Section IV: Preventive Medicine and Public Health Services

A US Army veterinarian from the 490th Civil Affairs Battalion Functional Specialty Team and a community animal health worker (second from left) work together to treat a young camel during an 8-day Veterinary Civic Action Program in Negele, Ethiopia, August 23, 2011. Using deployed US veterinary personnel helps develop the host nation's surveillance programs and laboratory capacity, which is not only critical to global zoonotic disease control and surveillance and preventive medicine programs, but also supports the concepts of One Health and nation-building.

Photograph: by US Air Force Captain Jennifer Pearson. Reproduced from: https://www.army.mil/article/65682/helping_an_ethiopian_community_survive_severe_drought. Accessed April 26, 2018.

Chapter 11

ZOONOTIC AND ANIMAL DISEASES OF MILITARY IMPORTANCE

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INTRODUCTION

The US military veterinarian is responsible for ensuring not only the health of the Department of Defense (DoD) private and military-owned animals but also the health of US service members and their family members. Although these two populations are distinctly different, they are closely linked, especially regarding infectious diseases. Globally, zoonoses account for over 60% of known human pathogens, and 75% of emerging or reemerging infectious diseases are zoonotic.[1]

The majority of military important diseases are also zoonotic. The *US DoD Tri-Service Reportable Events: Guidelines and Case Definitions* lists specific notifiable diseases that have clear case definitions and pose an "inherent, significant threat to public health and military operations."[2(p7)] Sixty-three of the 67 listed case definitions are of an infectious nature, and of these, 33 (52%) are true zoonoses in which animals either serve as the primary reservoir of the disease or as an important host or vector for the disease. In an additional seven other reportable diseases (eg, yellow fever), animals serve as incidental hosts or reservoirs. Taken together, zoonoses account for 63% of all infectious disease reportable medical events within the DoD. This number is even higher when some diseases that affect only humans but are theorized to have a zoonotic origin (eg, acquired immune deficiency syndrome or AIDS) are considered.[3]

Zoonoses in Service Members

Disease and nonbattle injury (DNBI) has historically been a significant source of mortality within the US military.[4] Prior to World War II, more US service members died from DNBI than from combat. During World War II, DNBI remained problematic, causing General Douglas MacArthur to famously lament: "This will be a long war if for every division I have facing the enemy I must count on a second division in hospital with malaria and a third division convalescing from this debilitating disease!"[5(p2)] Although advances in preventive medicine and treatment have helped to reduce the impact of DNBI on mortality, its effects are still felt, especially in terms of overall morbidity where DNBI still accounts for more lost duty days than battle injuries. Zoonoses such as *Brucella melitensis* and rabies continue to be associated with recent military operations in Afghanistan and Iraq, and others such as leishmaniasis have resulted in large outbreaks among deployed service members.[6–8]

Knowledge of local zoonotic diseases is necessary for early identification of these agents and appropriate treatment of infected patients. A retrospective serum study of hospitalized military personnel in Iraq found 10% of the sampled service members seroconverted to *Coxiella burnetii*, the causative agent of Q fever, as a result of their deployment to Iraq, suggesting a need to increase animal disease awareness among deployed physicians.[9] Awareness of local animal zoonoses is even more important for diseases that have long incubation periods or lack treatment options (eg, echinococcosis and bovine spongiform encephalopathy). Ignorance of the local zoonoses unnecessarily places service members at risk of infection through a failure to enact proper preventive measures. When infected with these diseases, service members may not develop symptoms until after they have redeployed or left military service, leading to potential misdiagnosis, improper case management, and development of long-term sequelae or death.

Knowledge of infectious diseases is also vital to maintaining military readiness. For centuries militaries have actively used biological agents during combat and have studied numerous other organisms as potential weapons, many of which are zoonotic. In fact, a 1997 report determined that 80% of the most likely biowarfare pathogens were zoonoses.[10]

One of the earliest examples of biowarfare involved the zoonotic organism *Yersina pestis*, the causative agent of plague. In 1346, near the end of a failed siege of Caffa, attacking Tatar forces catapulted plague-infected corpses over the city walls, which presumably triggered a bubonic plague epidemic among the defenders.[11] Centuries later, in 1710, Russian troops reportedly repeated this tactic against Swedish military forces.[12] During World War II, plague was again employed as a bioweapon, this time by the Japanese army in China where plague infected-flea vectors were examined as potential weapons against US troops.[13]

In the Cold War, the US and Soviet Union militaries both maintained substantial chemical and biological weapons programs. Although the US officially renounced the use of biological agents as offensive weapons in 1969 under President Richard M. Nixon, the United States continued defensive research, largely in response to the Soviet Union's ongoing bioweapons program.[14] Even with the end of the Cold War and the eventual scrapping of the Soviet Union's bioweapons program, biological threats persist (eg, the 2001 attacks on the US mail system with another common zoonotic agent, *Bacillus anthracis* or anthrax).[15] Biological threats throughout history are further discussed in Chapter 15, Veterinary Pathology.

Diseases in Military Animals

Infectious diseases are also of military concern even when only animals are infected. Up until the first part of the 20th century, many armies, including the US Army, relied heavily on horses, mules, and other animals for transportation, logistics, and combat. Outbreaks among these service animals had the same potential to negatively impact the operating force as outbreaks among soldiers themselves.

Consequently, just like their human counterparts, animals became biowarfare targets. In World War I, German agents reportedly used *B anthracis* and *Burkholderia mallei* to attack Allied equine supplies in Argentina, Spain, and the United States which subsequently affected military operations.[16,17] In a separate but related incident, German-sponsored saboteurs unsuccessfully attempted to attack Norwegian pack-reindeer with anthrax-laced sugar cubes to disrupt communication and transportation. Because of these historic incidents, during the Cold War, the United States maintained a defensive bioweapons program for animals at Plum Island, New York, similar to the human programs at Ft Detrick, Maryland, and other locations.[18]

The armies of today are much more mechanized and less reliant on animals for logistics; however, animals are still employed for military operations and therefore still affect human disease exposure, perhaps even more so. Today, military working animals (MWAs) are used by all five of the US armed services: (1) Army, (2) Navy, (3) Air Force, (4) Marines, and (5) Coast Guard. Military working dogs (MWDs), marine mammals, and solipeds (eg, horses and mules) routinely accomplish a wide variety of missions, including drug interdiction, mine detection, special operations, and general force health protection. Failing to prevent infections and maintain MWA health can detrimentally affect not only individual animals but also attending handlers, units, and entire missions.

Often the infectious agents that threaten MWA health are the same agents that threaten human health. A retrospective analysis of predeployment and postdeployment sera from US MWDs deployed to the Republic of Vietnam reported that a significant percentage of the dogs demonstrated seroconversion to Group B arboviruses, meliodosis (ie, *Burkholderia psuedomallei)*, and scrub typhus (ie, *Orientia tsutsugamushi*) as a result of their deployment.[19] A lesser percentage of the MWDs also demonstrated seroconversion against leptospirosis, plague, and *Rickettsia canada*. Another retrospective serum study examining French MWDs found that 9.7% of the dogs were seropositive for Q fever exposure, with those living close to sheep having a 6-fold increased risk of being seropositive.[20]

ZOONOSES OF IMPORTANCE IN THE VETERINARY TREATMENT FACILITY

Animals are the primary source of infection for several human diseases of military importance, including anthrax, brucellosis, hanta viruses, leptospirosis, Q fever, rabies, toxoplasmosis, tularemia, and viral hemorrhagic fevers (*Arenaviridae*, *Bunyaviridae*, and *Flaviridae*).[21] In several other military important diseases, infected animals generally pose little risk of directly infecting humans but may serve as important diseases reservoirs for arthropods and vector-borne disease transmission. Military important arthropod-borne zoonoses include the equine encephalitides (Eastern, Western, and Venezuelan encephalitis), ehrlichiosis, Japanese encephalitis, leishmaniasis, Lyme disease, plague, tick-borne encephalitis, trypanosomiasis, and typhus.[22]

Zoonoses of military importance and the associated diseases in humans are identified and comprehensively described in the *Military Preventive Medicine: Mobilization and Deployment, Volume 2* textbook.[23] This chapter's overview of some of these same diseases is more limited, given this textbook's military veterinary medicine point of view:

- Focus is on veterinary medicine applications and the pathogenesis and clinical features, diagnostic approaches, and recommendations for therapy, prevention, and control in animals. When human recommendations are presented, the focus is on how humans, including deployed veterinarians, can limit the spread of infections and disease through current therapy, prevention, and control methods.
- The overview is limited to those pathogens and disease carriers military veterinarians are most likely to see or treat when working with government, military, and privately owned animals on military installations. In other words, although diseases such as Japanese encephalitis have zoonotic reservoirs (eg, swine) and can produce serious or potentially fatal outcomes, swine are not typically seen at US military veterinary treatment facilities, stables (eg, Morale, Welfare and Recreation program facilities), or on military installations in

deployment areas; thus, Japanese encephalitis is not covered in this chapter. Lyme disease is covered in this chapter because military veterinarians may still see the disease among government, military, or privately owned animals, even though military veterinarians do not work with the reservoir population for this disease (ie, wild rodents).
- Rabies is not presented in this chapter because a thorough overview of this disease can be found in Chapter 12 of this textbook.

Since all diseases covered in this chapter appear in alphabetical order, readers should not infer importance by order of appearance.

Anthrax

Introduction and Military Importance

Anthrax, a disease of domesticated animals, wild animals, and humans caused by *B anthracis*, is especially pathogenic in most herbivorous animals (ie, goats, sheep, and cattle) and humans.[24] The clinical signs of disease vary with the species of animal infected and route of exposure.[24,25] The most lethal form of disease is inhalation of anthrax spores, with death occurring within a few days if clinicians fail to recognize the etiology and treat appropriately.[26]

Army veterinarians are concerned with anthrax because of its potential use as a biological weapon and the possibility of human and animal exposure from domestic and wild animals in places with inadequate anthrax eradication and control programs. The 2001 mail incident in which 22 US citizens were infected and 5 died of anthrax, despite treatment after exposure to contaminated letters, reveals the significant threat anthrax poses as a potential biological weapon.[27] Additionally, Army veterinarians, other service members, civilians, and MWAs deployed in Afghanistan, Iraq, and other foreign humanitarian assistance missions throughout the world face the risk of anthrax exposure from wild animals and unvaccinated livestock.

Description of the Pathogen

B anthracis is a large, Gram-positive bacillus measuring 1 to 1.5 by 3 to 10 μm.[24,25,28,29] The bacillus itself is virtually nontoxic; however, the organism produces an array of toxins that can kill animals, even after the organism's death from antibiotics.[24] The main toxins produced by *B anthracis* are three complementary components designated as lethal factor, edema factor, and protective antigen; factors I, II, III, respectively.[24–26,28–31] Lethal factor and edema factor bind to the protective antigen to form the two exotoxins, lethal toxin and edema toxin.[24,25,28] The combined effects of these toxins are impaired function of phagocytes, decreased coagulation, increased capillary permeability, and impairment of the complement system.[24,28,29,31]

The organism's vegetative form (ie, the one producing active disease) survives only a short time, which correlates with the short duration of animal and human infection. In carcasses, these vegetative forms are rapidly killed by putrefactive bacteria.[24,29,32] But, in live animals, vegetative forms (ie, antemortem bacilli) are expelled in all natural excretions and pathological exudates.[26,33,34] When exposed to oxygen, these expelled bacilli form spores that are very stable and can survive in the environment for decades. These dormant spores have minimal capacity for growth in the external environment, except under unique conditions of alkaline soil, abundant organic debris, intermittent periods of drought and rain, and constant temperatures above 15.5 °C.[26,34]

Epidemiology

Transmission. There is no definitive consensus on how animals acquire anthrax infection; however, most animals allegedly become ill after ingesting *B anthracis*. Herbivores (ie, goats, sheep, and cattle) ingest contaminated food and water; the bacteria likely enters the body through traumatized mucous membranes. Herbivores can also inhale spore-laden dust, although this route of infection is uncommon. Cutaneous infection in herbivores is also rare. In horses, anthrax infection is likely acquired from ingesting contaminated vegetation, but infection may also stem from blood-sucking insects. Dog and pigs may acquire anthrax by eating from an anthrax-infected carcass or ingesting the inadequately cooked meat of anthrax-infected animals.[24]

Humans contract various types of anthrax via many means, most commonly through the handling of spore-laden carcasses, hides, wool, hair, and bones. Such handling usually causes exposed body parts to become infected (eg, hands, arms, and neck) and develop cutaneous anthrax.[28,31,32] Humans, like animals, can also contract ingestional forms of anthrax by eating poorly cooked, contaminated meat.[32] But humans rarely develop deadly inhalation anthrax by inhaling anthrax spores. Inhalation infection usually occurs only in people who handle contaminated animal products (eg, wool and hides) in an enclosed space or people who are exposed to aerosolized anthrax spores in the form of a biological weapon.[28,31,32]

Geographic Distribution. Anthrax spores have been found worldwide, including the Americas, Europe, Africa, and Asia.[24,25,32–34] Spores have even been discovered in such extreme climates as Antarctica.[35] In countries with effective public health services and veterinary management, the incidence of anthrax is very low due to effective eradication programs and herd health management.[24,32–34] Anthrax exposure is always possible in countries without effective public health and veterinary management, especially in environments with temperatures above 15°C and a prolonged drought.[24]

Incidence. Within the United States and other developed nations, anthrax infection is exceedingly rare in domesticated and companion animals.[24,29] However, sporadic outbreaks do occur in some developed nations' wild animal populations.[32,34] During the 1990s, an average of one natural case of cutaneous anthrax a year occurred in humans within the United States,[32] and as mentioned previously in this chapter, anthrax spores have been used as a lethal biological weapon against US citizens.[27,30] This incident shows the dangers anthrax poses as a biological weapon.

