large percentage of hypothyroid dogs. These aberrations include increased serum cholesterol and triglycerides due to a decrease in lipolysis and decreased numbers of low-density lipopolysaccharide receptors (Peterson and Ferguson, 1989; Panciera, 1994). Normocytic, normochromic, nonregenerative anemia may be seen in approximately one-half of the cases (Avgeris *et al.*, 1990). Increased serum alkaline phosphatase and creatine kinase have also been reported in a significant number of hypothyroid dogs (Peterson and Ferguson, 1989; Panciera 1994). Neurologic signs of hypothyroidism, which include lameness, foot dragging, and paresis, may be caused by several mechanisms such as segmental nerve demyelination or nerve entrapment secondary to myxedema (Peterson and Ferguson, 1989). Mental impairment and dullness have also been reported in hypothyroid dogs, secondary to atherosclerosis and cerebral myxedema (Peterson and Ferguson, 1989). Hypothyroidism has been implicated in other neurological abnormalities such as Horner's syndrome, facial nerve paralysis, megaesophagus, and laryngeal paralysis; however, these conditions do not always resolve

with treatment (Bichsel *et al.*, 1988; Panciera, 1994), and a true causal relationship with hypothyroidism has not been completely defined (Panciera, 1994). Myopathies associated with hypothyroidism are caused by metabolic dysfunction and atrophy of type II muscle fibers and can present with signs similar to neurological disease (Peterson and Ferguson, 1989). Hypothyroidism can also cause abnormalities of the cardiovascular system including bradycardia, hypocontractility, increased vascular volume, and atherosclerosis (Seguin and Brownlee 2012). Abnormalities that may be detected by ECG include a decrease in P- and R-wave amplitude (Peterson and Ferguson, 1989) and inverted T waves (Panciera, 1994). These ECG abnormalities are caused by lowered activity of ATPases and calcium channel function.

An association between hypothyroidism and von Willebrand disease has been suggested. However, the relationship is probably one of shared breed predilection and not a true correlation. Contradictory studies have shown either deficient (Avgeris *et al.*, 1990) or normal (Panciera and Johnson 1994, 1996; Avgeris *et al.*, 1990) von Willebrand factor antigen and bleeding times in hypothyroid dogs. Most importantly, hypothyroidism does not appear to cause overt, clinical von Willebrand disease. However, it may exacerbate existing subclinical von Willebrand disease (Seguin and Brownlee, 2012).

Epizootiology The prevalence of hypothyroidism in the general canine population is reportedly less than 1% (Panciera, 1994). The disorder occurs most often in middle-aged, larger breed dogs (Avgeris *et al.*, 1990), and reports suggest a higher incidence of hypothyroidism in spayed, female dogs (Panciera, 1994; Peterson and Ferguson, 1989). Doberman pinschers and golden retrievers appear to have a higher incidence of hypothyroidism compared with other breeds (Panciera, 1994; Peterson and Ferguson, 1989; Scarlett, 1994). There have been several reports about hypothyroidism in laboratory colonies of beagles (Manning, 1979; Tucker, 1962; Beierwaltes and Nishiyama, 1968).

Diagnosis and Differential Diagnosis Because of the large number of clinical manifestations in dogs, the recognition of hypothyroidism is not always straightforward. Likewise, the diagnosis of hypothyroidism can be difficult because of the lack of definitive diagnostic tests available for the dog. A complete understanding of the diagnosis of hypothyroidism requires a familiarity with thyroid hormone metabolism and function that is beyond the scope of this writing. For additional information, the reader is referred to one of several manuscripts available (Peterson and Ferguson, 1989; Ferguson, 1994).

Currently, the ability to diagnose hypothyroidism relies heavily on the measurement of serum total T₄ (thyroxine) and free T₄ (Peterson and Ferguson, 1989; Ferguson, 1994). T₄ serves primarily as a precursor for T₃ and is heavily protein bound. Free T₄ represents the

unbound fraction that is available to the tissues (Peterson and Ferguson, 1989). The measurement of total T_4 carries a sensitivity of around 95% and can be used as a good screening tool. With the measurement of both serum total T_4 and free T_4 , hypothyroidism can usually be ruled out if the values are within the normal range or higher. If both hormone concentrations are low, it is highly likely that the patient has hypothyroidism, and a therapeutic trial may be in order (Peterson and Ferguson, 1989). However, nonthyroidal illnesses and some drugs (e.g., glucocorticoids, anticonvulsants, phenylbutazone, salicylates) can falsely lower these values (Peterson and Ferguson, 1989; Ferguson, 1994). Therefore, low values do not always indicate that hypothyroidism is present and animals should not be treated solely on the basis of serum hormone levels if clinical signs are not present. If the clinical signs are equivocal or only total T_4 or free T_4 is decreased, further diagnostic testing is warranted (Peterson and Ferguson, 1989). Although T_3 is the most biologically active form of thyroid hormone, the measurement of serum T_3 levels is an unreliable indicator of hypothyroidism (Peterson and Ferguson, 1989; Ferguson, 1994). Serum T_3 can be falsely lowered by many nonthyroidal illnesses and many drugs (see above). In addition, T_3 may be preferentially released and conversion of T_4 to T_3 may be enhanced by the failing thyroid (Peterson and Ferguson, 1989; Ferguson, 1994), particularly early in the disease. In one study, T_3 was within normal limits in 15% of the hypothyroid dogs (Panciera, 1994). Autoantibodies can be responsible for false elevations in the concentrations of T_3 and T_4 found in these respective assays. It has been recommended that free T_4 , measured by equilibrium dialysis, be assayed in dogs that are suspected of hypothyroidism and have autoantibodies with normal or high T_3 and T_4 . Autoantibodies have been found in less than 1% of the samples submitted to one laboratory (Kemppainen and Behrend, 2000).

Other means of diagnosing hypothyroidism have been described. In humans, endogenous thyroid-stimulating hormone (TSH) levels provide reliable information on thyroid status, and an assay is available for dogs. However, endogenous TSH levels can be normal in some dogs with hypothyroidism and high TSH levels have been noted in normal dogs and sick animals that are actually euthyroid. It is therefore recommended that TSH levels be considered along with other information (clinical signs, T_4) prior to diagnosis and treatment (Kemppainen and Behrend, 2000). TSH stimulation testing using exogenous bovine TSH provides a good and reliable method for establishing a diagnosis. Unfortunately, the availability and expense of TSH limit the use of this diagnostic tool (Peterson and Ferguson, 1989; Ferguson, 1994). Another drawback of TSH testing is that the test must be postponed for 4 weeks if thyroid supplementation has been given (Peterson

and Ferguson, 1989). When TSH is available for testing, there are several recommendations for dosage, routes of administration, and sampling times. One recommendation is 0.045 U of TSH per pound of body weight (up to a maximum of 5U) to be administered IV. For this protocol, blood samples are taken prior to administration of TSH and 6h after. A normal response to the administration of TSH should create an increase of T_4 levels at least $2\mu\text{g}/\text{dl}$ above the baseline levels or an absolute level that exceeds $3\mu\text{g}/\text{dl}$ (Peterson and Ferguson, 1989; Wheeler *et al.*, 1985).

Treatment The treatment of choice for hypothyroidism in the dog is L-thyroxine (sodium levothyroxine). A recommended dosing regimen is 0.01–0.02 mg/kg once a day (Avgeris *et al.* 1990). If drugs that decrease thyroxine levels are being administered concurrently, it may be necessary to divide the thyroxine dose for twice daily administration. After the supplementation has begun, the thyroid hormone level should be rechecked in 6–8 weeks, and blood samples should be drawn 4–8h after the morning pill. A clinical response is usually seen in 6–8 weeks and would include weight loss, hair regrowth, and resolution of other signs (Panciera, 1994). ECG abnormalities also return to normal (Peterson and Ferguson, 1989). For dogs with neurologic signs, the prognosis is guarded, because the signs do not always resolve with supplementation (Panciera, 1994).

2. Management-Related Issues

a. Obesity

Weight gain and eventual obesity are frequent findings in dogs in the research environment. Because obesity can adversely affect several body systems as well as general metabolism, the laboratory animal veterinarian must address obesity and its potential effects on animal welfare and research results.

Etiology Obesity is defined as a body weight 20–25% over the ideal. In general, obesity occurs when the intake of calories exceeds the expenditure of energy, the result of overeating or eating an unbalanced diet. Overeating is a common cause of obesity in pet dogs and may be triggered by boredom, nervousness, or conditioning (MacEwen, 1992). In addition, pet animals are often subjected to unbalanced diets supplemented with high-fat treats. In the laboratory animal setting, overeating is less likely than in a household because access to food is more restricted, and diets are usually a commercially prepared balanced ration. However, obesity can still be a problem if specific guidelines for energy requirements are not followed. In addition, the necessary caging of dogs in the research environment and limitation to exercise reduces energy expenditure. It is also important to realize that other factors may predispose dogs to obesity, even when guidelines for caloric intake and energy

expenditure are followed (Butterwick and Hawthorne, 1998). As in humans, genetics plays an important role in the development of obesity in dogs, and certain breeds are more predisposed toward obesity. In a study of dogs visiting veterinary clinics in the United Kingdom, Labrador retrievers were most likely to be obese. Other breeds affected included Cairn terriers, dachshunds, basset hounds, golden retrievers, and cocker spaniels. The beagle was also listed as a breed predisposed to obesity in the household environment (Edney and Smith, 1986).

Several metabolic or hormonal changes are also associated with obesity. It has been well established that neutering promotes weight gain. In one study, spayed female dogs were twice as likely to be obese compared with intact females (MacEwen, 1992). The authors proposed that the absence of estrogen promotes an increase in food consumption. A similar trend toward obesity was found in castrated male dogs (Edney and Smith, 1986). In addition, hypothyroidism and hyperadrenocorticism may present with obesity as one of the clinical signs (MacEwen, 1992).

Differential Diagnosis The diagnosis of obesity is somewhat subjective and relies on an estimate of ideal body weight. The ideal body condition for dogs is considered to be achieved when the ribs are barely visible but easily palpated beneath the skin surface. When the ribs are not easily palpated and/or the dog's normal function is impaired by its weight, the animal is considered obese. There are few objective, quantifiable methods for establishing this diagnosis. Ultrasound has been evaluated for measurement of subcutaneous fat in dogs, and measurements taken from the lumbar area can be used to reliably predict the total body fat (Wilkinson and McEwan, 1991). After a diagnosis of obesity has been made, additional diagnostic tests should be performed to determine if there is an underlying cause for the problem. A complete physical exam should be performed to look for signs of concurrent disease and to establish if obesity has adversely affected the individual. Serum thyroid hormones should be evaluated (see above), and serum chemistry may reveal an increased alkaline phosphatase associated with hyperadrenocorticism.

Treatment Restricting food intake readily treats obesity, and this is easily done in the research setting. It has been suggested that a good weight loss program involves restriction of intake to 60% of the calculated energy requirement to maintain ideal body weight. It has been shown that restriction of calories down to 50% produces no adverse health effects. However, T₃ levels will decrease in direct proportion with caloric intake. Ideally, weight loss will occur at a rate of 1–2% of body weight per week (Laflamme *et al.*, 1997). With more severe calorie restriction and more rapid weight loss, the individual is more likely to have rebound weight gain after restrictions are relaxed.