In developing countries, accurately determining the exact incidence of anthrax is impossible because of insufficient reliable data. Army veterinarians deployed to areas without anthrax eradication programs should be vigilant about the possible presence of *B anthracis* in the environment, livestock, and wild animal populations.

Pathogenesis and Clinical Findings

Anthrax pathogenesis depends on the primary route of infection: cutaneous, ingestion (ie, gastrointestinal or GI), or inhalation. Cutaneous anthrax, the most common form of disease in humans, is less common in animals and is named for the Greek word "anthrax," meaning "charcoal" or "coal."[28,30,31] Cutaneous anthrax develops when organisms enter a cut or abrasion in the skin, begin to multiply, and produce toxins within the infected skin, resulting in vesicle formation that becomes necrotic and blackens. Cutaneous anthrax usually resolves itself without medical treatment; however, antibiotic administration is recommended to prevent potential fatal septicemia.[28,30–32]

Gastrointestinal anthrax develops when anthrax organisms are ingested and is most common in carnivores and omnivores.[24,25] The toxins of the swallowed organism first cause local inflammation, necrosis, and edema in the upper GI tract (ie, oropharynx and esophagus) with swelling of the head and neck and resultant anorexia.[25] The bacteria travel to local lymph nodes via afferent lymph, and septicemia develops.[24,25,28,29] Bacteria then infect the lower GI tract, resulting in a hemorrhagic gastroenteritis and subsequently, dysentery, and diarrhea.[24,25,30-32] Anthrax can also spread from the stomach and intestines to the mesenteric lymph nodes, spleen, and liver, with concurrent or subsequent development of septicemia.

Figure 11-1. Ruminant: Marked congestion and enlargement of the spleen ("blackberry jam") with infection from *Bacillus anthracis*.
Photo courtesy of Bruce H. Williams, Doctor of Veterinary Medicine, Senior Pathologist, Department of Defense Joint Pathology Center, Silver Spring, Maryland.

Inhalation anthrax occurs when inhaled spores within alveolar lumina proliferate and are phagocytized by alveolar macrophages.[26,36,37] These macrophages travel to local lymph nodes (ie, tracheobronchial lymph nodes) where they continue to proliferate. Toxins produced by bacteria in the lungs and local lymph nodes cause massive pulmonary edema, pleural effusion, edema within the mediastinum, and necrohemorrhagic lymph nodes with resultant dyspnea, coughing, and fever.[24,25,28–32] From the lymph nodes, bacteria enter the blood and infect the spleen and other organs.

Septicemia may occur with all three routes of infection but is rarely reported with cutaneous infections.[24,25,28–32] Septicemia results when anthrax bacilli are present in large numbers within blood vessels. The three major toxins, factors I, II, and III, result in diffuse edema, hemorrhage, and congestion.[24,28,29,31,32] More specifically, parenchymatous organs swell and become congested; ecchymoses of mucous membranes, serosal surfaces, and subcutaneous tissues occur; and loose connective tissue (eg, adipose tissue) and body cavities may be filled with blood-tinged gelatinous fluid (Figure 11-1). The blood in septicemic animals will be thick, dark, and tarry and will either not clot or form soft friable clots that are easily separated.[24,29]

Often, especially with very susceptible species like ruminants, veterinarians will encounter animals that have died acutely from anthrax with few or no antemortem signs of disease. These animals show little or no rigor mortis; have dark, nonclotted blood oozing from the anus, mouth, and nasal cavity; and exhibit rapid decomposition, massive bloating, and a "saw horse" positioning of the legs. In endemic areas, Army veterinarians still consider anthrax on their differential diagnosis for acute death in animals, especially ruminants, because, as noted, even when indigenous, this disease is frequently asymptomatic.[26,31]

Diagnostic Approaches

Although anthrax can be diagnosed in many ways, a necropsy should never be performed on an infected animal because anthrax spores could be released into the environment.[24,25,29,32–34] The easiest method of field diagnosis is to collect a smear of the animal's blood or local exudate on a slide and stain the specimen with old methylene blue. *B anthracis* will stain pink and will usually be in pairs or short chains of three to four organisms with rounded free ends and square apposed ends.[24,25,26]

In people and animals with inhalation anthrax, nasal swab smears, pleural effusion smears, or pulmonary biopsy specimens also can be examined with direct fluorescent antibodies to *B anthracis* to confirm a diagnosis.[25] Polymerase chain reaction (PCR) can be used to confirm a diagnosis with sterile body fluids (ie, blood, abdominal effusions, and pleural effusions).[25,30,31] Serologic testing using enzyme-linked immunosorbent assay (ELISA) with a 4-fold increasing titer also might confirm a diagnosis; serologic testing is sensitive but not specific for anthrax.[24,25,30–32]

Because strict guidelines mandate the handling of shipments thought to contain *B anthracis*, veterinarians must alert shippers and laboratories when sending them such specimens.[33,34] If applicable, veterinarians also must notify public health authorities or the appropriate chain of command in theater of possible anthrax cases.

If a necropsy is inadvertently performed, extensive gelatinous edema is present within the mediastinum with inhalation anthrax or along the GI tract from the oral cavity to the rectum with GI anthrax.[24,25,28,29,32,36,37] Associated thoracic or GI lymph nodes are enlarged, edematous, and hemorrhagic. If the animals are septicemic, splenomegaly, an enlarged friable liver, and petechial hemorrhages on serosal surfaces are visible. If the brain is exposed, diffuse meningeal congestion, often referred to as a "cardinal's cap," is present.[28,30,31,36,37]

Recommendations for Therapy, Prevention, and Control

Therapy. Cephalosporin antibiotics and potentiated sulfonamides are ineffective against anthrax. The traditional treatment of choice for anthrax is penicillin G, but the treatment of a known attack with anthrax spores should be based on antibiotic sensitivity testing because weaponized spores may be resistant to traditional antibiotic treatments.[25,27,30–32] *B anthracis* is also susceptible to ciprofloxacin, enrofloxacin, doxycycline, and tetracycline. Initially, antibiotics should be administered intravenously (IV) to mitigate the potential for development of bacteremic or septicemic anthrax. Additional supportive care may be necessary to include fluid therapy, oxygen, vasopressors, and anti-inflammatory drugs.[25,30,32]

As noted earlier in this chapter, death from anthrax can occur despite effective killing of organisms by antimicrobials due to the persistence of bacterial toxins.[26,27,30,31,38] Antitoxins are being developed to combat these effects and will likely be utilized in the future in conjunction with antibiotics and supportive care to reduce the number of deaths incurred from anthrax exposure.[39] In all cases of anthrax exposure, IV antibiotics should be continued for 7 to 14 days and postexposure prophylaxis with oral antibiotics should be administered for at least 60 days to combat the possibility of environmental or pulmonary spore persistence.[25,27,30,31,38]

Cutaneous exposure to anthrax should be immediately treated by cleaning of the exposed area with soap and water.[25,32] A solution of 0 .5% sodium hypochlorite can be used initially to treat cutaneous exposures because of its effectiveness in killing spores, but caution is warranted because prolonged use is caustic to the skin.[25,30,31] IV antibiotics should also be used as well to prevent the development of bacteremia or septicemia. Any bandages or dressings should be treated as biohazardous waste and autoclaved or incinerated.[24,32]

Prevention. All service members on deployment should take basic precautions to avoid exposure to anthrax. First, service members should avoid all contact with animals, hides, or products made from hides.[30–34] Second, all meats acquired from local host country sources should be thoroughly cooked. Third, all service members deployed to endemic areas should be vaccinated with anthrax vaccine at 0, 2, and 4 weeks and 6, 12, and 18 months to provide immunity, and they should receive annual booster injections for maintenance.[25,30–34] Finally, military personnel responding to a suspected anthrax terrorist attack should wear proper protective clothing and a respirator.[25,27,32–34] While on deployment, MWD handlers should ensure their MWDs are protected from local animals and products made from local animals and feed their MWDs an approved diet.

Control. Samples from sick or dead animals suspected of being infected with anthrax should be tested to confirm the diagnosis; veterinarians or other trained personnel should wear necessary protective gear such as gloves and surgical masks when taking test samples to prevent accidental contamination (eg, unintentional contact with broken or exposed skin).[25,32] Animals that test positive should be incinerated, with remaining bones buried at least several feet deep; if incineration is not possible, animals should be buried at least 6 feet deep and covered with lime. All buildings and equipment exposed to infected animals should be cleaned and disinfected with 5% hypochlorite or 5% phenol.[25,33,34] Confirmed cases of anthrax should be reported to commanders and local public health agencies immediately. Decisions whether to implement anthrax eradication programs should be made by host nations, but commanders can implement controls for scavenging animals and insects.

Brucellosis

Introduction and Military Importance

Brucellosis, a zoonotic infection of wild and domesticated animals, is caused by one of several bacteria of the genus *Brucella*. Contact with contaminated animal tissues, inhalation of aerosolized bacteria, and ingestion of infected animal products such as unpasteurized milk cause human infection. Captain David Bruce, a Scottish pathologist and microbiologist, first isolated one causative agent of brucellosis in mammals, *B melitensis*. When Bruce sailed to Malta in 1857 to transport goats from Malta to the United States for the US government, all of his crew drank the local raw goat milk and became ill. Sailors stopped getting sick only after Malta's naval station imposed a moratorium on goat's milk. Disease associated with *B abortus,* another causative agent, was first recognized in the United States in a US Army officer who contracted the disease in Puerto Rico.[40]

The military importance of brucellosis is severalfold. First, it can cause an acute food-borne illness outbreak, especially when service members eat and drink products from locales that lack sanitary food systems. If many troops are exposed to contaminated food or liquids, an entire military unit and its mission can be impacted. Next, because the chronic complications of this disease are most commonly osteoarticular in nature, affected service members frequently lose duty time and may have to be medically discharged. Finally, the acute and chronic manifestations in combination with the highly infective nature of *Brucella* organisms and their ease of transmission via aerosol make them ideal bioterrorism agents.[41]

Description of the Pathogen

Brucella organisms are facultative, Gram-negative coccobacilli. Six species of *Brucella* that infect terrestrial mammals are traditionally classified as *B abortus, B canis, B melitensis, B neotomae, B ovis,* and *B suis.* Due to the homology between the species, there is an argument that the species should all be classified as *B melitensis* with the current species classified as biovars.[42] This discussion will maintain the traditional naming nomenclature of separate species. These species are broken down into two distinct types based on the presence of smooth lipopolysaccharide (LPS) on the outer cell membrane or a rough LPS. Smooth LPS species are zoonotic.

Each species contains a number of biovars; of the four zoonotic species, *B abortus* has seven biovars, *B melitensis* has three, *B suis* has five, and *B canis* has the one biovar that is defined by the species classification. These biovars have differing zoonotic potentials. Among the nonzoonotic species, *B neomotae* and *B ovis* are both made up of only one biovar each.[42]

Marine mammals have a distinct *Brucella* species that is classified in two ways. First, the species can be called *B maris* with two biovars based on the species

they infect. The other naming methodology identifies two species based on the animal reservoir: *B pinnipediae* and *B cetaceae*.[43]

The three species of *Brucella* that cause human disease in decreasing order of virulence are *B melitensis*, *B suis*, and *B abortus. B canis* can cause human infections but rarely causes disease. When cultured, species and biovars can be distinguished using biochemical tests.[44]

The focus of this chapter is the terrestrial *Brucella* species (ie, land animals).

Epidemiology

Transmission. Brucellosis is a pure zoonosis—meaning it can be transmitted from animals to humans with virtually no human to human transmission. Animals spread disease among themselves horizontally and vertically (ie, from mother to offspring) by direct and indirect contact. For example, suckling lambs can sometimes be infected while nursing an infected dam.[44] More commonly, the disease is spread by ingestion of grass or other feed contaminated by an infected animals' vaginal discharge, aborted material, or postparturient discharge. Sexual transmission is also possible and is most commonly seen with *B suis*, *B canis*, and *B ovis*.[42,44]

There are three primary routes of transmission of brucellosis to humans from infected animals: (1) through ingestion of contaminated dairy products, (2) direct contact, and (3) aerosolization. Despite the fact that many mammals carry *Brucella* species, hares, reindeer, and horses are not typically implicated in zoonotic transmission. Usually sheep, goats, cattle, and swine, and, rarely, dogs are implicated in human disease transmission.[40,45,46]

In general, *Brucella* species are primarily associated with their natural host mammalian species, but *B melitensis* and *B suis* infections can become established in cattle as well.[42] Also, camels can be infected with and shed *B melitensis* and *B abortus*.[40]

Geographic Distribution. The most widespread species, *B suis*, appears in many countries throughout the world; *B melitensis* is documented throughout Spain, much of Eastern Europe, effectively the entire Asian continent, and parts of South America; and *B abortus* is found in North America, South America, sub-Saharan Africa, south and southeastern Asia, and parts of Europe. Although *B canis* is also found throughout the world, especially in the Americas, Asia, Europe, and Africa, this species appears to be absent from Australia and New Zealand. The nonzoonotic species, *B ovis*, is found in most areas of the world that raise sheep, including New Zealand, Australia, the Americas, Central Asia, Russia, and Europe.[47]

Pathogenesis and Clinical Findings

Infection with zoonotic *Brucella* species is nearly always through intact mucous membranes, following exposure to feed or water contaminated with aborted materials. Although human infection from animal milk products is common, dam-to-progeny transmission via milk is not viewed as an important mechanism of transmission, even though this means may contribute to the spread of *B melitensis* among small ruminants.[42]

Pathogenic *Brucella* species of ruminants (*B melitensis* and *B abortus*) are intracellular organisms that evade the lysosomal activity of infected macrophages residing in lymph nodes or bone marrow, causing little to no pathology until pregnancy occurs. During times of stress, particularly pregnancy, *Brucella* organisms are able to lyse the macrophages by unknown mechanisms. This recurrent cell lysis causes febrile states in infected animals and individuals.[48]

Although these organisms can colonize anywhere in the body, they favor the reproductive tract and joint capsules. Specifically, *Brucella* species have a strong predilection for the ruminant placenta in naïvely infected animals and the udder in recurrently infected animals. The site of replication in the placenta is primarily at the cotyledons, which has been attributed to the unusual local production of erythritol. *Brucella* species proliferate wildly in the placenta, causing inflammation and necrosis of the cotyledons, resulting in fetal stress and abortion.[49] In aberrant hosts, such as humans, granulomas also form at the site of colonization.[50]

Adult animals in endemic areas may appear clinically normal. For acute infections, however, *Brucella* species can cause orchitis, epididymitis, testicular abscessation, and balanaposthitis in rams, boars, canine species, and bulls.[40] Among naïvely infected females, abortion is the more frequently observed manifestation, most frequently in late gestation. Aborted fetuses due to *B melitensis*, *B suis*, and *B abortus* are frequently intact but sometimes autolyzed. Abortions caused by *B ovis* are exceptionally rare, but mummified fetuses are sometimes reported. Placentae generally have fairly severe cotyledonary edema and necrosis and may have intercotyledonary inflammation, often described as "leathery thickening." Placentitis and abortion may also occur in pigs but usually earlier in gestation. Brucellosis also has been reported as a cause of fistulous withers and "poll evil" in horses.[51–54]

Diagnostic Approaches

For several reasons, definitive diagnosis using serology is exceptionally challenging and requires a high degree of technical expertise.[55] First, animals remain

seropositive for life, with a cross-reactivity between *B abortus* and *B melitensis* antibody. Next, antibody in smooth-phase and zoonotic species, such as *B melitensis*, *B abortus*, and *B suis*, is primarily generated against an O-chain polysaccharide on the LPS. All smooth strain species have this *O*-chain, but rough strain (ie, nonzoonotic) species, do not contain this *O*-chain on their LPS. The vaccine strains, strain 19 and Rev1 for *B abortus* and *B melitensis*, respectively, are both smooth strains. Thus, antibody induced by immunization with smooth-phase vaccines cannot easily be differentiated from antibodies produced by natural infection. RB51, a rough-phase *B abortus* strain, was developed to address this problem during the latter phase of the US brucellosis eradication program. False-positive serological reactions may also result from exposure to *Yersinia entercolitica* and some other similarly related Gram-negative organisms.[56]

Even when laboratory tests are performed correctly and results are determined accurately, astute judgment is required to interpret results and then the policy to take legal action such as quarantine or culling needs to be codified. With no firmly established international protocol for this process, individual government authorities must determine how to screen animals and what tests to use for definitive determination of individual animals or herds as "reactors." Guidance for establishing protocols is available in the terrestrial manual from the World Organization for Animal Health (OIE).[57]

Although *B melitensis* is the more serious animal and public health threat in most of the world, the US brucellosis eradication program has traditionally focused on *B abortus* because *B melitensis* has never been endemic in US livestock. The commonly used diagnostic tests and laboratory methods presented below are primarily used for *B abortus* diagnosis in cattle; they are generally applicable in sheep and goats using the same reagents but do require some procedural modifications. In other species, serosurveillance is generally not practiced.[57]

Screening Tests. Numerous screening tests approved for use in international trade by the OIE rely on antibody presence. Two common test types are (1) the buffered serum agglutination tests such as the rose bengal test (RBT) and the buffered plate antigen test and (2) the milk ring test. Both types of tests are excellent for screening and technically simple, but the milk ring test, which is useful in cattle, is ineffective in sheep and goats.[57]

For the RBT, the antigen is whole, killed *B abortus* cells conjugated to rose bengal, a purple-colored dye. Use of the RBT with sheep or goat serum for diagnosis of *B melitensis* in cattle requires individual laboratory validation and a slightly modified testing method, often referred to as the modified RBT. The buffered plate antigen test uses killed whole cells conjugated with brilliant green and crystal violet.[55]

The MRT allows testing of multiple animals at one time. The testing sample volume is increased as the herd increases. The antigen used is a whole, killed *B abortus* cell that is conjugated to haematoxylin stain.[57]

Traditional, unbuffered serum agglutination tests, including the tube or slide agglutination tests, do not meet international trade standards and are not required by either the OIE or the World Trade Organization. Still, these traditional tests are used by veterinarians in many national and local control programs. However, because these tests use killed whole cells and require subjective determination of agglutination, their utility highly depends on technical expertise.

Since all screening tests capture only animals that test positive, these tests have a higher rate of false-positives than more specific tests. A positive result from any screening test must be followed by a positive result on a confirmatory test to declare the animal positive.[58]

Confirmatory Tests. Confirmatory tests are used to validate screening test results. If both results are positive, then the sample is deemed positive, though consequent legal action (eg, indemnification) generally requires further confirmation such as a positive culture. Numerous tests, including serologic tests, can be used as confirmatory tests and are prescribed by the OIE for international trade.

The complement fixation test and the fluorescence polarization assay are two such confirmatory serologic tests (ie, tests detecting antibody presence in animal samples). The complement fixation test is a well-validated, universally accepted but technically challenging test requiring extensive laboratory support. The fluorescence polarization assay is simpler to run, using either a plate or a tube format or with specialized equipment in the field. This assay is also well validated and prescribed for international trade but is not widely used yet.

Many ELISA tests are also available for brucellosis testing. When run within OIE specifications, ELISA tests are well validated and considered to be confirmatory tests. In addition, the competitive ELISA or cELISA is identified as being able to differentiate vaccinated cattle (given S19 vaccine) from naturally exposed cattle based on titers.[55]

Antigen and Deoxyribonucleic Acid-Based Diagnostics. Blood culture of infected but apparently healthy adult animals is generally unrewarding because bacteremia is rarely pronounced (ie, the organism concentration in the blood is generally below detection limits) at the first signs of disease, often abortion, and is usually brief. Culture is still possible

following abortions if the aborting dam's fetal tissues (eg, abomasal contents, lung, liver, and spleen), abortive tissues (eg, placental cotyledons), milk, or vaginal swabs are used. Culturing these samples is relatively straightforward with appropriate selective media (eg, Casteñada's media, Farrell's media, and Thayer-Martin media) and a CO_2 incubator.[57]

However, because laboratory-acquired infection from cultures is a serious hazard and safer PCR tests can now do what only cultures could before, PCR is probably the better option. Numerous PCR assays (eg, BRUCE-ladder) are currently available for genus identification of *Brucella* that can distinguish between the species *B abortus, B melitensis, B ovis,* and *B suis*.[55,59]

Recommendations for Therapy and Control

Treatment is cost-prohibitive in animals because the disease commonly recrudesces, and acute disease is rarely recognized. Typical control programs are exclusion, movement control, vaccination, and test and slaughter. Exclusion requires that replacement animals are disease-free prior to being introduced to the herd; movement control limits the sharing of grazing land among herds and flocks of unknown disease status; vaccination is used for *B abortus* and *B melitensis*; and test and slaughter programs ensure seropositive adult animals are slaughtered to reduce overall disease prevalence. (Vaccination is effective but will not eradicate disease and does little once the prevalence falls below 2%. At this point, test and slaughter and disease-free replacements are used to maintain a disease-free flock or herd.[60,61]) Because many *Brucella* vaccines are available, only the most commonly used are overviewed in the last paragraphs of this section below.

Cattle vaccines include the S19 and RB51, with the latter replacing the former in many countries. The United States has converted to only using the RB51 vaccine on its replacement and heifer stock without any noticeable limitations to its effectiveness, though it is not clear if this is due to equivalent efficacy with the S19 vaccine or the United States practice of slaughtering all brucellosis-positive cattle to prevent disease spread. The RB51 vaccine is based on a rough strain mutant of *B abortus* and does not interfere with testing. On the other hand, S19 vaccination (ie, short-term with reduced doses) causes an immune reaction and antibody production that interferes with serologic testing, which can have an impact on prevalence studies and international trade.[62] In small ruminants such as goats or sheep, the Rev 1 strain vaccine is used to increase herd immunity against *B melitensis*. Rev 1 also interferes with serologic testing.[56] Vaccinations are approximately 60% to 70% effective but can cause abortion in pregnant animals.[57,61]

Equine Encephalitides

Introduction and Military Importance

Although the first-recorded epidemic of equine encephalitis occurred in the 1830s, it was not until 100 years later, in the 1930s, that three distinct but antigenically related virus complexes were recovered from horses with severe equine encephalitis[63]; the (1) western equine encephalitis virus complex (WEEV) was isolated in the San Joaquin Valley in California in 1930[64]; (2) eastern equine encephalitis virus (EEEV), in Virginia and New Jersey in 1933[65-67]; and (3) Venezuelan equine encephalitis virus complex (VEEV), in Venezuela in 1938. These three viruses, typically known as the equine encephalomyelitis viruses, are members of the genus *Alphavirus*.[68]

Natural infections with these three viruses are acquired by the bite of an infected mosquito. All these viruses cause similar clinical syndromes in horses; however, human disease manifestations vary by virus complex. For example, only about 4% to 5% of human EEEV infections result in encephalitis, but it is the most severe of the alphavirus encephalitides; case fatality rates range from 30% to 70%, with severe neurologic sequelae in those that survive.[69] Infection with WEEV results in encephalitis less often; case fatality rates in epidemics range from 8% to 15%.[70,71] VEEV epidemics are explosive, often resulting in thousands of cases, but it is the least neuroinvasive of the encephalitic alphaviruses. The vast majority of these human cases present as undifferentiated "flu-like" illness, with less than 1% of adults and 4% of children developing encephalitis.[72]

Alphaviruses are also highly infectious by aerosol. In fact, EEEV, VEEV, and WEEV possess many of the required characteristics for strategic or tactical weapon development, including ease of large-scale production, virus stability, potential for aerosolization, and virulence.[71] VEEV is of particular concern because it produces overt disease in nearly all human infections and can produce a self-sustaining natural outbreak. For these reasons, the encephalitic viruses are listed as Category B priority agents by the National Institute of Allergies and Infectious Diseases and the Centers for Disease Control and Prevention (CDC).[73] (See also Chapter 15, Veterinary Pathology, for more information on the biologic agents and toxins with the potential to endanger public health.)

Description of the Pathogen

Alphaviruses are single-stranded, enveloped, positive-sense ribonucleic acid (RNA) viruses that belong to the Togaviridae family. Currently, 28 virus

species are in the *Alphavirus* genus, which can be classified into at least seven groups based on antigenic complex homology. The EEEV complex is divided into four distinct lineages, I through IV, which differ in geographic, epidemiologic, phylogenetic, and pathogenic characteristics.[74,75]

Group I is composed of the strains enzootic along the eastern seaboard and Gulf Coast of North America and the Caribbean. The strains in this group are highly conserved, monophyletic, and temporally related and are responsible for the majority of human cases, with significant mortality rates in humans and equines. Groups II, III, and IV are composed of the strains enzootic in Central America and South America. The strains in these groups are highly divergent, polyphyletic, cocirculating, geographically associated, and primarily result in equine disease.[74]

The VEEV complex consists of six closely related subtypes that differ in respect to ecology, epidemiology, and virulence for humans and equines. Subtypes IA/B and IC are known as the epizootic strains and are responsible for large-scale epidemics in North, Central, and South America. Subtypes ID, IE, and IF are the enzootic strains, which may cause disease in humans but lack virulence for equines.[71,76]

The WEEV complex includes four viruses that differ in their ecology and virulence: WEEV, Highlands J virus, Ft Morgan virus, and Aura virus. Only WEEV causes disease in humans.[77]

Epidemiology

Geographic Distribution. Although the alphaviruses have worldwide geographic distribution, members of this genus have classically been described as Old World or New World viruses based on their predominant distribution. The Old World viruses, typically found in Africa and Asia, primarily cause a rash and arthritis. Examples include Chikungunya virus, O'nyong-nyong virus, and Ross River virus. The New World viruses, including EEEV, VEEV, and WEEV, are found in the Americas and can result in encephalitis. Based on phylogenetic analysis, alphaviruses most likely originated in the Americas and later spread to the rest of the world.[77]

Transmission and Incidence. Alphaviruses cycle between invertebrate insect vectors and vertebrate reservoir hosts. For most alphaviruses, the insect vectors are mosquitoes and the vertebrate hosts are birds and small mammals. In most cases, humans and equines are incidental hosts.