There has been a great deal of attention in humans as to the correct diet to encourage weight loss. Likewise, the type of diet fed to dogs has been examined. As mentioned above, the restriction of calories is most important, and feeding less of an existing diet can do this. Alternatively, several diet dog foods are available, and there is some evidence that these diets are superior to simple volume restriction (MacEwen, 1992). There has been much concern about the addition of fiber to the diet as a method for reducing caloric intake while maintaining the volume fed. Studies in dogs have examined the addition of both soluble and insoluble fibers to calorie-restricted diets. These studies have shown that the addition of fiber does not have an effect on satiety in dogs and therefore does not have a beneficial effect in weight loss protocols (Butterwick *et al.*, 1994; Butterwick and Markwell, 1997).

Research Complications It is important to control weight gain in research animals because of the association of obesity with metabolism. Although an association between obesity and reproductive, dermatologic, and neoplastic problems has been reported (MacEwen, 1992), this relationship is not consistently apparent (Edney and Smith, 1986). Joint problems including osteoarthritis and hip dysplasia have also been related to obesity (MacEwen, 1992; Kealy *et al.*, 1997). In addition, diabetes mellitus has been linked to obesity and obesity-induced hyperinsulinism in several experimental models (MacEwen, 1992). A recent study demonstrated metabolic disease, typified by hyperinsulinemia and hypoadiponectinemia, in approximately 20% of obese dogs (Tvarijonaviciute *et al.*, 2012). Research that requires anesthesia may be complicated by a greater risk of cardiovascular diseases (Edney and Smith, 1986) including hypertension and compromise to the respiratory tract.

C. Traumatic Disorders

1. Traumatic Wounds

Etiology In the laboratory setting, the majority of traumatic wounds will be small in size and quickly observed. Occasionally, dogs may sustain minor trauma during transport or have a small, previously undetected, chronic wound upon arrival at the facility. When dogs are group housed, they may sustain bite wounds during early socialization periods. Under these conditions, proper initial treatment will lead to uncomplicated wound healing. Complications such as infection and delayed healing arise when wounds are not noticed immediately or when the basic principles of wound management are not followed.

Clinical Signs The signs and appearance of a traumatic wound will vary with the cause and the duration of time since wounding. Abrasions, sustained by shear forces, are partial thickness skin wounds characterized

by minimal bleeding or tissue disruption. Puncture wounds have a small surface opening but penetrate into deep tissues with the potential for contamination. Lacerations are wounds caused by sharp separation of skin that may extend to deeper tissues. Acute wounds are characterized by bleeding tissue, sharp edges and no obvious devitalization. They have variable degrees of contamination. Chronic wounds generally do not exhibit active bleeding and will have curled or rounded edges. These wounds often have necrotic tissue and are considered contaminated.

Treatment To aid decision making about wound therapy, several classification systems have been developed for traumatic injuries. At one time, decisions about wound therapy were largely based upon the length of time since wounding, or the concept of a 'golden period.' It is now recognized that several factors must be considered prior to initiating wound care, including (but not limited to) the type and size of the wound, the degree of wound contamination, and the competence of the host's defense systems (Swaim, 1980; Waldron and Trevor, 1993). One of the most widely used classification systems is based upon wound contamination and categorizes wounds as clean, clean-contaminated, contaminated, or dirty (see Table 12.9).

The vast majority of the wounds seen in the laboratory setting will fall into the clean and clean-contaminated categories. These wounds may be treated with the basic wound care described below and primary closure of the wound. Contaminated and dirty wounds require more aggressive therapy. Postsurgical infections or complications of initial therapy would be considered dirty wounds. When in doubt as to the classification of a wound, the worst category should be presumed in order to provide optimal therapy and reduce the chance for complications.

The initial treatment of a wound is the same regardless of its classification. When first recognized, the wound should be covered with a sterile dressing until definitive treatment can be rendered. Bleeding should

be controlled with direct pressure; tourniquets are discouraged because of the complications that may arise with inappropriate placement (Swaim, 1980). It is best to avoid using topical disinfectants in the wound until further wound treatment (culture, debridement, lavage) has been performed (Swaim, 1980). Anesthesia or analgesia may be necessary and the choice of agent will depend on the size and location of the wound as well as the preference of the clinician. If the wound is contaminated or dirty, bacterial cultures, both aerobic and anaerobic, should be performed. Then a water-soluble lubricant gel may be applied directly to the wound to prevent it from further contamination during the hair removal process. A wide margin of hair should be clipped and a surgical scrub performed around the edges of the wound. Povidone-iodine alternating with alcohol or chlorhexidine gluconate scrub alternating with water is most often recommended for surgical preparation of the skin surface (Osuna *et al.*, 1990a, b). Simple abrasions that involve only a partial thickness of the skin do not generally require further treatment. Full-thickness wounds require further attention, including irrigation with large quantities of a solution delivered under pressure. Several irrigation solutions have been recommended (Lozier *et al.*, 1992; Waldron and Trevor, 1993; Sanchez *et al.*, 1988), but type may not be as important as the volume and pressure of delivery. It has been suggested that 8 psi is required to obtain adequate tissue irrigation, and this may be achieved by using a 35-ml syringe with an 18- or 19-gauge needle (Waldron and Trevor, 1993).

For wounds that are contaminated or dirty, debridement is an important part of initial therapy. Debridement usually proceeds from superficial to deeper layers. Skin that is obviously necrotic should be removed. Although it is often recommended to remove skin back to the point at which it bleeds, this may not be feasible with large wounds on the limbs. In addition, other factors such as edema or hypovolemia may reduce bleeding in otherwise viable skin (Waldron and Trevor, 1993). If one is unsure about tissue viability in areas that are devoid of

TABLE 12.9 Classification and Treatment of Traumatic Wounds^a

Classification	Description	Examples	Treatment options
Clean	Aseptic wound	Surgical incision	Aseptic, immediate closure
Clean-contaminated	Recent wound with minimal, easily removed contamination	Simple laceration, broken toenail	Wound lavage, debridement, ± immediate closure
Contaminated	Several hours since wounding; grossly contaminated	Bite wounds, old lacerations, fecal contamination	Wound lavage, debridement, ± drain placement, ± delayed closure
Dirty	Purulent exudate and infection already present	Infected bite wound, anal sac abscess, postsurgical infection	Wound lavage, debridement, drain placement, delayed or no closure

^aModified from Waldron and Trevor (1993).

extra skin, the tissue may be left (Swaim, 1980; Waldron and Trevor, 1993), and nonviable areas will demarcate within 2–3 days (Waldron and Trevor, 1993). Necrotic fat should be resected liberally, because it does not have a large blood supply and will provide an environment for infection. Often, resection of subcutaneous fat is necessary to remove debris and hair that could not be removed during wound irrigation. Damaged muscle should also be liberally resected (Swaim, 1980). The wound should be irrigated several times during debridement and again after completion.

After initial wound treatment, the options concerning wound closure must be weighed. The principles of basic surgery are discussed in several good texts, and readers are encouraged to pursue additional information. Primary wound closure is defined as closure at the time of initial wound therapy and is the treatment of choice for clean and clean-contaminated wounds. Closure is performed in two or more layers, carefully apposing tissues and obliterating dead space. If dead space will remain in the wound, a drain should be placed. Subcutaneous closure should be performed with absorbable suture such as polydioxanone, polyglactin 910, or polyglycolic acid. It is best to use interrupted sutures and avoid leaving excess suture material in the wound. It may be necessary to choose tension-relieving suture patterns, such as horizontal mattress. Skin closure is generally performed with nylon (3-0 or 4-0).

In situations where gross contamination cannot be completely removed, closure of the wound should be delayed or avoided. After debridement and irrigation, the wound should be bandaged. The wound may be covered by a nonadherent dressing such as vaseline-impregnated gauze (Swaim, 1980). The contact layer is covered by cotton padding, and the entire bandage is covered by a supportive and protective layer. The bandages should be changed once or twice daily, depending upon the amount of discharge coming from the wound. Wound closure within 3–5 days of wounding (prior to the formation of granulation tissue) is considered delayed primary closure. When the wound is closed after 5 days, this is considered secondary closure (Waldron and Trevor, 1993). Second-intention healing involves allowing the wound to heal without surgical intervention. This type of healing is often used on limbs when there is an insufficient amount of skin to allow complete closure (Swaim, 1980). It is important to note that second-intention healing will take longer than with surgical repair, and, in the case of large wounds, it will be more expensive because of the cost of bandaging materials.

Several factors must be weighed when considering the use of antibiotics in traumatic wound care, including the classification and site of the wound, host defenses, and concurrent research use of the animal. When wounds are clean or clean-contaminated, antibiotics are seldom

necessary unless the individual is at high risk for infection. When wounds have been severely contaminated or are dirty, antibiotics are indicated and the type of antibiotic will ultimately depend on culture and sensitivity results. Until such results are available, the choice of antibiotic is based on the most likely organism to be encountered. Topical application of bacitracin, neomycin sulfate, and polymixin B combinations may be used in wounds with minor contamination. In skin wounds with more extensive contamination, *Staphylococcus* spp. are generally of concern, whereas *Pasteurella multocida* should be considered in bite wounds. When systemic antibiotics are necessary, cephalosporins, amoxicillin-clavulanate, and trimethoprim sulfas are often recommended for initial antibiotic therapy (Waldron and Trevor, 1993).

Prevention In facilities with good husbandry practices and a diligent staff, potentially injurious equipment or surfaces are identified quickly. Appropriate attention to surgical technique and to initial wound care will generally reduce the occurrence of postprocedure wound infection.

2. Pressure Sores (Decubital Ulcers)

Etiology Pressure sores (decubital ulcers) can be a problem in long-term studies and housing situations that require chronic skin contact with hard surfaces. Decubital ulcers often develop over a bony prominence such as the elbow, tuber ischii, tarsus, or carpus. The compression of soft tissues between hard surfaces results in vascular occlusion, ischemia, and ultimately tissue death (Swaim and Angarano, 1990). Several factors that increase pressure at the site and/or affect the integrity of the skin will predispose an individual to develop pressure sores, including poor hygiene, self-trauma, low-protein diet, preexisting tissue damage, muscle wasting, inadequate bedding, and ill-fitting coaptation devices (Swaim and Angarano, 1990).

Clinical Signs Initially, the skin will appear red and irritated. Over time, constant trauma can result in full-thickness skin defects and can progress to necrosis of underlying tissues. The severity of the sores may be graded from I to IV according to the depth of the wound and the tissues involved, from superficial skin irritation to involvement of underlying bone (Waldron and Trevor, 1993).

Epizootiology The problem usually occurs in large dog breeds, but any type of dog can be affected.

Prevention and Control Minimizing or eliminating predisposing factors is important to both the prevention and treatment of this condition. If a dog will experience long periods of recumbency, adequate bedding or padding must be provided. Recumbent animals should be moved frequently, ideally every 2 h, to prevent continuous compression on a specific

area (Waldron and Trevor, 1993). Skin hygiene is of the utmost importance when trying to prevent or treat pressure sores. The skin should be kept clean and dry at all times. If urine scalding is a problem, the affected area should be clipped, bathed, and dried thoroughly at least once or twice daily. Finally, an appropriate diet to maintain body weight will minimize compressive forces experienced over areas susceptible to ulceration (Swaim and Angarano, 1990).