In North America, the enzootic cycle of EEEV is maintained in shaded swamps along the eastern seaboard, Gulf Coast, and Great Lakes region, where the virus cycles between ornithophilic mosquitoes, primarily *Culiseta melanura*, and passerine birds. Humans, horses, and other mammals are considered dead-end hosts and generally only become infected when bridge vectors (ie, zoophilic mosquitoes such as *Aedes* species and *Coquillettidia* species) feed on an infected bird and then a mammal. Outbreaks in humans, often seen in the late summer or early fall, are frequently preceded by cases of equine encephalitis and are usually associated with heavy rainfall and warmer water temperatures.[77,78] On average, six human cases of eastern equine encephalitis are reported in the United States per year. However, 2010 was a particularly bad year with 10 human cases, including five deaths, and over 200 equine cases, most of which resulted in death.[69,79]

Enzootic strains of VEEV, found primarily in Central America and northern South America, cycle between *Culex* mosquitoes and small mammals, especially rodents. While these strains can cause disease in humans, they are generally considered avirulent in horses. More importantly, horses are not amplifying hosts for enzootic VEEV. An epizootic or epidemic only occurs when a mutation in an enzootic strain develops into an epizootic strain of VEEV, allowing transmission to a bridge vector such as *Ochlerotatus* or *Psorophor*a mosquitoes and infection of both humans and horses.

Equids, especially horses, are very susceptible to epizootic VEEV, leading to high morbidity and mortality. Horses are also amplifying hosts for epizootic VEEV. The resulting viremia permits mosquito transmission and therefore fuels epizootics. Epidemics are the consequence of spillover during epizootics: humans become infected by mosquitoes that previously fed on infected horses.[76,80] The most recent significant outbreak occurred in Venezuela and Colombia in 1995, resulting in over 75,000 human cases and 300 deaths. The total number of equine cases was not reported but was probably similar in magnitude to human numbers. Epizootic VEEV has not been isolated in the United States since 1971.[81]

WEEV is widely distributed in the western plains and valleys of the United States and Canada, and in South America. The endemic cycle in North America is maintained in the *Culex tarsalis* mosquito and domestic and passerine birds, especially finches and sparrows. Historically, WEEV has caused epizootics and epidemics in the western United States; however, few cases have been reported in recent years. Nevertheless, morality can be as high as 20% to 40% in horses.[77] WEEV is the least virulent to humans. The elderly and infants are more susceptible groups to clinical illness with case fatality rates up to 10%.[71]

Pathogenesis and Clinical Findings

While encephalitic alphaviruses primarily cause disease in equids and humans, a number of other animals also are susceptible. Following natural infection, initial viral replication may occur at the site of inoculation or in secondary lymphoid tissue, depending on the virus's cellular tropism. The virus replicates within the cytoplasm of infected cells, shutting down host-cell protein and nucleic acid production, which causes cytopathic damage and often apoptosis. Viremia ensues, and, in most cases, neuroinvasion occurs by the vascular route. Typically, no gross lesions are evident; however, microscopic changes consist of suppurative or nonsuppurative encephalomyelitis, with a predilection to gray matter areas in the cerebrum and midbrain (ie, thalamus and hypothalamus).[82]

Initial clinical signs may consist of fever, anorexia, and depression. Not all cases result in neuroinvasion and encephalitis. When encephalitis occurs, central nervous system signs such as circling, ataxia, weakness, depression, paralysis or hyperexcitability, and convulsions may be observed. Depressed mentation, sometimes called sleeping sickness, can cause clinical signs such as head pressing, drooling, and drooping ears, lips, and eyelids.[82]

Clinical signs may appear as early as 2 days or up to 2 weeks following infection. Because the clinical signs for encephalitic alphaviruses are not specific, differential diagnoses should include infectious and noninfectious diseases such as equine herpesvirus-1, rabies, equine protozoal myeloencephalitis, West Nile virus, hepatic encephalopathy, and neurotoxins.[82,83]

Diagnostic Approaches

In horses, definitive diagnosis is commonly determined postmortem via virus isolation, PCR, or histopathologic analysis and immunohistochemistry. However, a few antemortem procedures facilitate a presumptive diagnosis of an alphavirus encephalitis infection, including cytologic evaluation of cerebral spinal fluid (CSF). In most cases, a mononuclear pleocytosis with an increased protein fraction is present; in acute cases, a neutrophilic pleocytosis may be observed. Serology is also a useful tool with presumptive diagnosis based on virus specific IgM antibody detection in the CSF. Although no hematologic or biochemistry values indicate alphavirus infection, neurologic signs from hepatic encephalopathy can be ruled out in the absence of liver enzyme value abnormalities.[82,83]

Recommendations for Therapy and Control

Treatment is often limited to supportive care, which may include IV fluids, corticosteroids, mannitol (to relieve cerebral edema), and flunixin meglumine (to reduce inflammation). In horses with neurologic symptoms, xylazine or detomadine may be used for sedation in order to minimize self-inflicted harm. There is limited data showing the reliability of antivirals and immunoglobulin therapies. Overall, the prognosis is poor; most horses die within 3 to 5 days of onset of clinical signs of encephalitis.[82]

Control and prevention are key to minimizing encephalitis. Environmentally, reducing mosquito breeding areas such as standing water decreases the number of competent vectors. Insecticides are an additional control measure; however, in large rural areas, logistical feasibility and effectiveness should be considered. Topical insect repellents also help reduce the incidence of horses being bitten by infected mosquitoes.

Vaccinating horses is another major factor in minimizing disease. Various formulations provide immunologic protection against eastern, western, and Venezuelan equine encephalitis. Current recommendations for unvaccinated horses 6 months and older include a primary series of two immunizations, with 4 to 6 weeks between doses and annual boosters thereafter. However, in areas where mosquitoes are active yearround, horses should be vaccinated every 6 months. Additionally, previously vaccinated pregnant mares should receive a booster 4 to 6 weeks prior to foaling. Previously unvaccinated pregnant mares should receive a two-dose primary series, with a 4-week interval between doses and a booster 4 to 6 weeks before foaling.[79]

Leishmaniasis

Introduction and Military Importance

Leishmaniasis is a vector-borne zoonotic disease caused by various species of the protozoan parasite *Leishmania*. Humans exhibit a variety of clinical disease manifestations, including visceral leishmaniasis, cutaneous leishmaniasis, and mucocutaneous leishmaniasis.[57] Domestic dogs are the principal reservoir host for human visceral leishmaniasis in the Mediterranean, parts of Asia, and Latin America.[84–86] Canines harboring the parasite may experience clinical or subclinical canine visceral leishmaniasis (CVL). MWDs may be exposed to *Leishmania* species during deployments or assignments to endemic areas. Thus, leishmaniasis is an important diagnosis to consider when a working dog that has been traveling presents with clinical signs characteristic of the disease.

Description of the Pathogen

Caused by *Leishmania infantum*, CVL is now known to be genetically indistinguishable from *Leishmania chagasi*, the commonly recognized agent in the Americas.[57,86,87] While other *Leishmania* species have been isolated from the host, *L infantum* is the most significant to canine disease.[57,84,85] *Leishmania* organisms are diphasic protozoa of the family Trypanosomatidae, order Kinetoplastida.[84,87,88] They parasitize the macrophage as amastigotes in the mammalian host and as extracellular flagellated promastigotes in the gut of the blood-sucking female vector, the phlebotomine sandfly.[85,87,88]

Epidemiology

Transmission. Leishmania species require both a vertebrate and an insect host to complete their diphasic life cycle. Vectors of leishmaniasis are phlebotomine sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World. The Old World vector is characterized by a crepuscular and nocturnal pattern of seasonal activity from late spring to late autumn. The New World vector is active year-round.[57,87,89]

After the vector's blood meal from an infected mammal, *Leishmania* organisms multiply in the sandfly's gut and migrate to the foregut where they become infective, nonreplicative promastigotes. When the infected female vector takes another blood meal from a minimally haired area of the mammalian host (eg, head, nasal bridge, ear pinnae, or inguinal and perianal areas), she inoculates promastigotes into the dermis.[85] Host dermal macrophages then phagocytize the parasites, transforming them into the nonflagellated amastigotes.[87]

Within the macrophage, *Leishmania* organisms are able to evade phagosomal elimination mechanisms, and replicate rapidly. Eventually, the cell ruptures, and the freed amastigotes undergo phagocytosis by additional macrophages. During subsequent bites, intracellular and extracellular amastigotes are ingested by the vector and transformed into promastigotes, perpetuating the *Leishmania* life cycle.[87,89] The parasite survives within infected dogs during winter; no transovarial has been documented.[87]

In addition to known vector-borne mechanisms, vertical transmission of leishmaniasis among specific breeds within North American dogs has been documented.[88,89] While four species of *Lutzomyia* are mammalian feeders in North America, no competent vector has been definitively identified.[87–89] A potential vector, *Lutzomyia shannoni*, is capable of harboring *Leishmania infantum* following a blood meal from an infected canine; however, whether these flies can complete the transformation of *L infantum* into infectious promastigotes is not known.[88,89] Confirmed *Leishmania* infections among North American canines, particularly in foxhound lines, suggest that vertical and horizontal transmission mechanisms exist.[87–90] Proposed vertical transmission mechanisms require *L infantum*-infected cells to be passed to pups via transplacental or transmammary routes. Exposure may also occur during parturition.[89,90] Horizontal transmission requires blood-to-blood contact; transmission via infected blood products has been documented.[87]

Geographic Distribution. In humans, leishmaniasis is considered the third most important vector-borne, parasitic disease after malaria and lymphatic filariasis. The disease is endemic in 88 countries and four continents, putting more than 350 million humans at risk.[87] *Leishmania* species known to infect dogs are present in the Mediterranean Basin (ie, Spain, Italy, and Portugal), the Middle East, Southwest and Central Asia (ie, Iran, Armenia, Afghanistan, and China), Central and South America, and East and North Africa. Animal importation or travel, along with disease propagation through nonvector-borne mechanisms (eg, direct contact and transplacental) may increase transmission in nonendemic areas.[85,87,90]

Incidence and Prevalence. In endemic areas, *Leishmania* transmission is sometimes focused, leading to dramatic variation in infection prevalence.[87] Several studies in the Mediterranean region show infection rates between 1.6% to 40% in the canine population. However, infection rates in endemic areas are much higher than actual clinical disease rates. For example, a combination of serology, cellular immunity testing, leishmaniasis antigen skin testing, and clinical evaluation in endemic areas reveals that 5% to 10% of *Leishmania*-infected dogs are sick, and 90% to 95% of infected dogs are clinically healthy.[84] Further, hypothetical models based on previous studies reveal that, in endemic areas, apparently healthy animals can be divided into approximately two-thirds infected and one-third uninfected.[87]

Pathogenesis and Clinical Findings

Host response plays a significant role in susceptibility or resistance to clinical leishmaniasis infection.[84,85,87] Activity of T-helper system CD4+ lymphocytes can shift the immune system toward a humoral (ie, Th2) or cell-mediated (ie, Th1) response, resulting in susceptibility or resistance to disease, respectively.[87,89] Compared to dogs with clinical disease, asymptomatic dogs tend to develop strong cell-mediated immune responses characterized by increased interleukin-12,

tumor necrosis factor, and interferon compared to dogs with clinical disease. Resistant dogs generally have low anti-*Leishmania* antibody production.[84,89]

Some dogs that lack an appropriate cell-mediated immune response mount an exaggerated humoral response to *Leishmania* infection. These dogs are typically symptomatic and clinical consequences are often a result of excessive immunoglobulin G production, autoantibody formation, and immune complex deposition.[84,85,87]

While significant research supports the cell-mediated mechanism of resistance versus susceptibility, whether other factors such as age, breed, gender, nutrition, host genetics, coinfections, immunosuppression, parasite burden, and virulence play a role in determining if a dog becomes clinically ill with leishmaniasis is not known.[87] Additionally, the complex interaction between host immunity and *Leishmania* infection creates the potential for immunosuppression to incite clinical disease in previously subclinical patients.[85]

Leishmaniasis affects many organ systems, but because divergent host immune responses determine the extent of clinical disease manifestations, clinical presentations vary greatly. In general, clinical signs are the result of immune complex deposition (ie, glomerulonephritis, polyarthritis, meningitis, vasculitis, and uveitis) and autoantibody production against platelets or RBCs (ie, thrombocytopenia, anemia, and coagulopathy).[85] Common findings on physical exam and patient history include skin lesions (Figure 11-2), generalized lymphadenomegaly, chronic weight loss, muscle atrophy, decreased appetite, lethargy, hepatosplenomegaly, polyuria and polydipsia, ocular lesions, epistaxis, onychogryphosis, lameness, vomiting, and diarrhea.[84,87,89,91]

Skin lesions are the most common manifestation of CVL in dogs admitted for treatment due to the disease.[87] In fact, in one study from endemic areas in Greece, over 80% of clinically affected dogs demonstrated cutaneous lesions of varying types.[84]

Fever, brittle or dull hair coat, distended abdomen, bilateral symmetrical alopecia, hyperkeratosis, excessive scaling, and depigmentation are also characteristic of the disease.[86] Laboratory abnormalities associated with leishmaniasis include nonregenerative anemia, hyperproteinemia with gammaglobulinemia, azotemia, isosthenuria, proteinuria, hyperphosphatemia, hypermagnesemia, and elevated alkaline phosphatase and alanine transferase.[84–86]

Diagnostic Approaches

Diagnosis of leishmaniasis in dogs requires an integrated approach. Patient history, signalment, age, clinical findings, basic laboratory findings, and specific test data should all be considered when determining if the canine's illness is leishmaniasis.