Treatment The treatment of pressure sores must involve care of the wound and attention to the factors causing the wound. The extent of initial wound management will largely depend on the depth of the wound. For simple abrasions and small wounds involving the skin only, simple wound cleansing and open-wound management provide adequate treatment. When wounds involve deeper tissues, including fat, fascia, or bone, more aggressive diagnostics and therapy must be performed. The affected area should be radiographed to assess bone involvement and the wound should be cultured. All of the damaged tissue should be debrided and basic wound management guidelines should be followed (see above). When a healthy granulation bed has formed over the entire wound, a delayed closure over a drain may be performed (Swaim and Angarano, 1990). With extensive lesions, reconstruction with skin flaps may be necessary (Waldron and Trevor 1993).

Bandaging should be performed on all full-thickness wounds; however, it is important to remember that ill-fitting or inadequately padded bandages or casts may worsen the problem. The area over the wound itself should not be heavily padded. The wounded area should be lightly covered and then a doughnut, created from rolled gauze or towel, should be fitted around the wound, in order to displace pressure over a larger area and onto healthier tissue. The doughnut is then incorporated into a padded bandage. If a cast has been applied to the area for treatment or research purposes, a hole can be cut over the wound to reduce pressure in that area and allow treatment of the wound (Swaim and Angarano, 1990). Bandages should be removed at least once or twice a day to allow wound care.

3. Acral Lick Granuloma

Etiology An acral lick granuloma is a skin lesion caused by self-trauma. In a few cases, the self-trauma is due to initial irritation caused by an identifiable neurologic or orthopedic condition (Tarvin and Prata, 1980). Allergy may also be a source of irritation that leads to self-trauma. However, the majority of cases begin because of repetitive licking by dogs that are confined and lack external stimuli (Swaim and Angarano, 1990). It has been theorized that the self-trauma promotes the release of endogenous endorphins, which act as a reward for the abnormal behavior (Dodman *et al.*, 1988).

The laboratory environment could promote the abnormal behavior and lead to acral lick granuloma.

Epizootiology The lesions associated with acral lick granuloma are seen most often in large dog breeds, particularly Dobermans. However, any type of dog can be affected (Walton, 1986).

Clinical Signs Early lesions appear as irritated, hairless areas usually found on the distal extremities (Swaim and Angarano, 1990). The predilection for the limbs may be due to accessibility or possibly a lower threshold for pruritus in these areas. As the lesions progress, the skin becomes ulcerated and the wound develops a hyperpigmented edge. The wounds may partially heal and then be aggravated again when licking resumes.

Diagnosis and Differential Diagnosis Acral lick granulomas must be differentiated from several other conditions, including bacterial or fungal infection, foreign bodies, and pressure sores. In addition, mast cell tumors and other forms of neoplasia can mimic the appearance of acral lick granuloma. Many of the aforementioned problems can be ruled out by the history of the animal. However, a complete history may be unavailable in the laboratory setting. Fungal cultures and allergy testing may aid in diagnosis. Biopsy of the affected area would rule out neoplasia. An uncomplicated acral lick granuloma would feature hyperplasia, ulceration, and fibrosis without evidence of infection or neoplasia (Walton, 1986).

Prevention and Control Behavior modification and relief of boredom are important aspects of preventing (and treating) acral lick granuloma. Environmental enrichment including exercise, co-housing and various toys is already a basic requirement and may be increased to combat self injurious behaviors.

Treatment Several treatments have been reported for acral lick granuloma and the selection of a treatment should be based on the underlying cause. One of the most important aspects of treatment is to break the cycle of self-trauma. Mechanical restraint with an Elizabethan collar is one of the easiest methods to accomplish this goal. Several direct treatments have been examined, including intralesional and topical steroids, perilesional cobra venom, acupuncture, radiation, and surgery (Swaim and Angarano, 1990; Walton, 1986). Opioid antagonists have been applied as treatments for acral lick granulomas and self-injurious behaviors with the theory that this will block the effects of endogenous opioids. Naltrexone and nalmefene have been used successfully to reduce excessive licking behaviors and resolve associated lesions. However, lesions did recur after the drugs were discontinued (Dodman *et al.*, 1988; White, 1990). The topical administration of a mixture of flunixin meglumine, steroid, and dimethyl sulfoxide has also been shown to be effective (Walton, 1986). In addition, psychoactive drugs have been suggested to relief of

boredom or anxiety. These have included phenobarbital, megestrol acetate, and progestins. However, side effects have been reported (Swaim and Angarano, 1990). Other behavior-modifying medications such as clomipramine may be effective in the treatment of compulsive anxiety disorder. The potential for effects that could interfere with experimental results must be determined prior to initiation of treatment. It is important to note that none of the above-mentioned treatments have been successful in all cases. The overall prognosis for acral lick granuloma should be considered guarded since the lesions often recur when treatment is discontinued.

4. Elbow Hygroma

Etiology Hygromas are fluid-filled sacs that develop as a result of repeated trauma or pressure over a bony prominence. The area over the olecranon is most frequently affected, but hygromas have been reported in association with the tuber calcis, greater trochanter, and stifle (Newton *et al.*, 1974).

Epizootiology Elbow hygromas are most frequently reported in large and giant breeds of dogs, less than 2 years of age (Johnston, 1975; White, 2003; Cannap *et al.*, 2012). Elbow hygromas are seen infrequently in the laboratory animal setting because the commonly affected breeds are seldom used in research. However, the housing environment of research dogs, especially cage bottoms and cement runs, may predispose them to hygromas. For this reason, laboratory animal veterinary and husbandry staff should be familiar with this condition.

Clinical Signs The clinical presentation will depend upon the chronicity of the problem. A dog with an elbow hygroma usually presents with a painless, fluctuant swelling over the point of the elbow without signs of lameness. The condition may be unilateral or bilateral. Over a long period of time, elbow hygromas may become inflamed and ulcerated. If the hygroma becomes secondarily infected, the animal may exhibit pain and fever (Johnston, 1975; White, 2003).

Pathology The fluid-filled cavity in the hygroma is lined by granulation and fibrous tissue. Hygromas lack an epithelial lining and therefore are not true cysts. The fluid within the cavity is a yellow or red serous transudate. This fluid is less viscous than joint fluid and elbow hygromas do not communicate with the joint (Johnston, 1975).

Treatment The treatment of elbow hygromas should be conservative whenever possible. Conservative management of the elbow hygroma is aimed at relieving the source of pressure at the point of the elbow. In early and mild cases, simply providing padding to cover hard surfaces will result in resolution of the hygroma. A soft padded bandage or doughnut bandage around the affected site may also be of benefit. Neoprene/polyester

sleeves that cover the elbows and fit over the shoulders are also available as an option for either prevention or treatment of hygromas (Cannap *et al.*, 2012). More aggressive therapies, including needle drainage and injection of corticosteroids into the hygroma, have been described but are not recommended due to the risk of infection (Johnston, 1975). Surgical options should be reserved for complicated or refractory cases. Even simple excision can be associated with complications such as wound dehiscence and ulceration (Johnston, 1975) due to the location of the bony prominence at the surgical site. This issue may be avoided by using a skin advancement flap (White, 2003) that allows intact, healthy skin to cover the bony prominence. A muscle advancement flap has also been described (Green *et al.*, 2008). Regardless of the method used to treat an elbow hygroma, recurrence of the problem is likely unless the predisposing factors are identified and relieved.

5. Corneal Ulcers

Etiology In the research environment, corneal ulcers are most often associated with direct trauma, contact with irritating chemicals, or exposure to the drying effects of air during long periods of anesthesia. Chronic or recurrent corneal ulcers may also be associated with infection or hereditary causes in some breeds of dogs; however, these would be rare in the laboratory setting.

Clinical Signs The signs of corneal ulceration are blepharospasm, epiphora, and photophobia. The eye may appear irritated and inflamed. In minor cases, the cornea may appear normal however, in cases of deeper ulceration, the cornea may appear roughened or have an obvious defect. In addition, the periocular tissues may be swollen and inflamed because of self-inflicted trauma from rubbing at the eye.

Diagnosis A tentative diagnosis of corneal ulcer or abrasion may be based on the clinical signs. A definitive diagnosis of corneal ulcers is made by the green appearance of the cornea when stained with fluorescein dye. When a corneal ulcer has been diagnosed, the eye should be inspected for underlying causes such as foreign bodies, abnormal eyelids, or aberrant cilia.

Treatment The treatment of corneal ulcers will depend on the depth and size of the affected area, as well as the underlying cause. Superficial abrasions are generally treated with topical application of antibiotics. A triple antibiotic ointment that does not contain steroids given three times a day for 2–3 days usually provides adequate treatment. Simple corneal ulcers are restained with fluorescein after 3 days and should show complete healing at that time. If the ulcer is not healed, this may indicate that the ulcer has an undermined edge impeding proper healing. Topical anesthetic should be applied to the eye, and a cotton-tipped applicator can be rolled over the surface of the ulcer toward its edge.

This will remove the unattached edge of the cornea and healing should progress normally after debridement. Deep ulcers may require further debridement and primary repair. In such cases, a third eyelid or conjunctival flap may be applied to the eye until experienced help can be obtained. In all cases, an Elizabethan collar or other restraint may be necessary to prevent additional trauma to the eye. Ulcers caused by entropion, ectropion, or dystichiasis will not resolve until the condition is repaired, and descriptions for this can be found elsewhere.

Prevention The proper application of lubricant eye ointment at the time of anesthesia will prevent drying due to exposure and may also protect the eye from scrub solutions applied near the eye. Early treatment of superficial ulcers should prevent self-trauma and progression of the wound.

D. Iatrogenic Diseases

1. Implant and Catheter Infections

Etiology Research protocols often require the placement of chronic implants. Implants such as cardiac or other biomedical devices may be the primary focus of the research study. Implants may also be used as chronic monitoring devices, for delivery of compounds, or to collect serial samples. Infection may occur at the time of implant. Alternatively, the implant may serve as a nidus after hematogenous spread from other sources. One of the most common sources of infection is from colonization of the device from an external component, which is a frequent complication with indwelling catheters.

The actual incidence of complications associated with indwelling vascular catheters in dogs is unknown. One study (Hysell and Abrams, 1967) examined the lesions found at necropsy in animals with chronic indwelling catheters, which included traumatic cardiac lesions, visceral infarcts, and fatal hemorrhages. These lesions were primarily associated with catheter-induced trauma or secondary to embolization of fibrin. In a veterinary clinical setting, infections in peripheral catheters were more likely when the catheters were used for blood collection immediately after placement and when a 'T' connector rather than a 'Y' connector was used. (Jones *et al.*, 2009).

Intestinal access ports have been used to study the pharmacokinetics of drugs at various levels in the intestinal tract. These catheters are usually vascular access ports with several modifications to allow secure placement in bowel (Meunier *et al.*, 1993). The most frequently reported complication associated with these catheters is infection around the port site (Meunier *et al.*, 1993; Kwei *et al.*, 1995).