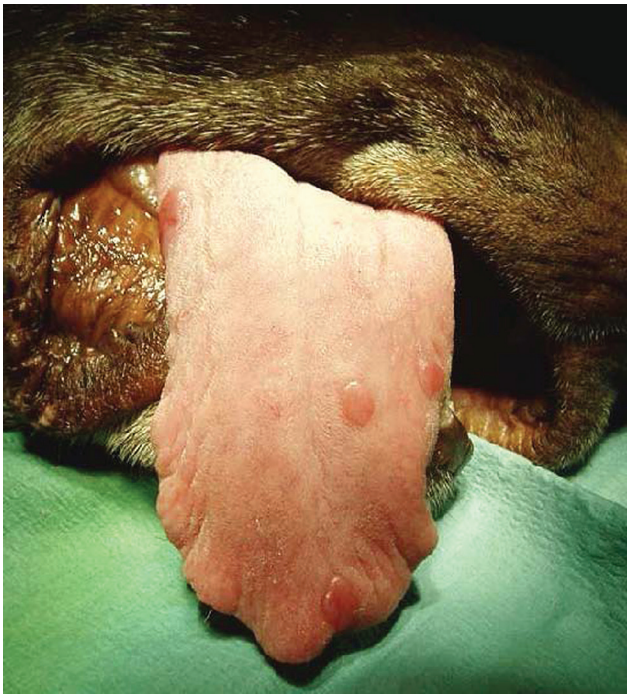

Figure 11-2. Canine tongue: Glossitis due to infection with *Leishmania donovani infantum*.
Photo courtesy of Dr Elvio Lepri, Faculty of Veterinary Pathology, University of Perugia, Italy.

Gathering an accurate travel history is essential, especially when clinical signs consistent with the disease present after travel to endemic areas. Signalment also provides practitioners valuable diagnostic information because some breeds may be predisposed to developing overt disease secondary to *Leishmania* infection, including German shepherd dog, Rottweiler, cocker spaniel, and boxer breeds.[85,87] Prevalence of infection within certain age groups has a bimodal distribution, with peaks at less than 3 years and greater than 8 years.[85,87,92]

When clinical signs and clinicopathologic data are characteristic of leishmaniasis, the simplest way to confirm the diagnosis is through cytologic demonstration of the parasites in stained smears of affected tissues (Figure 11-3).[85] Unfortunately, detection of the *Leishmania* organisms may be difficult using simple cytology; even dogs with significant clinical disease may be harboring low numbers of detectable parasites.[86,87]

Biological fluids can also be obtained for analysis if clinical signs suggest they may be affected (synovial fluid in the case of arthritis and CSF in the case of neurologic disease). Fine needle aspiration should be performed on any clinically affected tissues such

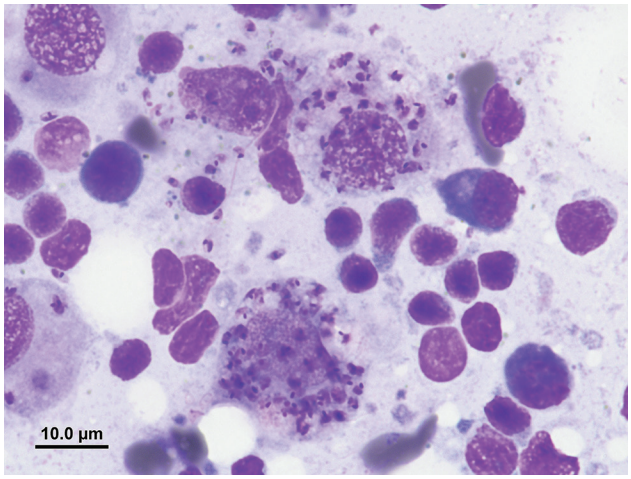

Figure 11-3. Intracellular leishmaniasis amastigotes within macrophages.
Photo courtesy of Jennifer Scruggs, Doctor of Veterinary Medicine; Resident, Clinical Pathology, University of Tennessee, Knoxville, Tennessee 37996.

as papular, nodular, or ulcerative skin lesions; bone marrow (in the case of anemia); and lymph nodes. If no clear lesions exist, tissues most likely to contain *Leishmania* organisms besides bone marrow and lymph nodes include spleen, skin, and buffy coat from whole peripherally obtained blood.[57,87] Heavily infected cells may burst, and extracellular amastigotes may be observed along with those found within the macrophage.[85]

Histologic sections obtained from affected tissues may reveal the parasite using routine hematoxylin and eosin staining. Additionally, pathologic changes noted in affected tissues can increase the clinical suspicion of this illness even in the absence of observable parasites.[85] Lymphoplasmacytic or granulomatous-pyogranulomatous inflammation and lymphoid hyperplasia of reticuloendothelial organs are all characteristic of leishmaniasis.[85,87] When parasites are not observed but strong clinical suspicion remains, immunoperoxidase staining may also improve the diagnostic value of histologic samples.[85]

Available molecular methods that detect *Leishmania* organisms are particularly useful when cytology is negative and there are no obvious lesions to histologically sample. Quantitative PCR, a highly sensitive and specific molecular model, detects extremely low parasitic loads and, due to the quantitative nature of its results, is used to monitor treatment.[57,85,87] Various biological samples can be used for such molecular diagnosis, including (in decreasing order of sensitivity) bone marrow, lymph nodes, spleen, skin, conjunctiva, buffy coat, and whole peripherally obtained blood.[57,85,87]

Because *Leishmania*'s deoxyribonucleic acid (DNA) can be detected in clinically healthy, even seronegative, dogs living in endemic areas,[87,89] PCR results must be interpreted cautiously; some clinically healthy PCR-positive animals do not require treatment. However, use of molecular diagnostic methods in clinically healthy dogs is appropriate in cases of importation to nonendemic areas with competent vectors or in screening for blood donor suitability.[85,87]

Serologic methods also can be used to diagnose CVL, although some limitations exist. Typically, seroconversion in naturally infected dogs occurs between 1 and 22 months (median 5 months). Several reference and commercial tests for detection of serum antibodies against *Leishmania* are available, including the immunofluorescent antibody test (IFA), ELISA, and rapid immunochromatographic strip test. The IFA delivers highly sensitive and specific results; in fact, the OIE recommends the IFA as the reference serologic method.[85]

Quantitative results provide useful information for distinguishing subclinically infected dogs (ie, usually low titers) from those with dissemination and clinical disease (ie, usually high titers).[85,87,93] (High titers can be reasonably defined as at least 2 to 4 times the reference positive value.[85]) In sick patients, presence of a high-level antibody titer generally confirms the diagnosis of leishmaniasis[87]; however, cross-reactivity due to exposure to similar organisms, especially *Trypanasoma cruzi*, in areas where both parasites are present (eg, South and Central America and southern parts of the United States) is possible.[86,87] Especially in patients with low antibody titers, additional diagnostics (see below) are required to confirm clinical disease.[85]

The Canine Leishmaniasis Working Group has developed a step-by-step diagnostic approach for dogs with clinical signs and pathologic changes consistent with leishmaniasis:

- Cytologic evaluation should be performed first; direct observation of parasites in and around infected macrophages confirms the diagnosis, and these patients should be classified as sick from leishmaniasis.[85]
- In the case of negative cytology, quantitative serology should be performed next. Patients with negative or doubtful antibody titers should be evaluated for other differential diagnoses. In very rare cases, an infected patient may be evaluated prior to seroconversion.[87]
- If a patient is seronegative, but there is a very strong suspicion of leishmaniasis, it is reasonable to pursue additional diagnostic testing.

Patients with high antibody titers (as defined by the testing laboratory) should be classified as sick from leishmaniasis. Patients with positive but low antibody titers require additional testing.[85]

- In patients with cutaneous lesions, biopsies can be sent for histologic evaluation with or without immunohistochemical staining.[85]
- PCR can also be performed on biopsy samples. In dogs without cutaneous lesions, PCR should be performed on bone marrow, lymph node tissue, or other high-yield biological samples associated with clinical signs. Patients with positive PCR or histopathologic findings should be considered infected, but clinical signs may be attributable to other disease. Patients with negative PCR or histopathologic findings should be considered exposed to, but not sick from, leishmaniasis.[85]

To guide classification further, the Canine Leishmaniasis Working Group has proposed the following four-stage clinical system:

1. *Stage A (Exposed):* These dogs have negative cytologic findings and negative histologic or molecular results, despite positive but low-level serum antibody titers. These dogs may be clinically healthy. Clinical signs exhibited by these patients are attributable to other disease.
2. *Stage B (Infected):* These dogs have negative cytologic findings and positive histologic or molecular diagnostic results with low-level serum antibody titers. These dogs may be clinically healthy, and any clinical signs are likely associated with other disease.
3. *Stage C (Sick):* These dogs have either positive cytologic findings, regardless of serologic results, or high serum antibody titers. Rarely, infected dogs with lower titers will be classified as sick from leishmaniasis. These dogs display one or more clinical signs consistent with leishmaniasis.
4. *Stage D (Severely sick):* These are sick dogs with one or more additional clinical signs, including proteinuric nephropathy; chronic renal failure; ocular disease with functional loss; severe joint disease that requires immunosuppressive therapy; concomitant neoplastic; metabolic or endocrine disease; or unresponsiveness to repeated courses of anti-*Leishmania* drugs.[85]

Recommendations for Therapy, Prevention, and Control

Treatment for CVL is rarely curative. Treatment objectives vary by stages, but all are aimed at clinical remission by reducing parasite load, treating organ damage secondary to *Leishmania*, restoring effective immune response, and treating clinical relapse.[86,87,93]

Stage A dogs (exposed) do not require treatment. Serology should be repeated 2 to 4 months following the initial evaluation. Any change in titer or clinical condition warrants reevaluation and possibly reclassification.[85,83] Stage B dogs (infected) require treatment only if parasites detected by direct methods cause a rise in antibody response, as evidenced by an increased titer on repeat serology 2 weeks following initial results. Stage C dogs (sick) require treatment by anti-*Leishmania* drugs and may also require ancillary treatment targeted to clinically affected systems. Stage D dogs (severely sick) require both anti-*Leishmania* therapy and one or more ancillary therapies targeted at reducing severe clinical disease.[85]

The most widely used treatment protocol combines a pentavalent antimonial compound (eg, megulmine antimoniate) with allopurinol.[87,93] Meglumine antimoniate selectively inhibits leishmanial glycolysis and fatty acid oxidation, leading to a reduction of parasite load and a temporary restoration of cell-mediated immune response. Allopurinol inhibits the enzyme xanthine oxidase, which catalyzes hypoxanthine to xanthine and xanthine to uric acid. When incorporated by *Leishmania* organisms, allopurinol is converted into a toxic compound that kills the parasite.[93] When these two drugs are used in combination, dogs experience longer remission times than when treated with either drug alone.[87,93]

Therapy with megulmine antimoniate typically lasts 4 weeks; however, lifelong allopurinol therapy may be required to maintain clinical remission.[87] The first line of treatment for canine leishmaniasis should include meglumine antimoniate at 75 to 100 mg/kg/day subcutaneously for 4 to 8 weeks, with allopurinol 10 mg/kg every 12 hours orally for several months.[85] Other treatment methods exist, although this combination is most commonly used.[87,93]

Prognosis for CVL is difficult to establish because no controlled studies have evaluated prognostic factors. However, based on the clinical staging system, a reasonable assumption would be that more severe clinical and clinicopathologic derangements carry a less favorable prognosis.[87]

CVL prevention strategies are aimed at avoiding sandfly bites. Employing a combination of preventive measures (eg, reducing outdoor activity from dusk to

dawn, reducing sandfly microhabitat, using environmental insecticide treatments, and using individual topical insecticide treatments) achieves the greatest results.[87] Indoor residual spraying with organophosphate, carbamate, or pyrethroid compounds is effective against the sandfly vector. Topical insecticide use is effective if the vector spends sufficient time on the reservoir to absorb a lethal toxic dose of the chemical, or if the vector becomes disoriented and irritated during short contact with the reservoir, leading to reduced feeding rate.[91]

Deltamethrin-impregnated protector bands also have proven effective in preventing CVL in field studies.[91,94] One study suggests a 72.3% protection rate in kenneled dogs[92]; other studies suggest 80% to 96% protection.[89,91] Deltamethrin-impregnated collars release the pyrethroid slowly, distributing it within the animal's subcutaneous adipose tissue conferring full protection after 1 week.[87,91] Under optimal conditions, this treatment is effective for up to 6 months with continual collar use.[87]

Permethrin-based spot-on treatments offer high levels of protection as well. The maximum effect is achieved between 24 and 48 hours following application and extends for 3 to 6 weeks, depending on permethrin concentration.[87,91] Typically, spray application of permethrin is more immediately effective but may have equal,[89] or shorter, duration (ie, approximately 2 weeks).[87]

When a dog is living in an endemic area, deltamethrin collars should be applied 2 weeks prior to travel and changed every 5 months. Spot-on treatments should be applied 2 days prior to travel and repeated every 2 to 3 weeks, depending on the product (ie, spray versus spot-on) and concentration.[87]

Leptospirosis

Introduction and Military Importance

Leptospirosis, an emerging infection affecting humans and over 150 different species of animals,[95] is caused by highly motile, obligate aerobic spirochetes of the genus *Leptospira* and is one of the most widespread and prevalent zoonotic diseases in the world.[96] Geographically, most human disease occurs in tropical areas of Asia and South America, as well as, to a lesser extent, Eastern Europe.[97] Among various animal populations, the disease appears worldwide in its distribution.[98] All domesticated animals are potential disease hosts, although cats are particularly resistant.[98,99] The disease was first described in humans in 1886[100] and in animals in 1899.[97] Three characteristics of this disease underscore its military relevance: (1) Military expeditions into endemic areas have historically been linked to the incidence of disease in service members[21,100,101]; (2) MWDs can potentially be infected; and (3) human infection from contact with wild and domestic animals, including pets and MWDs, is also possible.

Description of the Pathogen

Leptospires are flexible, highly motile, helicoidal rods with one or both ends hooked in shape. Darkfield microscopy or special staining techniques are necessary for visualization (Figure 11-4). As of 2013, nearly 300 antigenically distinct pathogenic serovars have been identified, with more being discovered continuously.[96,97]

The taxonomy of the genus *Leptospira* is complex due to separate and coexisting methods for classifying the organisms. Genotypic classification is based on DNA hybridization studies, grouping the genus into species defined as being at least 70% DNA-related with no more than 5% divergence.[102] Antigenic classification is more useful from a clinical perspective; this method organizes the organisms into serological groups based on the lipopolysaccharide structure present on the organism's outer envelope. Under the latter system, pathogenic leptospires are typically classified as serovars and serogroups within *Leptospira interrogans* sensu lato. Similarly, several saprophytic leptospires have been identified in the environment, and may be described either as separate species or as a complex of strains under a single species (eg, *Leptospira biflexa* sensu lato).[101]

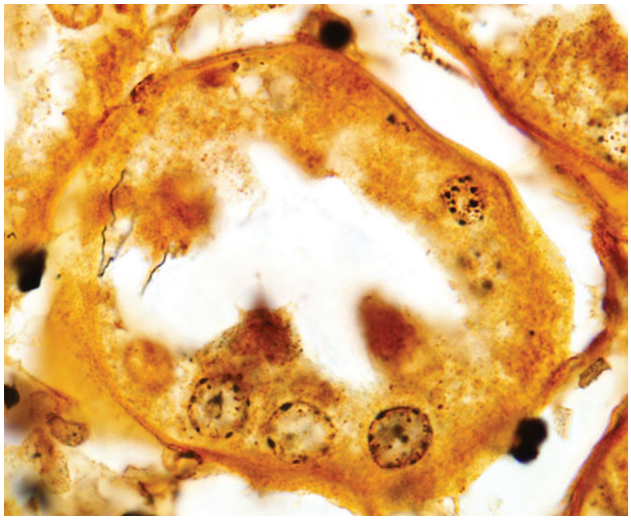

Figure 11-4. Spirochetes (*Leptospira*).
Photo courtesy of Jeremy Bearss, Doctor of Veterinary Medicine, Chief–Resident Training, Department of Defense Joint Pathology Center, Silver Spring, Maryland.

Epidemiology

Transmission. After exposure, pathogenic leptospires colonize the proximal tubules of a wide variety of wild and domestic animal species; however, transmission potential varies by host type (eg, primary, carrier, and incidental hosts). Primary hosts (ie, those that harbor mature or adult parasites) do not typically develop clinical signs of disease, but they may become chronic shedders. For example, a 2003 study from Kansas assessed 500 seemingly healthy dogs and revealed 41 to be PCR-positive for leptospires via urine sample.[103] Because contact with infected primary animal hosts is one potential route of transmission to humans, animal-intensive professions such as farming, slaughterhouse work, and veterinary medicine have long been identified as occupational risk factors.

There are two other types of infections, depending on the host pathogen's degree of adaptation. Hosts that are evolutionarily well adapted to the serovar they have been infected with are called "carrier hosts." Carrier hosts are usually not harmed by the infection but are an important source of infection of other more susceptible hosts. Hosts that are not as well adapted to a particular serovar are called "dead-end" or "incidental" hosts. Incidental hosts often develop clinical disease and either clear the infection or perish from it; they rarely continue to excrete the pathogen and are generally not an important transmission source.[97]

A 2004 study from California found that 10% of the human cases of leptospirosis in California over the preceding 20 years had resulted from contact with pets.[104] This finding is somewhat at odds with a 2011 study of 91 German veterinary clinic staff exposed to dogs clinically diagnosed with leptospirosis, in which none of the 91 humans developed antibodies to any leptospiral serovars.[105] In both studies, infected humans excreted leptospires for weeks to months after infection but remained incidental hosts. Thus, humans are not considered an important source of further infection.[101]

Domestic animals and MWDs most commonly come into contact with leptospires through contact with soil or water contaminated with urine from infected wild animals. After urinary excretion, pathogenic leptospires can survive in stagnant water or damp soil for up to 6 months[21] and last in undisturbed liquid culture for years.[101] Abrasions to a host animal's skin promote likely infection, although the organism is also able to enter the body through intact mucous membranes. Additionally, leptospires may be transmitted directly between hosts through venereal routes, placental transfer, bites, or ingestion of infected tissue.[97]

Geographic Distribution. Leptospirosis has a global distribution, with human and animal cases occurring in nearly every country.[21] The incidence of infection is higher in the tropics than in temperate regions, likely due to the organism's inability to survive freezing temperatures. In humans, recent epidemics have occurred in Nicaragua in 2007, Sri Lanka in 2008, and the Philippines in 2009, each affecting several thousands of people and causing hundreds of deaths.[96] Within the United States, the majority of cases have been noted in Hawaii and the southeastern states.[21]

A clear link also exists between increased rainfall and increased incidence of disease; flooded areas are especially prone to outbreaks.[97] A recent study found a 4-fold increase in the incidence of leptospirosis in Guadeloupe from 2002 to 2004, when the El Niño phenomenon produced heavy rainfall.[106]

Interestingly, one factor may limit the number of pathogenic serovars in an environment: the potential host population's biodiversity. For example, on small islands and in urban environments, the number of native infectious serovars would be expected to be relatively low when compared with environments such as the Amazon Basin or Southeast Asia that have a richly biodiverse host population.[101]

Incidence and Prevalence. Incidence of leptospirosis is grossly underreported in both humans and animals. In humans, mild cases are often misdiagnosed as nonspecific influenza-like illness. In animals serving as asymptomatic, primary hosts for their particular serovars of *Leptospira*, the true incidence or prevalence is even more difficult to define.

However, recent estimates do exist from a few specific animal populations. For instance, a 2003 study of the prevalence of positive antibody titers in beef cattle within a Texas slaughterhouse was found to be 22% (262 of 1200 tested). Of these infected animals, approximately 35% tested positive by PCR for urinary shedding of leptospires.[107] A similar 2011 study of 478 beef cattle from western Canada determined the prevalence of positive antibody titers in unvaccinated cattle to be between 6.1% to 9.6% for the various serovars tested.[108] A 2002 study of data from 22 veterinary teaching hospitals across North America determined the prevalence of clinical leptospirosis among dogs in the United States and Canada to be 37 cases per 100,000 dogs.[109] Finally, a 2007 study conducted among veterinary clinics in the lower peninsula of Michigan found a 24.9% prevalence of leptospiral antibodies among healthy dogs.[110]

Pathogenesis and Clinical Findings

Leptospirosis can develop in dogs of any signalment, although working dogs may be at increased risk compared with other breeds, and male dogs are more commonly affected than female dogs.[109] (This same sex association is noted in humans.[100])

After the leptospire's initial entry into the host's body through mucous membranes or abraded skin, the organism disseminates throughout the body. Many organs may be affected, but the two most classically associated with this disease are the kidneys and liver. Incidental hosts that survive and clear the infection are unlikely to maintain *Leptospira* anywhere in their bodies afterwards, but primary hosts often maintain a clinically silent infection within their renal tubular epithelium. When present, clinical signs vary from mild fever to severe kidney, liver, and pulmonary disease.[98]

A wider spectrum of signs is possible in secondary host infections. Typically, dogs initially present with nonspecific signs such as anorexia, depression, tachycardia, tachypnea, pale mucous membranes, and vomiting, which can progress quickly into a uremic crisis in 80 to 90% of cases characterized by dehydration, lumbar pain from renomegaly and nephritis, and tongue-tip ulceration and necrosis. Nonuremic dogs will generally develop icterus and bilirubinuria, suggestive of cholestasis or hepatic necrosis.[111]

In dairy cattle, sometimes a sudden drop in milk production (by as much as 75%) or change in milk viscosity signals leptospirosis. The infected cow's milk may become clotted, thick, and blood-tinged, with a high somatic cell count. Cattle are also the definitive host for serovars *pomona* and *hardjo*; infections, with these strains typically manifesting as abortion and stillbirths. Thus, within a breeding herd, an "abortion storm" may be another indicator of leptospirosis infection, given that mild initial renal and hepatic signs often pass unnoticed. In endemically infected herds, abortions are sporadic and occur mostly in younger animals.[111]

Diagnostic Approaches

The microscopic agglutination test (MAT) is a widely available and inexpensive diagnostic test for leptospirosis. Three factors limit the MAT's usefulness, however: (1) false-negative results in early disease progression, (2) lack of specificity between serovars, and (3) potential hazards from maintaining a necessary stock of live leptospires in the testing laboratory. Nonetheless, the current advantages of the MAT, coupled with the large amount of historical data regarding its use, make the MAT the most frequently used method of diagnosis to date.[98]

Other diagnostic tests such as darkfield microscopy, silver staining, and immunohistochemistry are used less frequently in veterinary medicine. Organism culture from blood or urine may be useful from a herd health perspective; however, this method requires an incubation period of up to 6 months, which is not practical for diagnosing individual animals.[98] PCR tests, recently developed for individual leptospiral serovars, will probably replace the MAT as the preferred diagnostic test in the future.[97]

Recommendations for Therapy, Prevention, and Control

Treatment of leptospirosis in dogs consists of supportive care, treatment of specific renal or hepatic manifestations, and antibiotics. Antibiotics should be administered as soon as disease is suspected and samples have been drawn. Rapid treatment eliminates the potential for bacteremia and live organisms in the urine that pose a zoonotic risk to humans.[97] Antibiotic therapy should also be started before diagnosis confirmation because treatment initiated after 4 to 7 days of illness is less effective at promoting clinical recovery.[98]

The antibiotic of choice is doxycycline at 5mg/kg either orally or intravenously, twice daily for 2 weeks. Concurrent aggressive intravenous fluid therapy is critical to prevent further kidney damage. Hemodialysis also benefits dogs that develop anuria or oliguria.[98]

Prevention of leptospirosis in both humans and domestic animals centers on animal vaccination and avoidance of contaminated water sources. Although no effective vaccine for humans is currently available,[100] animal vaccines for serovars *icterohemorrhagiae*, *canicola*, *grippotyphosa*, and *pomona* have been accessible for many years and provide good protection for at least 12 months.[97] Vaccination of domestic animals provides a buffer between humans and wild animals where leptospiral infection is likely to be endemic.

Leptospires are also susceptible to ultraviolet radiation, desiccation, and routine disinfectants, although they may be able to survive and remain infective in urine-soaked hair or bedding materials. Caution is recommended, particularly on the part of pregnant or immunocompromised humans, when handling animals suspected of infection. Bleach solutions (ie, 10%), iodine-based disinfectants, accelerated hydrogen peroxide, and quaternary ammonium have all effectively inactivated the pathogen on surfaces, as has normal laundering of potential fomites (eg, soiled bedding).[98]

Lyme Disease

Introduction and Military Importance

Lyme disease is caused by the bacterium *Borrelia burgdorferi* and is transmitted to humans through the bite of an infected tick. Symptoms in humans include fever, headache, fatigue, and a characteristic skin rash called erythema migrans (EM). If left untreated,

infection can spread to the joints, heart, and nervous system.[112] All symptoms cause loss of productive man-hours, which is disruptive to military training, deployment, and operations. Because of its military importance and its potential to become a significant public health threat, Lyme disease is listed as one of the DoD triservice reportable events.[2]

In humans, Lyme disease is diagnosed on the basis of physician-observed clinical manifestations and a history of probable exposure to infected ticks. Laboratory tests are neither suggested nor required to confirm diagnosis for patients with recent onset (ie, 2–3 weeks) of a characteristic EM rash.[113] Unfortunately, symptoms and physical findings can be vague, and knowledge of tick exposure is often unreliable or unavailable. Despite these limitations, the *Annual Lyme Disease Report* compiled by the Armed Forces Health Surveillance Center in 2011 showed that case counts increased over 1.5 times from 2001 to 2007, posing a concern for military communities, especially in Germany and the Northeastern United States.[114,115]

Domestic dogs are also susceptible to Lyme disease. In fact, given their higher tendency to be in close proximity with ticks, canine seroprevalence has been proposed as a sensitive and independent measure of human Lyme disease risk.[116] The CDC published a Lyme disease study in September 2011 corroborating this proposed measure. The study showed a positive correlation between canine seroprevalence and human incidence, suggesting that regions with high canine seroprevalence may anticipate increased human infection rates.[117]

Description of the Pathogen

Lyme disease (SYN Lyme borreliosis or borreliosis) is caused by infection with a Gram-negative spirochetal bacterium of the genus *Borrelia.* The genus *Borrelia* contains at least 31 species and is further divided into specific genospecies. *Borrelia burgdorferi sensu strict*, the primary isolate found in the United States, is the one associated with disease among humans and companion animals.[118] In Eurasia, the three main isolates are (1) *Borrelia garinii*, (2) *Borrelia afzelii*, and (3) *B burgdorderi sensu stricto*. The greater diversity among Eurasian species suggests that the organisms may have originated there.[119]

Epidemiology

Transmission. Borreliae cannot survive as free-living organisms in the environment; they are host-associated and require a hematophagous arthropod vector for transmission to the vertebrate reservoir host. The principal vectors are the slow-feeding hard ticks of the *Ixodes* family, and their distribution is directly associated with the prevalence of disease. In the United States and Canada, these ticks include the black-legged *Ixodes scapularis, Ixodes pacificus,* and *Ixodes neotomae*. There are approximately 50 to 80 competent vertebrate hosts across North America. Nymphal and larval stages prefer small mammals in the north and lizards in the south; adults prefer deer and other larger mammals.[118]