Clinical Signs Dogs with implant infections may not exhibit signs initially (Jones *et al.*, 2009). Localized swelling around the implant may occur. In the case of indwelling catheters, signs may include redness and

swelling of the skin around the external port or discharge from the skin wound. Vascular access ports may develop fluctuant subcutaneous abscesses. In more severe cases, systemic signs may be noted (Bach *et al.*, 1998; Hysell and Abrams, 1967). The systemic signs of infection are covered elsewhere in this chapter.

Treatment The treatment of catheter infections almost invariably requires removal of the catheter, as demonstrated in both dogs and monkeys (Ringler and Peter, 1984; DaRif and Rush, 1983). Superficial wound irritation or infection may be treated locally with antibiotic ointment, sterile dressing changes, and efforts to minimize catheter movement; however, more extensive problems require aggressive therapy. Localized abscesses or sinus tracts may be managed by establishing drainage and copious flushing. Aerobic and anaerobic cultures of blood and locally infected sites should be performed prior to initial treatment (Ringler and Peter, 1984). Systemic antibiotic therapy should be initiated for a 10-day period. The choice of drug will ultimately be based on previous experience and culture results. If retention of a catheter is important, the catheter lumen may be safely disinfected with chlorine dioxide solution (Dennis *et al.*, 1989). The solution is removed after 15 min and replaced with heparinized saline. All of the extension lines and fluids used with an infected catheter should be discarded. The blood cultures should be repeated 3 days after the antibiotic therapy has ceased. If bacteria are still cultured, the catheter must be removed.

Prevention It is highly desirable to prevent complications that may result in loss of an implanted device. Catheters and other implants should be made of non-thrombogenic material and be as simple as possible. A catheter with extra ports or multiple lumens requires additional management and supplies more routes for infection. The initial placement of an indwelling catheter must be done under aseptic conditions by individuals who are familiar with the procedure. Intravenous catheters that are used for delivery of drugs or blood sampling should be positioned in the vena cava and not in the right atrium, thereby minimizing trauma to the tricuspid valve. Ideally, catheters are secured to reduce movement and irritation of the skin, which may predispose to infection around external ports. The use of vascular access ports that lie entirely under the skin eliminates many problems with infection. It has also been found that long extension tubing connected to the port may actually reduce the potential for infection of the catheter (Ringler and Peter, 1984). For intestinal access ports, catheter security may be improved with a synthetic cuff added to the end of the catheter allowing better attachment to the intestine (Meunier *et al.*, 1993). After any catheter placement, animals should be observed daily for signs of either local or systemic infection. The catheter entry site should be disinfected,

coated with antibiotic ointment, and rebandaged every other day. Once a month, the catheter line may be disinfected with chlorine dioxide. In addition, a solution of the antibiotic ceftazidime used on alternate days with the heparin locking solution has been shown to effectively reduce infections in indwelling vascular catheters (Bach *et al.*, 1998).

Throughout the life of the catheter, injections into and withdrawals from the catheter should be done in a sterile manner, and the number of breaks in the line should be kept to a minimum. Periodically, the placement of an indwelling catheter may be verified by radiography. When placed and managed correctly, catheters and ports of any kind may remain in place for months without complications.

2. Sepsis

Etiology Sepsis is defined as the systemic response to infection caused by bacteria (gram negative and/or gram positive), fungi, or viruses. In laboratory animals, sepsis is most often seen as a complication of surgical procedures or associated with chronic implants. Sepsis may also be seen as a complication of infectious diseases such as parvovirus.

Clinical Signs The signs of sepsis can vary, depending on the source of the infection and the stage of the disease. Early in the course, dogs may present with signs of a hyperdynamic sepsis, including increased heart rate, increased respiratory rate, red mucous membranes, and a normal-to-increased capillary refill time. Systemic blood pressure and cardiac output will be increased or within the normal range. The animals will often be febrile. Later in the course of the syndrome, the animals may show classic signs of septic shock including decreased temperature, pale mucous membranes, and a prolonged capillary refill time. Cardiac output and blood pressure are decreased as shock progresses. Peripheral edema and mental confusion have also been reported (Hauptman and Chaudry, 1993).

Pathogenesis The pathophysiology of sepsis is complex and is mediated by immune responses involving mediators such as cytokines, eicosinoids, complement, superoxide radicals, and nitric oxide. The body responds to overwhelming infection with an attempt to optimize metabolic processes and maximize oxygen delivery to tissues. However, if inflammation is left unchecked, the system may be unable to compensate, and the result is cardiovascular collapse.

Diagnosis In general, a presumptive diagnosis of sepsis is made based on the occurrence of several in a group of signs, including altered body temperature, increased respiratory and/or heart rate, increased or decreased white blood cell (WBC) count, increased number of immature neutrophils, decreased platelet count,

decreased blood pressure, hypoxemia, and altered cardiac output. However, extreme inflammation without infection (e.g., pancreatitis, trauma) may create similar signs. One study examined the diagnosis of sepsis in canine patients at a veterinary hospital based on easily obtainable physical and laboratory findings. That study found that septic individuals had higher temperatures, WBC counts, and percentage of band neutrophils than nonseptic individuals, whereas platelet counts were lower in the septic dogs. There were no differences in respiratory rate or glucose levels between the groups. Using these criteria, the results had a high sensitivity and a tendency to overdiagnose sepsis (Hauptman *et al.*, 1997). Ultimately, the presence of a septic focus simplifies diagnosis greatly; however, the focus may not be obvious. If the signs of sepsis are evident but the focus is not, several areas should be evaluated for infection, including the urinary, reproductive, respiratory, alimentary, and cardiovascular systems, as well as the abdominal cavity (Kirby, 1995).

Treatment The treatment of sepsis has three aims. The first aim is to support the cardiovascular system. All septic animals should be treated with fluids to replace deficits and to maximize cardiac output. Crystalloids are most frequently used to maintain vascular volume, primarily because of their low cost. Colloids offer the advantage of maintaining volume without fluid overload and may have other positive effects on the cardiovascular system. Acid-base and electrolyte imbalances should also be addressed.

After the animal has stabilized, the treatment of sepsis should be aimed at removing the septic focus. Obvious sources of infection should be drained or surgically removed. If an implant is infected, it should be removed. Antibiotic therapy should also be instituted. The choice of antibiotic will ultimately depend upon the results of culture; however, the initial choice of antibiotics is based on previous experience, source of infection, and gram stains. The organisms associated with sepsis are often gram-negative bacteria of gastrointestinal origin or are previously encountered nosocomial infections. Ideally, the antibiotic chosen for initial therapy should be a broad-spectrum, bactericidal drug that can be administered intravenously. Second- or third-generation cephalosporins provide good coverage, as does combination therapy with enrofloxacin plus metronidazole or penicillin.

Finally, the treatment of sepsis is aimed at blocking the mediators of the systemic response. This category of sepsis treatment is the focus of much research. Several studies have examined the effects of steroids, nonsteroidal anti-inflammatory drugs, and antibodies directed against endotoxin, cytokines, or other mediators of the inflammatory response; however, none of these treatments have proven greatly effective in clinical trials.

Consequently, there is no 'magic bullet' for the treatment of sepsis at this time. Successful therapy remains dependent on aggressive supportive care coupled with identification and elimination of the inciting infection.

3. Aspiration Lung Injury

Etiology In research animals, aspiration may occur accidentally during the oral administration of various substances or by the misplacement of gastric tubes. Aspiration of gastric contents may also occur as a complication of anesthesia. In pet animals, aspiration is often seen as a result of metabolic and anatomical abnormalities; however, such occurrence would be rare in the research setting.

Pathogenesis Aspirated compounds can produce direct injury to lung tissue, but more importantly, the aspiration provokes an inflammatory response, probably mediated by cytokines. The result is a rapid influx of neutrophils into the lung parenchyma and alveolar spaces. The inflammation leads to increased vascular permeability with leakage of fluid into the alveolar spaces and can eventually lead to alveolar collapse. If the condition is severe, it may result in adult respiratory distress syndrome and respiratory failure. It should be noted that infection is not present in the early stages of this condition but may complicate the problem after 24–48 h.

Clinical Signs The severity and clinical manifestation of aspiration lung injury are dependent upon the pH, osmolality, and volume of the aspirate. The signs of aspiration lung injury may include cough, increased respiratory rate, pronounced respiratory effort, and fever. When respiration is severely affected, the oxygen saturation of blood will be decreased. The diagnosis of this problem is based on witness of aspiration, history consistent with aspiration, and/or the physical findings. Classically, radiographs of the thorax demonstrate a bronchoalveolar pattern in the cranioventral lung fields. However, these lesions may not appear for several hours after the incident of aspiration. In addition, the location of the lesions may be variable, depending on the orientation of the animal at the time of aspiration.

Treatment The treatment of aspiration lung injury is largely supportive and depends upon the severity of the inflammation and the clinical signs. If the aspiration is witnessed, the mouth and, ideally, the upper airway should be cleared of residual material. When small amounts of a relatively innocuous substance (e.g., barium) have been aspirated, treatment may not be necessary. When severe inflammation is present, systemic as well as localized therapy may be necessary. Oxygen therapy may be instituted; however, the concentration and time frame are controversial, because lung injury may be exacerbated by long-term administration of oxygen at high concentrations (Nader-Djahal *et al.*, 1997).

Fluid therapy may also be necessary in severe cases; however, cardiovascular support should be performed judiciously as fluid overload could lead to an increase in pulmonary edema. The use of colloids is also controversial because of the increase in vascular permeability that occurs in the lungs. Several studies have addressed the use of anti-inflammatory agents to reduce lung injury associated with aspiration; however, none are used clinically in human or veterinary medicine at this time. Corticosteroids are contraindicated (Raghavendran *et al.*, 2011).

In humans, antibiotics are reserved for cases with confirmed infection, in order to prevent the development of antibiotic-resistant pneumonia. It has been suggested that dogs should be immediately treated with antibiotics when the aspirated material is not acidic or has potentially been contaminated by oral bacteria associated with severe dental disease. Amoxicillin-clavulanate has been recommended as a first line of defense, reserving enrofloxacin for resistant cases (Hawkins, 2000). The presence of pneumonia should be verified by tracheal wash and cultures.

Prevention Aspiration of drugs and other compounds may be avoided through careful administration of oral medications by experienced individuals. Likewise, gavage or orogastric administration of liquids should be performed by experienced individuals, and the procedure should be aborted if coughing or other respiratory signs occur. The aspiration of stomach contents can largely be avoided by appropriate fasting prior to anesthesia for at least 12 h for food and 2 h for water. If appropriate fasting times are not observed, anesthesia should be postponed whenever possible, particularly if intended procedures require manipulation of the viscera or head-down positioning of the dog. If anesthesia cannot be avoided, it should be rapidly induced and the dog should be intubated. During recovery from anesthesia, the endotracheal tube should be removed with the cuff partially inflated and with the dog in a head-up position (Haskins, 1993).