In Europe, borreliosis appears to be associated with *Ixodes ricinus* ticks, and eastward in Eurasia, it appears to correspond with *Ixodes persulcutus*. Studies show that together these ticks parasitize over 200 vertebrate species, with mice, voles, rats, squirrels, hedgehogs, shrews, and birds all playing an important role as reservoir hosts.[118]

Ixodes ticks have a 2-year life cycle and maintain infection in nature by harboring the organism over the winter in their larval form.[120] Direct transmission of borreliae between reservoir hosts is unlikely, and transovarial transmission in ticks is practically nonexistent.[121]

Nymphs are thought to be the most important life stage for transmission to humans. Often less than 2 mm in size, these tiny vectors feed relatively undetected, which is important because they must be attached for at least 36 to 48 hours before the borreliae bacterium can be transmitted. Adult female ticks tend to be the ones transmitting the infection to larger mammals such as white-tailed deer, dogs, and livestock.[118]

Geographic Distribution. In general, Lyme disease occurs throughout the Northern Hemisphere in temperate latitudes with cooler climatic conditions. In North America, the majority of canine and human cases have been reported in the mid-Atlantic to New England coastal states, northeastern states into southern Canada, and upper midwestern states.[113,122] Specifically, a serosurvey of dogs in the United States showed overall positive prevalence rates were highest in the Northeast (11.6%), followed by the Midwest (4.0%), West (1.4%), and Southeast (1%).[122] In Europe, most cases have been documented in the Scandinavian countries and in central Europe in areas with moderate temperature and moderate humidity (eg, Austria, Belgium, Croatia, Czech Republic, parts of France, Germany, Hungary, Netherlands, Portugal, Slovenia, and Switzerland).[118] Data derived from the United States military Defense Medical Surveillance System mirror these distributions, showing a high number of Lyme disease cases reported from military medical facilities in the northeastern part of the United States and Germany.[114]

Incidence. Lyme borreliosis is still a relatively young disease. Since first being described in 1977, it has become the most commonly reported vector-borne

illness among humans in the United States, with over 30,000 cases reported in 2010. From 1992 to 2006, the number of reported cases more than doubled. The majority of cases are in children and young males, with most new infections occurring in the spring and early summer when the ticks reemerge and seek their blood meals.[112]

However, divergent surveillance practices limit the reliability of state incidence reports. Because cases are reported based solely on where the patient lives (ie, state residence) it is difficult to definitively determine where the patient was exposed (ie, in which state, perhaps not same as the state of residence). Also, reporting practices and case definitions between states are not uniform, making determinations of true incidence difficult.[112,113]

Many prevalence studies have been conducted on canine populations, primarily to determine their effectiveness as sentinel animals when assessing human disease risk.[116,122–125] These canine studies demonstrate a correlation with Lyme disease incidence in humans, given three limiting factors. First, like collected human surveillance data, these canine studies only indicated the state the animals were tested in, not the state the animals were exposed in; dogs testing positive may have been exposed elsewhere.

Second, the testing conducted only determined antigen or antibody presence in the animal, not the existence of the agent in that particular area. Third, in many studies, only distinct subsets of dogs were sampled, namely those that were brought to a veterinarian and whose owners opted to have their animals tested.

Pathogenesis and Clinical Findings

The borrelia spirochete is injected into the vertebrate host's tissues through the infected tick's saliva. Organisms replicate from the tick bite site and migrate through skin and connective tissues, later colonizing many different tissues, including the joints. In some humans and animals, the host immune response reduces spirochete numbers to nondetectable levels within a few weeks of infection, even without the aid of antibiotics. In others, the immune reaction may actually be strong enough to prevent infection altogether.[118] However, in some instances, *B burgdorferi* acts as a persistent pathogen, evading host antibodies by varying its immunoreactive proteins and existing extracellularly in protected tissues. This may explain why *B burgdorferi* can still persist and be detected in tissues by PCR or occasionally culture months after antibacterial treatment.

Clinical disease associated with *B burgdorferi* results from the host's own inflammatory response. The clinical hallmark of Lyme disease in humans is the characteristic bull's eye lesion around the tick bite (ie, EM). As noted earlier in this section, documentation of this characteristic lesion categorizes a patient as positive for Lyme disease in existing human surveillance programs.

Animals, on the other hand, do not reliably develop any dermatological lesions, and when they do, their hair coat often masks it. The most frequently reported presenting clinical sign is an acute mono- or oligoarthritis in large joints such as the shoulder, elbow, or knee combined with lymphadenopathy in the draining local lymph node.[126,127] Affected joints are very painful, swollen, and warm, but often the lameness is transient and resolves within 3 to 5 days, regardless of treatment.[126,127] (In studies, animals developed signs of arthritis approximately 2 to 3 months post known exposure and generally occurred in the joints closest to the original tick bite.[127])

Protein-losing glomerulopathy and acute renal failure have also been reported.[125] Often, the addition of acute systemic signs such as fever between 39.5°C to 40.5°C, anorexia, and general malaise raise a clinician's suspicion of Lyme disease; however, the combination of these signs is equally observed in dogs with and without *B burgdorferi* specific antibodies. Therefore, these symptoms should be considered nonspecific indicators of Lyme disease.[118,126]

Diagnostics Approaches

No specific hematologic or biochemical changes are associated with borreliosis. Cerebrospinal fluid, joint fluid, and urine may show evidence of inflammatory changes, and hematologic abnormalities can include leukopenia and thrombocytopenia. Unfortunately, these changes may also be observed with other tick-borne diseases and may even be attributed to coinfection with these other pathogens.[118]

There is also no pathognomonic test for Lyme borreliosis. The presence of an elevated antibody titer to *B burgdorferi* signifies exposure to the spirochete but does not prove that the current clinical illness is caused by the organism. In endemic areas, asymptomatic animals are often seropositive, possibly from an adequate host immune response, exposure to a nonpathogenic form of *B burgdorferi*, or exposure to a closely related nonborrelia spirochete organism.[118,126]

The first available immunodiagnostic tests were done with antigens from whole spirochete preparations. Unfortunately, these tests were not standardized, had a high level of cross-reactivity with other bacteria (eg, *Leptospira*), and could not differentiate between vaccinated and naturally exposed dogs. Because of these shortcomings, most of these serologic

tests have been discontinued. ELISA and IFA tests for immunoglobulin M and immunoglobulin G (IgM and IgG, respectively) also are available, but because dogs don't develop clinical signs of Lyme disease early in the course of infection, the usefulness of testing for these is questionable as well. In general, the acute rise in IgM is missed altogether, and because clinical signs develop so late in disease (well after seroconversion), paired IgG titers are not useful.[126]

In 2001, the SNAP® 3DX (IDEXX Laboratories, Westbrook, Maine) point-of-care test became commercially available for Lyme disease testing. This test was designed to detect the presence of serum antibody to C_6, a synthetically produced peptide encoded by specific surface lipoproteins of *B burgdorferi*. Because this surface lipoprotein's genes (ie, IR_6) are only expressed during infection of and replication of the spirochete in the mammalian host, the presence of serum antibodies to C_6 indicates host invasion and infection with *B burgdorferi,* allowing differentiation between vaccination and true infection. The IR_6 surface protein is also genetically, structurally, and antigenically highly conserved among many *B burgdorferi* strains. Experimentally, the C_6 antibody response is detectable 3 to 5 weeks postinfection and stays positive for at least 69 weeks. Test positivity occurs earlier than with conventional assays and even before onset of clinical lameness.[128,129]

In 2006, with the addition of *Anaplasma phagocytophilum* , the SNAP® 3DX test became known as the SNAP® 4DX. Unlike previous ELISA tests, the C_6 antibodies detected in the SNAP® 4DX® are not increased in dogs infected with dirofilariasis, babesiosis, ehrlichiosis, Rocky Mountain spotted fever (RMSF), or leptospirosis.[126,128]

Even though currently available disease screens are growing increasingly more useful in diagnosing clinical illness from *B burgdorferi* infection, they are still not conclusive. Thus, the American College of Veterinary Internal Medicine consensus statement notes that the presumptive diagnosis of Lyme disease should include (*a*) evidence of exposure to *B burgdorferi,* (*b*) clinical signs consistent with Lyme disease, (*c*) consideration (and exclusion of) other differentials, and (*d*) response to treatment.[126]

Recommendations for Therapy and Control

Because of the difficulty obtaining an accurate diagnosis, antibacterials are often given empirically to try to make a therapeutic diagnosis. Extrapolating from human medicine, the animal drugs of choice are tetracycline derivatives or amoxicillin. Most clinicians, including a survey of American College of Veterinary Internal Medicine diplomats, recommend using doxycycline at 10 mg/kg orally, once daily, for a minimum of 1 month.[126]

However, clinical improvement should be viewed with caution for several reasons. First, an animal's improvement does not confirm that the clinical illness is caused by *B burgdorferi*. Second, the intermittent nature of the acute arthropathy often resolves spontaneously within days to weeks, regardless of antimicrobial therapy. Third, doxycycline has antiflammatory properties and may work at resolving the clinical signs of lameness, regardless of cause. Finally, doxycycline is effective against other diseases with similar clinical signs such as RMSF, anaplasmosis, and ehrlichiosis, and they may actually exist as coinfections with Lyme disease.[126]

Whether antimicrobial therapy truly clears the *B burgdorferi* organism is also still debatable. Research shows that although clinical signs improve and antibodies decrease with antimicrobial therapy, study-infected dogs were still PCR-positive for the organism in various tissues. The research also showed that suppressing the host immune system with administered corticosteroids caused some dogs to develop Lyme arthritis.[127]

Prevention. The mechanism behind the Lyme vaccinations is to prevent the *Borrelia* spirochete from infecting the host. When ticks feed, outer surface lipoprotein (Osp) A converts to OspC, allowing the spirochete to detach from the tick's midgut and migrate to its salivary gland and, subsequently, to the host. OspC may also help the spirochete avoid detection by the host's immune system. The vaccine-induced immune protection begins in the tick before spirochetes even enter the host.[118]

Four Lyme vaccines are available in the United States: (1) monovalent bacterin, (2) bivalent bacterin, (3) nonadjuvanted recombinant (r) OspA, and (4) adjuvanted rOspA.[118,126] Both the bacterin and the rOspA vaccines induce anti-OspA antibodies in the host, but the bacterin vaccines are also marketed as stimulating the production of anti-OspC antibodies, thereby proclaiming higher preventive efficacy.[126] The downside is that bacterin-containing vaccines may put dogs at higher risk of developing immune-mediated reactions and adverse effects.[118]

Lyme vaccine effectiveness studies are difficult to interpret mainly because making an accurate diagnosis of Lyme disease is also difficult. Studies report preventive fraction ranges from 92% with whole spirochete bacterin vaccines to 60.3% with OspA vaccines. However, the enhanced resistance to infection seen in dogs vaccinated before infection is not seen in dogs that have recovered from natural infection, and

vaccinating infected dogs does not help clear infection. Dogs should be selected for vaccination based on geographic location (ie, where they reside or travel) and by their outdoor activities and habits. For example, dogs that participate in outdoor activities in known high tick-exposure areas should receive priority for vaccination.[118]

Depending on the product, vaccines are recommended for use beginning at 6 to 12 weeks of age. Primary vaccination schedules consist of two inoculations 3 weeks apart. Higher antibody titers are induced in dogs given a third dose, so extra-label recommendations in highly endemic areas may include the use of a third immunization 6 months after the two initial doses. Annual boosters are recommended in high-risk dogs because vaccinations do not sustain protective titers. Because heterologous vaccines produce weak antibody cross-reactivity, species-specific vaccines are probably necessary for adequate protection.[118,126]

In Lyme-endemic areas, tick control measures are not only important to prevent Lyme disease, but also to prevent the many other tick-borne diseases. The carriers, *Ixodes* ticks, are field ticks that look for hosts by waiting in leaf litter and overgrown lawns, low-lying vegetation, overhanging branches, and wooded and brushy areas. Tick control begins with avoidance of these tick habitats, careful landscaping when possible, and routine checks for ticks after being in or around such environments.[118]

Because of the relatively long duration of attachment required for the transmission of *B burgdorferi* (at least 36 to 48 hours) any product that effectively reduces the duration of attachment can be effective in reducing transmission. Tick control products include fipronil, amitraz collars, permethrin with imidacloprid, and other permethrin-containing products. Many veterinarians recommend the combination of amitraz collars with fipronil in endemic areas.[122] Using these collars is not without risk, though; they are very toxic when ingested, and veterinarians should have the antidote yohimbine on hand when recommending this product. Fipronil and permethrin with imidacloprid also have been shown to be effective, and they are not washed away by swimming or bathing.[126]

Public Health Considerations. Although Lyme borreliosis is classified as a zoonosis, animals and humans are incidental hosts for a sylvan cycle that exists in nature. Dogs do not appear to be a source for infection in humans because dogs do not excrete infectious organisms in their fluids. In addition, because ticks do not refeed after detachment, the risk of a pet bringing infected ticks home to their owners is minimal. However, because of their greater exposure risk, dogs may be very useful sentinel hosts for human infection.