4. Burn Wounds

Based on the source of energy, burn wounds may be categorized into four groups: thermal, chemical, radiation, and electrical. In laboratory animals, accidental burns are usually the result of thermal injury (heating pads, water bottles), chemicals (strong alkalis, acids, disinfectants, drugs), or experimental irradiation protocols.

a. Thermal Injury

Etiology Inappropriate use of external heating devices is the most common cause of burns in laboratory animal medicine. The insult to the skin results in desiccation of the tissue and coagulation of proteins.

In addition, the severely injured area is surrounded by a zone of vascular stasis, which promotes additional tissue damage. Even small burns can result in significant inflammation that could affect the outcome of some research investigations and cause considerable discomfort to the animal. The proper and immediate treatment of burn wounds can reduce the effects of the injury on both the individual and the research.

Clinical Signs The clinical signs vary with the depth, location, and surface area of burn injury. Classification systems for thermal burns are generally based on the depth of the injury, varying from superficial involvement of only epidermis to complete destruction of skin and subcutaneous tissues (Bohling, 2012). Superficial burns appear erythematous and inflamed. In some cases, matting of the overlaying hair with exudate may be the first sign of a previously undetected skin lesion. Progressive hair and skin loss may be evident over the first few days after injury (Johnston, 1993). Although blistering is a characteristic of partial thickness burns in humans, this is rarely seen in dogs (Bohling, 2012). Uncomplicated, superficial burn wounds heal by reepithelialization within 3–5 days. Deeper burn wounds are characterized by a central area of nonviable tissue surrounded by edematous, inflamed tissues. A thick eschar, composed of the coagulated proteins and desiccated tissue fluid, develops over deep burn wounds. These wounds heal by granulation under the eschar, which will eventually slough.

The amount of pain associated with burns depends upon several factors including the depth and area of the wound, procedural manipulations, and movement at the affected site (Bohling, 2012). Pain associated with superficial burn wounds usually subsides in 2–3 days. Theoretically, deep burns destroy nerve endings and result in less pain than superficial burns. However, inflammatory pain may still be present due to the tissue reaction around the necrotic site. In addition, sharp procedural pain and breakthrough pain have been described in humans during the healing phases of burn injuries and should be considered as potential complications in dogs as well (Bohling, 2012).

Severe and widespread accidental burn injury can result in clinical signs associated with multiple organs including the pulmonary, gastrointestinal, hematopoietic, and immune systems. In addition, extensive burn injury can predispose to infection and even sepsis. This type of injury with the associated complications would be extremely rare in the laboratory setting.

Treatment Appropriate and timely treatment of a burn wound will reduce the extent of tissue damage and associated pain. Thermal injuries should be immediately exposed to cool water (15°C) to reduce edema and pain. Exposure to very cold water and ice does not improve outcomes (Bohling, 2012). Topical wound dressings are

recommended in the early stages of treatment for both partial- and full-thickness burns that are of small size.

Systemic antibiotics are unable to penetrate eschar and are not adequately distributed through the abnormal blood supply of burned tissues. Therefore, a thin film of a water-soluble, broad-spectrum antibiotic ointment should be applied to the wound surface. Silver sulfadiazine has a broad spectrum, penetrates eschar, and is often the preparation of choice for burn wound therapy. Povidone-iodine ointment will also penetrate thin eschar and provides a broad spectrum. Mafenide has a good spectrum that covers gram-negative organisms well and is often used to treat infected wounds, although it has been associated with pain upon application (Demling and Lalonde, 1989). Once a topical antibiotic has been applied, a nonadherent dressing should be placed on the wound. Burn wounds covered in such a manner tend to epithelialize more rapidly and are less painful than uncovered wounds (Demling and Lalonde, 1989; Bohling, 2012). After the initial treatment, burn wounds should be gently cleansed two to three times a day, followed by reapplication of the topical antibiotic and rebandaging (Demling and Lalonde, 1989). Systemic antibiotics are indicated in cases where local or systemic infection is present and their ultimate selection should be based on culture results. Burn wounds can be extremely painful, and analgesia should be instituted immediately and adjusted accordingly throughout the treatment period.

Surgical intervention may be necessary in some cases. With small or moderately sized wounds, the eschar over the burn wound may actually impede wound contraction and reepithelialization. In such cases, once the eschar has become fully defined, a complete resection may improve wound healing. With large and severe burn wounds, repeated debridement by surgery or other means might be necessary. In the laboratory setting, a decision to pursue extensive surgical intervention would be dependent upon full consideration of the effects on animal welfare and research results.

Prevention Thermal burns can be prevented in the research setting. Electric heating pads and heat lamps should be avoided if possible. Only heated water blankets or circulating warm air devices should be used to provide warmth to the animals. In rare instances, heated water blankets have also caused burns; therefore, these devices should be carefully monitored. As a precaution, a thin towel may be placed between the animal and the water blanket. Basic fire prevention precautions should be taken particularly around oxygen sources and flammable agents

b. Chemical Injury

Etiology Chemical injury may be due to skin contact with concentrated solutions such as disinfectants or

inadvertent exposure to laboratory chemicals. In addition, perivascular injection of certain drugs (pentobarbital, thiamylal, thiopental, thiacetarsemide, vincristine, vinblastine, and doxorubicin) have been associated with extensive tissue damage (Swaim and Angarano, 1990; Waldron and Trevor, 1993). The mechanism of action will vary depending upon the pH, osmolality, and chemical composition of the agent and may include oxidation, reduction, disruption of lipid membranes, or other reactions (Bohling, 2012; Swaim, 1990; Waldron and Trevor, 1993).

Clinical Signs Surface contact with chemicals may result in mild irritation and redness of superficial layers of the skin. However, many agents may cause progressive injury until the chemical reaction has been neutralized. This may result in tissue necrosis and secondary infection. The immediate signs of perivascular injection are withdrawal of the limb or other signs of discomfort and swelling at the injection site. The area may appear red, swollen, and painful as inflammation progresses. There may eventually be necrosis of the skin around the injection site. In cases of doxorubicin extravasation, signs may develop up to a week after the injection, and the affected area may progressively enlarge over a 1- to 4-month period. This is because the drug is released over time from the dying cells (Swaim and Angarano, 1990).

Treatment In cases of skin contact with chemical agents, the affected area should be thoroughly and repeatedly lavaged with warm water to dilute or remove the substance. The material safety data sheet for the substance should be consulted for any possible neutralization protocol. Additional treatment will depend upon the severity of the tissue damage and will follow the same guidelines as for the thermal injury described earlier. For the treatment of perivascular injections, dilution of the drug with subcutaneous injections of saline is recommended. In addition, steroids may be infiltrated locally to reduce inflammation. Topical application of dimethyl sulfoxide (DMSO) may also be helpful in reducing the immediate inflammation and avoiding the development of chronic lesions. The addition of lidocaine to subcutaneous injections of saline has been used in cases of thiacetarsemide injection (Hoskins, 1989). The local infiltration of hyaluronidase accompanied by warm compresses has been suggested for perivascular vinblastine (Waldron and Trevor, 1993) and for doxorubicin. The use of DMSO or another free radical scavenger, dexrazoxane, infused at the site has also been suggested for doxorubicin toxicities. Despite these treatments, necrosis of skin may be observed and would require serial debridement of tissues with secondary wound closure or skin grafting. In cases of doxorubicin extravasation, early excision of affected tissues is advocated to prevent the progressive sloughing caused by sustained release of the drug from dying tissues (Swaim and Angarano, 1990). In all

cases, the condition can be painful and analgesia should be addressed.

Prevention Prior to the use of any substance, the investigator should be aware of its chemical composition and the potential for problems. The material safety data sheets should be available for all compounds and storage recommendations followed closely. For intravenous administration of toxic compounds, insertion of an indwelling catheter is extremely important. Prior to the injection, the catheter should be checked repeatedly for patency by withdrawal of blood and injection of saline. Any swelling at the catheter site or discomfort by the subject indicates that the catheter should not be used. Access to a central vessel such as the cranial or caudal vena cava is preferred over the use of peripheral vessels. When peripheral catheters are used, the injection should be followed by a vigorous amount of flushing with saline or other physiological solution and removal of the catheter. Additional injections are best given through newly placed catheters in previously unused vessels. The repeated use of an indwelling peripheral catheter should be approached cautiously and done only out of necessity.

c. Radiation Injury

Etiology Radiation burns are generally a complication of therapeutic administration and are a result of free oxygen radical formation (Waldron, 1993). The severity of radiation burns and their treatment will depend upon the dose, frequency, total surface area, and location of the radiation. Damage to epithelial layers of the skin can lead to desquamation. Direct injury to fibroblasts results in decreased collagen production and poor wound healing. In addition, there may be fibrosis of blood vessels (Pavletic, 2010) and subsequent hypoxia causing necrosis of deeper tissues.

Clinical Signs The tissues most often affected are the skin and mucus membranes. With superficial injury, affected skin may exhibit hair loss and erythema, and produce a clear exudate. The intensity of the inflammation may increase for 1–2 weeks after the completion of radiation treatment. Deeper and more serious injury manifests with subcutaneous fibrosis and can lead to disfigurement (Johnston *et al.*, 1993). The skin and underlying deep structures including the bone may become necrotic over several weeks (Pavletic, 2010). These deeper injuries are prone to infection due to their lack of blood supply. Systemic signs such as vomiting are rare in dogs unless there has been direct radiation treatment to organs (Johnston *et al.*, 1993).

Treatment With superficial skin burns, the wound should be kept clean and should be covered if possible. In cases of oral mucous membrane damage, there may be special feeding requirements. When wounds are ulcerated, avascular tissues should be excised. Treatments

with silver sulfadiazine, mafenide acetate, or other topical agents are recommended to control infection. In addition, infection is avoided by closure of the wound as soon as possible. The goal of surgery is to cover the wound with healthy tissue to promote vascularization of the area. In some cases, this may require muscle and/or skin grafts.

Prevention Radiation burns can be limited by selection of appropriate, fractionated therapy and application of shielding to reduce exposure. Prompt treatment of the injuries can reduce the occurrence of infection. Since radiation is associated with poor wound healing, complications may arise when additional procedures are required. It is recommended to wait at least 1 week (Pavletic, 2010) or even longer (Laing, 1990) prior to administering radiation to a surgical site. After radiation, routine surgeries should be avoided for 1–2 months (Pavletic, 2010).

E. Neoplastic Diseases

1. Introduction

The prevalence of cancer in the general canine population has increased over the years (Dorn, 1976). This can be attributed to the longer life spans resulting from improvements in nutrition, disease control, and therapeutic medicine. Because of these changes, cancer has become a major cause of death in dogs (Bronson, 1982).

In a lifetime cancer mortality study of intact beagles of both sexes, Albert *et al.* (1994) found death rates similar to the death rate of the at-large dog population (Bronson, 1982). Approximately 22% of the male beagles died of cancer. The majority of the tumors were lymphomas (32%) and sarcomas (29%), including hemangiosarcomas of the skin and fibrosarcomas. Of the female beagles dying of cancer (26% of the population studied), three-quarters had mammary cancer (40%), lymphomas (18%), or sarcomas (15%). Of the sarcomas in females, one-third were mast cell tumors. In addition to these tumors that cause mortality, the beagle is also at risk for thyroid neoplasia (Hayes and Fraumeni, 1975; Benjamin *et al.*, 1996).

Because of the popularity of the beagle as a laboratory animal, discussion of specific neoplasms will focus on the tumors for which this breed is at risk, as well as tumors that are common in the general canine population. A complete review of clinical oncology in the dog is beyond the scope of this chapter but can be found elsewhere (Withrow *et al.*, 2013).

2. Biopsy Techniques

Fine-needle aspirates are generally the first diagnostic option for palpable masses, because they can easily be performed in awake, cooperative patients. This technique allows for rapid differentiation of benign and neoplastic processes. In cases where cytologic results from

fine-needle aspirates are not definitive, more invasive techniques must be used.

Needle-punch or core biopsies can also be performed in awake patients with local anesthesia. An instrument such as a Tru-Cut[®] needle (Travenol Laboratories, Inc., Deerfield, Illinois) is used to obtain a 1-mm × 1–1.5-cm biopsy of a solid mass. A definitive diagnosis may be limited by the size of the sample acquired using this technique.

Incisional and excisional biopsies are utilized when less invasive techniques fail to yield diagnostic results. Excisional biopsies aid in histopathological examination and are the treatment of choice when surgery is necessary, because the entire mass is removed. Surgical margins should extend at least 1 cm around the tumor and 3 cm if mast cell tumors are suspected (Morrison *et al.*, 1993). Incisional biopsies are performed when large soft-tissue tumors are encountered and/or when complete excision would be surgically difficult or life-threatening. When performing an incisional biopsy, always select tissue from the margin of the lesion and include normal tissue in the submission.

3. Neoplastic Disease

a. Lymphomas

Etiology Lymphomas are a diverse group of neoplasms that originate from lymphoreticular cells. Canine lymphoma represents 5–7% of canine tumors and a majority (85%) of canine hematopoietic disease (Ettinger, 2003; Vail and Young, 2013). Whereas retroviral etiologies have been demonstrated in a number of species (e.g., cat, mouse, chicken), conclusive evidence of a viral etiology has not been established in the dog. In humans, data implicate the herbicide 2,4-dichlorophenoxyacetic acid as a cause of non-Hodgkin's lymphoma, but studies in dogs with similar conclusions have come under scrutiny (MacEwen and Young, 1991). In addition, tobacco smoke, environmental chemicals, and waste emissions are considered possible risk factors (Marconato *et al.*, 2009; Gavazza *et al.*, 2001)

Clinical Signs Multicentric high-grade lymphoma (MHGL) accounts for the majority of reported cases of canine lymphoma. Depending upon grade, immunophenotype, and location involved, dogs with MHGL usually present with painless, enlarged lymph nodes and nonspecific signs such as anorexia, weight loss, polyuria, polydipsia, fever, and lethargy. When the liver and spleen are involved, generalized organomegaly may be felt on abdominal palpation.

Less commonly, dogs develop alimentary, mediastinal, cutaneous, and extranodal lymphomas. Alimentary lymphoma is associated with vomiting and diarrhea, in addition to clinical signs associated with MHGL. Dogs with mediastinal lymphoma often present with respiratory signs (dyspnea and exercise intolerance) secondary to pleural effusion or cranial vena caval syndrome.

Hypercalcemia is most frequently associated with this form of lymphoma and may result in polyuria, polydipsia, and weakness. Cutaneous lymphoma is an uncommon epitheliotrophic form of lymphoma. It is often referred to as mycosis fungoides and is typically of a CD8+ T-cell immunophenotype. It varies in presentation from solitary to generalized and may mimic any of a number of other inflammatory skin disorders including oral mucosal lesions. The lesions may occur as erythema, plaques, erosions, scales, nodules, crusts, hypopigmentation, and alopecia (Fontaine *et al.*, 2009). Approximately half of the cases are pruritic. A number of extranodal forms of lymphoma have been reported, including tumors affecting the eyes, central nervous system, kidneys, or nasal cavity. Clinical presentation varies, depending on the site of involvement (e.g., nervous system: seizures, paresis, paralysis).

Epizootiology The incidence of lymphoma is highest in dogs 5–11 years old, accounting for 80% of cases. Although the neoplasm generally affects dogs older than 1 year, cases in puppies as young as 4 months have been reported (Dorn *et al.*, 1967). No gender predilection has been reported.

Diagnosis and Pathologic Findings A fine-needle aspirate is initially performed on accessible lymph nodes. Thoracic radiographs and abdominal ultrasound \pm fine needle aspiration of the liver or spleen can be used if mediastinal or abdominal involvement is suspected. Additional staging can be determined through complete blood counts, serum biochemistry, flow cytometry for immunotyping, bone marrow aspiration, or surgical lymphadenectomy and histology. Enlarged neoplastic lymph nodes vary in diameter from 1 to 9 cm and are moderately firm. Some may have areas of central necrosis and are soft to partially liquefied. The demarcation between cortex and medulla is generally lost, and on cut section, the surface is homogenous. The spleen may have multiple small nodular masses or diffuse involvement with generalized enlargement. The enlarged liver may have disseminated pale foci or multiple large, pale nodules. In the gastrointestinal tract, both nodular and diffuse growths are observed. These masses may invade through the stomach and intestinal walls.

Flow cytometry and lymphoblastic markers (CD34) can aid in diagnosis and subtyping of tumors. In addition, positron emission tomography is being explored for detection of extranodal and metastatic lymphoma (LeBlanc *et al.*, 2009; Marconato, 2011; Elstrom *et al.*, 2003).

Classification of lymphoma types is based upon cytological, morphological, and immunological characteristics using the Kiel classification criteria (Vail and Young, 2013). Histologically, the most common lymphomas are classified as intermediate to high grade and of large-cell (histiocytic) origin. The neoplastic lymphocytes typically obliterate the normal architecture of the lymph

nodes and may involve the capsule and perinodal areas. Lymphoma subtypes can be further characterized based upon genetic, molecular, and immunological criteria (Ponce *et al.*, 2004).

Pathogenesis All lymphomas regardless of location should be considered malignant. A system for staging lymphoma has been established by the World Health Organization. The average survival time for dogs without treatment is 4–6 weeks. Survival of animals undergoing chemotherapy is dependent on the treatment regimen as well as the form and stage of lymphoma (MacEwen and Young, 1991). Median survival time with aggressive therapy is generally less than 12 months.

Hypercalcemia is a paraneoplastic syndrome frequently associated with lymphoma. The pathogenesis of this phenomenon is not fully understood but may be a result of a parathormone-like substance produced by the neoplastic lymphocytes.

Differential Diagnosis Differential diagnoses for multicentric lymphoma include systemic mycosis; salmon-poisoning and other rickettsial infections; lymph node hyperplasia from viral, bacterial, and/or immunologic causes; and dermatopathic lymphadenopathy. Alimentary lymphoma must be distinguished from other gastrointestinal tumors, foreign bodies, and lymphocytic–plasmacytic enteritis. In order to make a definitive diagnosis, whole lymph node biopsies and full-thickness intestinal sections for histopathologic examination may be needed.

Treatment Therapy for lymphoma primarily consists of one or a combination of several chemotherapeutic agents. In addition, radiation therapy and bone marrow transplantation have been utilized. The treatment regimen is based on the staging of the disease, the presence of paraneoplastic syndromes, and the overall condition of the patient. Although treatment may induce clinical remission and prolong short-term survival, most treatment is palliative and aimed at improving quality of life. A thorough discussion of therapeutic options for the treatment of lymphomas in the dog can be found elsewhere (Chun, 2009; Marconato, 2011). Future directions include development of molecular and cellular targeted therapies to enhance traditional chemotherapy treatment, prolong remission, and treat immunologic subtypes of lymphoma (e.g., T-cell lymphoma).

Research Complications Given the grave prognosis for lymphoma with or without treatment, euthanasia should be considered for research animals with significant clinical illness.

b. Mast Cell Tumors

Etiology Mast cells are derived from CD34+ bone marrow progenitor cells. Neoplastic proliferations of mast cells are the most commonly observed skin tumor of the dog and may account for up to 21% of canine skin

tumors (Bostock, 1986; Welle *et al.*, 2008). Mast cells are normally found in the connective tissue beneath serous surfaces and mucous membranes, and within the skin, lung, liver, and gastrointestinal tract. Current research has linked mast cell tumor development to multifactorial causes including breed predisposition and a genetic component, chronic inflammation, and mutations in the surface growth factor, *c-kit* (Ma *et al.*, 1999; Reguera *et al.*, 2000; Webster *et al.*, 2006).

Clinical Signs Well-differentiated mast cell tumors are typically solitary, well-circumscribed, slow-growing, 1- to 10-cm nodules in the dermis and subcutaneous tissue. Alopecia may be observed, but ulceration is not usual. Poorly differentiated tumors grow rapidly, may ulcerate, and may cause irritation, inflammation, and edema to surrounding tissues. Mast cell tumors can be found on any portion of the dog's skin but frequently affect the trunk and hind limb extremities along with perineal and preputial areas. The tumors usually appear to be discrete masses, but they frequently extend deep into surrounding tissues. Abdominal organs are rarely involved but may be associated with anorexia, vomiting, melena, abdominal pain, and gastrointestinal ulceration. Mast cell tumors have also been reported in extracutaneous areas such as the salivary glands, larynx, nasopharynx (London and Thamm, 2013) and conjunctiva (Fife *et al.*, 2011). Mast cell tumors within the perineal, preputial, or inguinal areas are associated with a greater predilection for recurrence or metastasis (Misdorp, 2004).

Epizootiology These tumors tend to affect middle-aged dogs but have been observed in dogs ranging from 4 months to 18 years (Pulley and Stannard, 1990).

Pathologic Findings Because of the substantial variation in histologic appearance of mast cell tumors, a classification and grading system described by Patnaik *et al.* (1986) has become widely accepted. In this system, grade I has the best prognosis and are well differentiated, with round to ovoid, uniform cells with distinct cell borders. The nuclei are round and regular, the cytoplasm is packed with large granules that stain deeply, and mitotic figures are rare to absent. Grade II (intermediately differentiated) mast cell tumors have indistinct cytoplasmic boundaries with higher nuclear–cytoplasmic ratios, fewer granules, and occasional mitotic figures. Grade III (anaplastic or undifferentiated) mast cell tumors have the worst prognosis. The cells contain large, irregular nuclei with multiple prominent nucleoli and few cytoplasmic granules. Mitotic figures are much more frequent. Cells are pleomorphic with indistinct borders. In addition to associated skin lesions (e.g., ulceration, collagenolysis, necrosis, and infection), mast cell tumors have been associated with gastric ulcers in the fundus, pylorus, and/or proximal duodenum, most likely secondary to tumor production of histamine. Histamine stimulates the H₂ receptors of the gastric parietal cells,

causing increased acid secretion. Gastric ulcers have been observed in large numbers (>75%) of dogs with mast cell tumors (Howard *et al.*, 1969).

Pathogenesis Although all mast cell tumors should be considered potentially malignant, the outcome in individual cases can be correlated with the histologic grading of the tumor. Grade III tumors are most likely to disseminate internally. This spread is usually to regional lymph nodes, spleen, and liver, and less frequently to the kidneys, lungs, and heart.

Diagnosis and Differential Diagnosis Using fine-needle aspiration, mast cell tumors can be distinguished cytologically from other round cell tumors (such as histiocytomas and cutaneous lymphomas) by using toluidine blue to metachromatically stain the cytoplasmic granules red or purple. Mast cell granules can also be stained with Wright's, Giemsa, and Romanowsky stains. In addition, mast cells may contain tryptase, chymase, or both (Fernandez *et al.*, 2005). Histological evaluation is generally required for grading. Examination of regional lymph nodes may be warranted if metastatic or systemic disease is suspected. In addition, radiographs and ultrasound with guided aspirates of the liver, spleen, or sublumbar lymph nodes can be used to determine metastatic disease.

Treatment Depending upon the grade, initial treatment for mast cell tumors is generally wide surgical excision (3-cm margins), which may be followed by radiation, chemotherapy, or glucocorticoid therapy. Aspiration or surgical removal of regional lymph nodes is recommended if lymphatic tumor drainage is suspected. If the tumor is not completely resectable or is grade II or III (moderately to undifferentiated), then debulking surgery and adjunct therapy may be used. Treatment algorithms are outlined elsewhere (Withrow *et al.*, 2013)

Research Complications Because of the potential for systemic release of substances such as histamine, vasoactive substances, heparin, eosinophilic chemotactic factor, and proteolytic enzymes, along with the possibility of delayed wound healing and tumor recurrence, dogs with mast cell tumors are not good candidates for research studies. Grade I mast cell tumors may be excised, allowing dogs to continue on study; however, monitoring for local recurrence should be performed monthly. Grade II tumors are variable; animals that undergo treatment should be monitored for recurrence monthly, and evaluation of the buffy coat should be performed every 3–6 months for detection of systemic mastocytosis. Because of the poor prognosis for grade III tumors, treatment is unwarranted in the research setting.

c. Canine Transmissible Venereal Tumors

Etiology Also known as infectious or venereal granuloma, Sticker tumor, transmissible sarcoma, and contagious venereal tumor, the canine transmissible venereal tumor (CTVT) is transmitted horizontally to the

genitals by coitus (Nielsen and Kennedy, 1990). CTVT is a 'parasitic-like' tumor that appears to have originated from dogs or wolves thousands of years ago and despite immense mutation, CTVT adapted, survived, and spread across multiple continents making it the oldest known continuously passaged somatic cell line (Rebbeck *et al.*, 2009; Murchison *et al.*, 2014; Murgia *et al.*, 2006). It has been described as a round cell tumor of histiocytic origin. Although this tumor has been reported in most parts of the world, it is most prevalent in tropical or temperate climates (MacEwen, 1991).

Clinical Signs The tumors are usually cauliflower-like masses on the external genitalia, but they can also be pedunculated, nodular, papillary, or multilobulated. These friable masses vary in size up to 10cm, and hemorrhage is frequently observed. In male dogs, the lesions are found on the caudal part of the penis from the crura to the bulbus glandis or on the glans penis. Less frequently, the tumor is found on the prepuce. Females typically have lesions in the posterior vagina at the junction of the vestibule and vagina. When located around the urethral orifice, the mass may protrude from the vulva. These tumors have also been reported in the oral cavity, skin, and eyes.

Epizootiology and Transmission CTVTs are most commonly observed in young, sexually active dogs. Transmission takes place during coitus when injury to the genitalia allows for exfoliation and transplantation of the tumor. Genital to oral to genital transmission has also been documented (Nielsen and Kennedy, 1990). Extragenital lesions may be the result of oral contact with previously traumatized areas.

Pathologic Findings Histologically, cells are arranged in compact masses or sheets. The cells are round, ovoid, or polyhedral, and have large, round nuclei with coarse chromatin. The cytoplasm is eosinophilic with small vacuoles arranged in a 'string of pearls' pattern.

Pathogenesis Tumor growth occurs within 2–6 months after mating or implantation, and then growth generally slows. Metastasis is rare (<5–17% of cases) but may involve the superficial inguinal and external iliac lymph nodes as well as distant sites. Spontaneous regression may occur within 6–9 months of tumor development.

Diagnosis and Differential Diagnosis Transmissible venereal tumors have been confused with lymphomas, histiocytomas, mast cell tumors, and amelanotic melanomas. However, cytological examination of impression smears, swabs, and fine-needle aspirates generally provide a definitive diagnosis. Although not usually required, histopathology of a biopsy from the mass can aid in diagnosis.

Prevention Thorough physical examinations prior to bringing new animals into a breeding program should prevent introduction of this tumor into a colony.

Control Removing affected individuals from a breeding program should stop further spread through the colony.

Treatment Surgery and radiation can be used for treatment, but chemotherapy is the most effective. Vincristine (0.5–0.7 mg/m²) IV once weekly for four to six treatments will induce remission and cure in greater than 90% of the cases (MacEwen, 1991).

Research Complications Experimental implantation of CTVTs has been shown to elicit formation of tumor-specific IgG (Cohen, 1972). This response may occur in natural infections and could possibly interfere with immunologic studies.

d. Mammary Gland Tumors

Etiology Dogs are susceptible to a wide variety of mammary gland neoplasms, most of which are influenced by circulating reproductive steroidal hormones.

Clinical Signs Single nodules are found in approximately 75% of the cases of canine mammary tumors. The nodules can be found in the glandular tissue or associated with the nipple. Masses in the two most caudal glands (fourth and fifth) account for a majority of the tumors. Benign tumors tend to be small, well circumscribed, and firm, whereas malignant tumors are larger, invasive, and coalescent with adjacent tissues. Inflammatory mammary carcinomas may mimic mastitis or severe dermatitis and must be ruled out to prevent misdiagnosis.

Epizootiology Mammary tumors are uncommon in dogs under 5 years of age with the incidence rising sharply after that. The median age at diagnosis is 10–11 years. A longitudinal study of a large beagle colony showed that significant risk for development of mammary tumors begins at approximately 8 years of age (Taylor *et al.*, 1976). Mammary tumors occur almost exclusively in female dogs, with most reports in male dogs being associated with endocrine abnormalities, such as estrogen-secreting Sertoli cell tumors.

Pathologic Findings The T (tumor size), N (lymph node involvement), and M (metastasis) system is commonly used to stage mammary tumors. Based on histologic classification of mammary gland tumors, approximately half of the reported tumors are benign (fibroadenomas, simple adenomas, and benign mesenchymal tumors), and half are malignant (solid carcinomas, tubular adenocarcinomas, papillary adenocarcinomas, anaplastic carcinomas, sarcomas, and carcinosarcomas) (Bostock, 1977). Histopathologic grades are scored based upon tubule formation, nuclear pleomorphism, and mitosis (Elston and Ellis, 2002). Extensive discussions of classification, staging, and histopathologic correlations can be found elsewhere (Moulton, 1990; Sorenmo *et al.*, 2011).

Pathogenesis Mammary tumors of the dog develop under the influence of hormones. Receptors for both

estrogen and progesterone can be found in 60–70% of tumors. Malignant mammary tumors typically spread through the lymphatic vessels. Metastasis from the first, second, and third mammary glands is to the ipsilateral axillary or anterior sternal lymph nodes. The fourth and fifth mammary glands drain to the superficial inguinal lymph nodes where metastasis can be found. Many mammary carcinomas will eventually metastasize to the lungs and extraskeleton.

Diagnosis and Differential Diagnosis Both benign and malignant mammary tumors must be distinguished from mammary hyperplasia, mastitis, and severe dermatitis. Cytological evaluation from fine-needle aspirates correlates well with histological examination of benign and malignant tumors (Simon *et al.*, 2009). Radiographs and possibly ultrasound should be performed to rule out metastatic disease prior to surgery.

Prevention The lifetime risk of developing mammary tumors can effectively be reduced to 0.5% by spaying bitches prior to the first estrus (Schneider *et al.*, 1969). This is commonly done in the general pet population at 6 months of age. The protective effects of early spay rapidly decrease after several estrus cycles. Dogs spayed prior to the first estrus had a risk of 0.8%, whereas dogs spayed after the first and second estrus had risks of 8% and 26%, respectively.

Treatment Surgery is the treatment of choice for mammary tumors, because chemotherapy and radiation therapy have not been reported to be effective. The extent of the surgery is dependent on the area involved. Single mammary tumors should be surgically removed with 2-cm lateral margins or margins wide enough for complete resection. Deep margins may include removing sections of abdominal fascia or musculature *en bloc* with mammary tumor. Multiple mammary tumors should be removed via regional or unilateral chain mastectomies. Bilateral, staged mastectomies are reserved for more aggressive tumors. There is insufficient evidence at this time to recommend routine complete unilateral or bilateral chain mastectomies. At the time of surgery, axillary lymph nodes are removed only if enlarged or positive on cytology for metastasis. Sorenmo *et al.* (2013) provide a thorough review of canine mammary gland neoplasia.

Research Complications Treatment of early-stage or low-grade mammary tumors may be rewarding, allowing dogs to continue on study. If removed early enough, malignant masses could yield the same results. All dogs should be monitored regularly for recurrence and new mammary tumors.

F. Miscellaneous Diseases

1. Congenital Disorders

Beagles are subject to many of the inherited and/or congenital disorders that affect dogs in general. In a

reference table on the congenital defects of dogs (Hoskins, 2000), disorders for which beagles are specifically mentioned include brachyury (short tail), spina bifida, pulmonic stenosis, cleft palate–cleft lip complex, deafness, cataracts, glaucoma, microphthalmos, optic nerve hypoplasia, retinal dysplasia, tapetal hypoplasia, factor VII deficiency, pyruvate kinase deficiency, pancreatic hypoplasia, epilepsy, GM₁ gangliosidosis, globoid cell leukodystrophy, XX sex reversal, and cutaneous asthenia (Ehlers–Danlos syndrome). Other defects observed include cryptorchidism, monorchidism, limb deformity, inguinal hernia, diaphragmatic hernia, hydrocephaly, and fetal anasarca. Each of these other congenital defects occurred at less than 1.0% incidence.

2. Age-Related Diseases

a. Benign Prostatic Hyperplasia

Etiology Benign prostatic hyperplasia (BPH) is an age-related condition in intact male dogs. The hyperplasia of prostatic glandular tissue is a response to the presence of both testosterone and estrogen.

Clinical Signs BPH is often subclinical. Straining to defecate (tenesmus) may be seen because the enlarged gland impinges on the rectum. Urethral discharge (yellow to red) and hematuria can also be presenting clinical signs.

Epizootiology and Transmission BPH typically affects older dogs (>4 years), although glandular hyperplasia begins as early as 3 years of age. Approximately 95% of intact male dogs will develop BPH by 9 years of age (Smith, 2008).

Pathologic Findings In the early stages of BPH, there is hyperplasia of the prostatic glandular tissue. This is in contrast to human BPH, which is primarily stromal in origin. Eventually, the hyperplasia tends to be cystic, with the cysts containing a clear to yellow fluid. The prostate becomes more vascular with a honeycomb appearance (resulting in hematuria or hemorrhagic urethral discharge), and BPH may be accompanied by mild chronic inflammation.

Pathogenesis BPH occurs in older intact male dogs because increased production of estrogens (estrone and estradiol), combined with decreased secretion of androgens, sensitizes prostatic androgen receptors to dihydrotestosterone. The presence of estrogens may also increase the number of androgen receptors, and hyperplastic prostate glands also have an increased ability to metabolize testosterone to 5 α -dihydrotestosterone (Kustritz and Klausner, 2000) mediating BPH.

Diagnosis and Differential Diagnosis BPH is diagnosed in cases of nonpainful symmetrical swelling of the prostate gland in intact male dogs, with normal hematologic profiles and urinalysis that may be characterized by hemorrhage. A prostatic biopsy can be performed to

confirm diagnosis. Differential diagnoses include squamous metaplasia of the prostate, para-prostatic cysts, bacterial prostatitis, prostatic abscessation, and prostatic neoplasia (primarily adenocarcinoma). These differential diagnoses also increase in frequency with age and, except for squamous metaplasia, can also occur in castrated dogs. As such, these conditions do not necessarily abate or resolve with castration.

Prevention Castration is the primary means for prevention of benign prostatic hyperplasia.

Treatment The first and foremost treatment for BPH is castration. In pure cases of BPH, castration results in involution of the prostate gland detectable by rectal palpation within 7–10 days. For most dogs in research studies, this is a viable option to rapidly improve the animal's condition. The alternative to castration is hormonal therapy, primarily with estrogens. This may be applicable in cases where semen collection is necessary from a valuable breeding male (e.g., genetic diseases). If the research study concerns steroidal hormone functions, then neither the condition nor the treatment is compatible. Finasteride, a synthetic 5 α -reductase inhibitor, has been used in dogs to limit the metabolism of testosterone to 5 α -dihydrotestosterone. Treatment at daily doses of 0.1–0.5 mg/kg orally for 16 weeks was shown to reduce prostatic diameter and volume without affecting testicular spermatogenesis (Sirinarumitr *et al.*, 2001). Upon discontinuation of finasteride, the prostate generally returns to its pretreatment size within several months (Smith, 2008). Gonadotropin-releasing hormone analogs such as desorelin inhibit production of testosterone and estrogen via negative feedback on the hypothalamus–pituitary axis. This is available in a sustained release subcutaneous implant, which has demonstrated efficacy in reducing prostatic size in dogs (Junaidi *et al.*, 2009). However, medical therapy has not shown to be as advantageous as castration.

Research Complications BPH can cause complications to steroidal hormone studies, in that the condition may be indicative of abnormal steroidal hormone metabolism, and neither castration nor estrogen therapy is compatible with study continuation. The development of tenesmus as a clinical sign may also affect studies of colorectal or anal function.

Research Model Older dogs with benign prostatic hypertrophy are used in research to evaluate the use of ultrasonic histotripsy as a precise nonsurgical urethral-sparing alternative to prostate surgery (Lake *et al.*, 2008; Hall *et al.*, 2009; Schade *et al.*, 2012).

b. Juvenile Polyarteritis Syndrome

Etiology Juvenile polyarteritis syndrome (JPS), also known as steroid-responsive meningitis-arteritis, is a painful disorder seen in young beagles (occasionally reported in other breeds) caused by a systemic

necrotizing vasculitis. The cause of the vasculitis has not been established but appears to have an autoimmune-mediated component and may have a hereditary predisposition.

Clinical Signs Clinical signs of JPS include fever, anorexia, lethargy, and reluctance to move the head and neck. The dogs tend to have a hunched posture and/or an extended head and neck. Most dogs seem to be in pain when touched, especially in the neck region. Neurological examination may reveal proprioceptive deficits, paresis, or paralysis. The syndrome typically has a course of remissions and relapses characterized by 3–7 days of illness and 2–4 weeks of remission (Scott-Moncrieff *et al.*, 1992). There may be a component of this condition that is subclinical, given that vasculitis has been diagnosed postmortem in beagles that had no presenting signs.

Epizootiology and Transmission JPS typically affects young beagles (6–40 months), with no sex predilection. JPS has been reported in other breeds including sibling Welsh Springer Spaniels (Caswell and Nykamp, 2003).

Pathologic Findings On gross necropsy, foci of hemorrhage can be seen in the coronary grooves of the heart, cranial mediastinum, and cervical spinal cord meninges (Snyder *et al.*, 1995). Local lymph nodes may be enlarged and hemorrhagic. Histologically, necrotizing vasculitis and perivasculitis of small to medium-sized arteries are seen. These lesions are most noticeable where gross lesions are observed, but they may be seen in other visceral locations. Arterial fibrinoid necrosis leading to thyroid gland hemorrhage and inflammation was also reported (Peace *et al.*, 2001). The perivasculitis often results in nodules of inflammatory cells that eccentrically surround the arteries. The cellular composition of these nodules is predominantly neutrophils, but it can also consist of lymphocytes, plasma cells, or macrophages (Snyder *et al.*, 1995). Fibrinous thrombosis of the affected arteries is also seen. A subclinical vasculitis has also been diagnosed in beagles post mortem; it is not known whether this subclinical condition is a different disorder or part of a JPS continuum. This subclinical vasculitis often affects the coronary arteries (with or without other sites).

Pathogenesis The initiating factors for JPS are unknown. It was once presumed to be a reaction to test compounds by laboratory beagles, but this may have been coincident to the fact that the beagle is the breed most often affected with JPS. Immune mediation of JPS is strongly suspected, because the clinical signs have a cyclic nature and respond to treatment with corticosteroids, and the affected dogs have elevated α_2 -globulin fractions and abnormal immunologic responses. There may be hereditary predisposition, given that pedigree analysis has indicated that the offspring of certain sires

are more likely to be affected, and breeding of two affected dogs resulted in one in seven affected pups (Scott-Moncrieff *et al.*, 1992).

Diagnosis and Differential Diagnosis Differential diagnoses include encephalitis, meningitis, injury or degeneration of the cervical vertebrae or disks, and arthritis. In the research facility, the disorder may be readily confused with complications secondary to the experimental procedure, or with postsurgical pain. Beagles with JPS that were in an orthopedic research study were evaluated for postsurgical complications and skeletal abnormalities prior to the postmortem diagnosis of systemic vasculitis (authors' personal experience).

Prevention and Control No prevention and control measures are known at this time.

Treatment Clinical signs can be abated by administration of corticosteroids. Prednisone administered orally at 1.1 mg/kg, q12h, was associated with rapid relief of clinical symptoms. Maintenance of treatment at an alternate-day regimen of 0.25–0.5 mg/kg was shown to relieve symptoms for several months. However, withdrawal of corticosteroid therapy led to the return of clinical illness within weeks.

Research Complications Because of the potentially severe clinical signs and the need for immunosuppressive treatment, JPS is often incompatible with use of the animal as a research subject. It is unknown whether subclinical necrotizing vasculitis causes sufficient aberrations to measurably alter immunologic responses.

3. Other Miscellaneous Diseases

a. Interdigital Cysts

Etiology Interdigital cysts are chronic inflammatory lesions (not true cysts) that develop in the webbing between the toes. The cause for most interdigital cysts is usually not identified unless a foreign body is present. Bacteria may be isolated from the site, but the lesions may also be sterile (hence the synonym 'sterile pyogranuloma complex').

Clinical Signs Dogs with interdigital cysts are usually lame on the affected foot, with licking and chewing at the interdigital space. Exudation may be noticed at the site of the lesion. The lesion appears as a cutaneous ulcer, usually beneath matted hair, with possible development of sinus tracts and purulent exudate.

Epizootiology and Transmission Interdigital cysts are common in a variety of canine breeds, including German shepherds. Beagles have been affected in the research setting. Interdigital cysts usually occur in the third and fourth interdigital spaces (Bellah, 1993). A retrospective study of beagles housed in a research industry setting, linked development of interdigital cysts to body condition score, age, location of cyst, and type of caging, and may result from chronic interdigital dermatitis (Kovacs *et al.*, 2005).

Pathologic Findings Histopathologically, interdigital cysts are sites of chronic inflammation, typically described as pyogranulomatous.

Pathogenesis Initial development of the cysts is unknown, except for those cases in which a foreign body can be identified.

Diagnosis and Differential Diagnosis Bacterial culture swabs and radiographs should be taken of the cysts to rule out bacterial infection and radiopaque foreign bodies or bony lesions, respectively. A biopsy should be taken if neoplasia is suspected.

Treatment If a foreign body is associated with the lesion, then removal is the first order of treatment. If biopsy of the site provides a diagnosis of sterile pyogranuloma complex, then systemic corticosteroid therapy (e.g., prednisolone at 1 mg/kg q12h) can be initiated and then tapered once the lesion heals. Interdigital cysts that are refractory to medical therapy require surgical removal. Excision includes the removal of the lesion and the interdigital web, and a two-layer closure of the adjacent skin and soft tissues is recommended (Bellah, 1993). The foot should be put in a padded bandage and a tape hobble placed around the toes to reduce tension when the foot is weight-bearing. The prognosis for idiopathic interdigital cysts is guarded, because the cysts tend to recur (Bellah, 1993).

Research Complications Research complications from the cysts are minimal, unless the dogs need to be weight-bearing for biomechanical or orthopedic studies. Treatment with systemic steroids could be contraindicated with some experimental designs.

b. Hyperplasia of the Gland of the Nictitating Membrane

Etiology 'Cherry eye' is a commonly used slang term for hyperplasia and/or prolapse of the gland of the nictitating membrane (third eyelid). This is not considered a congenital anomaly, but there is breed disposition for this condition, including beagles. A specific etiology is not known.

Clinical Signs The glandular tissue of the nictitating membrane protrudes beyond the membrane's edge and appears as a reddish mass in the ventromedial aspect of the orbit. Excessive tearing to mucoid discharge can result, and severe cases can be associated with corneal erosion.

Pathologic Findings Typically, the glandular tissue is hyperplastic, possibly with inflammation. Rarely is the tissue neoplastic.

Pathogenesis Prolapse of the gland may be a result of a congenital weakness of the connective tissue band between the gland and the cartilage of the third eyelid (Helper, 1989).

Prevention Hyperplasia of the third eyelid cannot be prevented, but dogs that develop this condition

unilaterally should have the other eye evaluated for potential glandular prolapse. Preventative surgical measures might be warranted.

Treatment Corticosteroid treatment (topical or systemic) can be used to try to reduce the glandular swelling. However, surgical reduction or excision of the affected gland is typically required to resolve the condition. In the reduction procedure, the prolapsed gland is sutured to fibrous tissue deep to the fornix of the conjunctiva (Helper, 1989). If reduction is not possible (as with deformed nictitating cartilage) or is unsuccessful, removal of the gland can be performed. Such excision is fairly straightforward and can be done without removal of the nictitating membrane itself. The gland of the third eyelid is important in tear production; although the rest of the lacrimal glands should be sufficient for adequate tear production, keratoconjunctivitis sicca is a possible consequence after removal of the gland of the nictitating membrane.

Research Complications In most cases, research complications would be minimal, especially if treated adequately. Either the presence of the hyperplastic gland or its removal might compromise ophthalmologic studies.

Acknowledgments

The authors thank Dr. Bambi Jasmin of Marshall Farms for contributions on preventive health practices for Class A dogs.

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