Plague

Introduction and Military Importance

Plague, an infectious disease of animals and humans, is caused by the Gram-negative bacterium *Yersinia pestis* that circulates in the environment among susceptible rodent species, including rats, rock squirrels, ground squirrels, and prairie dogs.[130,131] Humans are usually infected with bubonic, primary septicemic, or pneumonic plague from flea bites during an epizootic event.[131] However, they can also be infected by other means, including exposure to blood or tissues of infected rodents, rabbits, or domestic cats[132]; exposure to infectious aerosol droplets, generally from infected humans or household cats[132]; or through laboratory exposures.[131] Carnivores such as dogs, cats, coyotes, raccoons, and skunks also can become infected, but clinical signs rarely appear in species other than cats.[131] Cats develop clinical manifestations of bubonic, pneumonic, or septicemic plague, with 50% mortality rates in untreated animals.[132]

Because plague is a life-threatening disease that can be spread through aerosol transmission, the US military is concerned with plague as an endemic disease and a biological warfare threat.[131] All suspected or confirmed plague cases, in animals and humans, must be reported to local or state health departments. Plague is classified as a Category A critical biological agent because of its potential as a bioterrorism agent.[73] (See Chapter 15, Veterinary Pathology, for additional details on agent categorizations by the CDC and the National Institute of Allergy and Infectious Diseases.) In order to facilitate prompt diagnosis and treatment, veterinarians must understand preventive medicine concepts, including the plague's natural mechanisms of transmission.[133]

Description of the Pathogen

Y pestis is a Gram-negative, nonmotile member of the family Enterobacteriaceae. When stained with Wright, Geimsa, or Wayson stains, it takes on a characteristic "safety pin" bipolar staining effect.[131,132] Y pestis can grow at a wide range of temperatures in the laboratory, although optimal growth occurs at 28°C.[131] The bacterium is relatively slow growing, with pinpoint colony growth requiring more than 24 hours' incubation. If cultures are discarded prior to 48 hours, a diagnosis of plague may be missed.[132] Despite the slow growth, Y pestis readily grows on standard laboratory media, including sheep blood agar, MacConkey agar, nutrient broth, and unenriched agar.[131,132]

Epidemiology

Transmission. More than 200 species of mammals and 150 species of fleas are capable of transmitting *Y pestis*. While the Oriental rat flea (*Xenopsylla cheopis*) has been responsible for large bubonic plague outbreaks, the most important vector in the United States appears to be *Oropsylla montana*, which is commonly found on rock squirrels and California ground squirrels.[130,131]

Although plague has two distinct patterns—epizootic or enzootic outbreaks—it generally occurs in the enzootic form where a stable cycle of rodents infecting fleas exists. Questions still remain about how the enzootic form is maintained; however, there is thought to be no excess mortality in a largely resistant population.[131] Epizootics occur about every 5 years, where climatic or environmental conditions result in a higher-than-normal host susceptibility and corresponding high mortality. Under such circumstances, fleas are more likely to migrate and, subsequently, encounter and bite humans and other nonrodent animals.[134]

Generally, plague is also a seasonal disease, with most reported human cases occurring between March and October.[134] However, cases associated with domestic cats occur year-round, without a seasonal pattern.[130,133]

Almost any mammal can become infected by plague, but most species do not show clinical disease signs. For example, *Y pestis* infections are rarely identified in ungulates (eg, bison, deer, pigs) in the United States, and these animals probably pose relatively little risk to humans.[132] However, because of their interaction with wildlife during hunting behaviors,[130] both domestic dogs and cats are epidemiologically important sources of human plague cases. Although dogs seldom exhibit clinical disease signs, they pose a potential human health risk because they may transport fleas into homes.[131,132]

Cats pose a double risk: not only do they show clinical signs, but they are also particularly efficient at transmitting disease to humans.[131] Although there were no reported cases of human-to-human transmission since 1924, 7.7% of the 297 US human plague cases from 1977 to 1998 were associated with transmission from cats.[130,133]

Geographic Distribution. Plague occurs in various regions of all continents, except Australia. It is endemic in the former Soviet Union, the Americas, Asia, and especially in parts of Africa. In fact, the World Health Organization reports from 2003 indicate that over 95% of cases worldwide came from Africa.[131,135]

In the United States, plague is endemic in the western states, with most human cases coming from New Mexico, Arizona, Colorado, and California. In the early 1900s, most cases in the United States were found in urban areas. Since then, the distribution pattern has altered: 80% of cases reported since 1925 are sylvatic or peridomestic. The World Health Organization notes that plague distribution also coincides with the geographical distribution of its natural foci.[135,136]

Incidence. Historically, plague has resulted in significant human loss, often impacting entire civilizations. In the 1300s, plague (ie, the "Black Death") killed an estimated 30 percent or more of the population of Europe. Since these times, improved sanitation standards and antibiotics have reduced infection and mortality rates, lessening the plague's dramatic impact. Globally, the World Health Organization reported a total of 2,118 plague infections in 2003, including 182 deaths.[135] In the United States, human cases are relatively rare, with the CDC reporting less than 15 cases annually.[130,136]

Pathogenesis and Clinical Findings

As few as 1 to 10 bacilli can cause plague infection.[131] The organisms are susceptible to phagocytosis and killing by neutrophils; however, some bacteria may proliferate in tissue macrophages. In humans, clinical plague infection occurs in three forms, depending on the route of infection: (1) bubonic, (2) septicemic, and (3) pneumonic. Infected cats present with similar manifestations of disease, and bubonic plague is the most commonly observed form of plague in cats and humans, affecting 53% of cats with plague in a New Mexico clinical survey.[132]

A primary risk factor is hunting behavior in plague-endemic areas. When killed infected rodents are ingested, *Y pestis* organisms inoculate the cat's oral lacerations or interdental crevices, resulting in swollen submandibular and cervical lymph nodes. Although approximately 75% of plague-infected cats show submandibular lymphadentitis, abscessed lymph nodes may be clinically indistinguishable from abscesses caused by different means (eg, bite wounds).[133] Other, more distinguishable, initial symptoms include fever, lethargy, and anorexia. Cats also can develop pneumonia or disseminated intravascular coagulopathy, multiorgan failure, and other complications associated with a Gram-negative septic condition. Like human cases, untreated bubonic plague in cats frequently progresses to septicemic or pneumonic plague.[133,137]

Pneumonic plague in cats is a serious and rapidly progressive disease. The incubation period ranges from 3 to 4 days, and symptoms include fever, cough, and, frequently, bloody sputum.[138] Cats infected with pneumonic plague pose a serious hazard to owners, veterinarians, and others who handle or have close

contact with these animals because feline plague infection is a risk factor for human plague infection.[139] Between 1977 and 1998, 23 cases of cat-associated human plague were identified in eight states: New Mexico, Colorado, California, Arizona, Nevada, Oregon, Utah, and Wyoming.[133] Five of these human cases (21.7%) resulted in fatal infections. Six of the 23 cases (26.1%) occurred in veterinarians or their staff,[133] suggesting that veterinary staff in plague-endemic areas may be at increased risk of occupationally acquired infection.[130]

If used as a biological weapon, the plague's pathogenesis and clinical manifestations must be altered from those of naturally occurring disease. In humans, primary pneumonic plague would result from inhalation of aerosolized *Y pestis*. The time from exposure to clinical signs would likely range from 2 to 4 days. Initial symptoms would include a fever with a cough and dyspnea, progressing rapidly to a severe progressive pneumonia similar to secondary pneumonic plague. An intentional aerosol release of *Y pestis* also causes feline primary plague cases, especially among exposed feral or free-roaming cats.[137]

Diagnostic Approaches

Because early plague cases foreshadow a larger epidemic, laboratory or clinical suspicions of plague must be immediately reported to appropriate health professionals. Definitive tests can be arranged through a state reference laboratory or the CDC, and early interventions can be implemented, even though no rapid assays for plague are widely available. Antigen detection, IgM enzyme immunoassay, immunostaining, and PCR (all human confirmatory tests) are available at some state health departments, the CDC, and military laboratories. Confirmatory testing for feline cases can be most effectively achieved by performing fluorescent antibody testing on lymph node aspirates.[130,138]

If possible, diagnostic samples should be taken prior to administering antimicrobials. Samples should be placed on ice or frozen (not in preservatives) and shipped overnight to a reference laboratory.[130] However, the state health department must be notified prior to shipment of any plague suspect's biological samples.

Recommendations for Therapy, Prevention, and Control

Therapy. While bubonic plague in either cats or humans can be successfully treated with antibiotics if diagnosed early,[131] pneumonic plague is one of the most deadly infectious diseases. Fatality rates approach 100 percent in untreated pneumonic plague cases, and mortality rates depend on how soon treatment is started. In fact, patients with primary pneumonic plague are unlikely to survive if antibiotic treatment is not initiated within 18 hours of symptom onset. Further, most plague fatalities are a result of a delay in appropriate antimicrobial therapy.[136] The drug of choice for human plague is streptomycin[131,137]; however, this drug is not available for veterinary use. Alternative drugs for veterinary use include gentamicin, doxycycline, tetracycline, and chloramphenicol.[130]

Prevention. Plague prevention in domestic cats is critically important because the disease can rapidly kill cats and trigger human plague. Although feline plague's clinical signs may be similar to those of other diseases, a high fever, especially when coupled with lymphadenopathy or sublingual abscesses, in a free-roaming cat from the western United States is a strong indicator of plague.[137]

Pet owners who live in plague-endemic areas should exercise the following precautions to prevent plague infection: (*a*) Cats should be prevented from free-roaming behaviors; (*b*) all domestic cats and dogs should be regularly treated for fleas, especially during the summer months; (*c*) cats and other mammalian pets should not share sleeping areas with family members to avoid potential flea bites; and (*d*) outdoor areas providing harborage for rodents, such as wood piles or junk piles, should be eliminated.[133]

Veterinarians and their staffs, especially those in plague-endemic areas, also need to remain vigilant to protect animal and human health. Personnel in contact with an infected cat should consult their physicians and local or state health departments, and they should advise owners of cats with suspected plague to do the same.[132,133] Staff should immediately treat cats with suspected plague for fleas and isolate these animals to prevent human contact with infectious exudates or respiratory aerosols.[132] All personnel also should use a respirator mask—preferably one approved by the US National Institute of Occupational Safety and Health—and gloves when handling live or dead cats suspected of plague infection.[138]

In the event of a bioterrorism event, feral or free-roaming cats may become infected,[132] so staff members should notify animal shelters and control facilities of potential feline infection and associated human health implications. To the extent possible, the staff should also advise their pet owners and local animal providers that cats and other animals remain indoors until qualified experts complete environmental safety assessments.

Q Fever

Introduction and Military Importance

During World War II, US troops and other militaries experienced multiple, large outbreaks of "Query fever" (Q fever) that sometimes affected more than 1,700 troops at a time, causing manpower losses between 23% to 77%.[140] These outbreaks occurred primarily in the European theater of war and were associated with exposures to contaminated farm buildings, straw, and hay in agricultural areas where sheep and goats were raised. In the 1950's and 1960's, additional outbreaks were reported in US and allied troops in Europe and northern Africa. During and after the Persian Gulf War, four US cases were reported from the region while supporting Operation Desert Storm, with one case identified from Saudi Arabia.[140,141]

Multiple articles and case reports also have been published on the diagnosis of Q fever in military personnel returning from and serving in the Middle East during Operation Iraqi Freedom and Operation Enduring Freedom.[9,142–148] For example, in early 2007, the US Army Public Health Command initiated a Q fever surveillance program that identified more than 135 US military cases between January 2007 and January 2011 (S Scoville, DrPH, epidemiologist, US Army Public Health Command, unpublished data, February 2011). Exposures occurred in various occupational specialties, including administrative, aviation, and infantry personnel.

In 2010, the CDC published the following guidance in a health advisory to enable healthcare providers to capture and better identify returning military individuals who might be exhibiting symptoms and signs of Q fever: "Healthcare providers in the United States should consider Q fever in the differential diagnosis of persons with febrile illness, pneumonia, or hepatitis who have recently been in Iraq or the Netherlands."[149(p1)]

Description of the Pathogen

Q fever was first described in 1937 as "query" or "Q" fever, a disease produced by an unnamed pathogen infecting abattoir (ie, slaughterhouse) workers during outbreaks in Brisbane, Queensland, Australia, in 1935.[150,151] During 1935, a similar organism was found in ticks collected from Nine Mile Creek, Montana, and was isolated by using guinea pigs.[152–154]

Additional research was performed on the guinea pigs that recovered from the illness produced from the agent isolated from the ticks. In 1938, these guinea pigs were challenged with, and demonstrated protective immunity against, the Q fever agent from Australia.[155] In 1948, the organism was named *Coxiella burnetii* after the two researchers who were instrumental in identifying the new species causing Q fever: Herald Cox (United States) and Macfarlane Burnet (Australia).[156]

C burnetii is a Gram-negative coccobacillus and an obligate intracellular organism. Historically, it has been categorized as *Rickettsia*-like, but phylogenetic analysis reveals a closer relationship to *Legionella* and *Francisella*. The organism has two distinct forms or life cycles: (1) vegetative form—large cell variant where the organism resides and replicates in monocytes and macrophages; and (2) infective form—small cell variant where the organism is extracellular and spore-like.[157–158]

Epidemiology

Transmission. The reservoir of *C burnetii* for human disease is commonly found in food animals, including cattle, sheep, and goats; however, it is also found in a wide range of other domestic and wild mammals (eg, cats), arthropods, and birds. The organism is shed in infected animals' milk, urine, and feces and in higher concentrations in their placenta and amniotic fluids. Contact with just one of these infected cells can cause infection in humans and other animals. The infective form, which is resistant to drying and most disinfectants, also can remain viable and stable in most environments for a long time.[157]

The primary modes of transmission to humans are inhalation of aerosolized bacteria (eg, infected barnyard dust) and direct contact with the infective form in droplets and fomites. Ingestion of the organism in unpasteurized or uncooked animal products (eg, raw milk), infected blood transfusion, sexual transmission, and tick bites are rarer human transmission modes. Ticks can also transmit the organism between animals.[158]

Geographic Distribution. Q fever became a notifiable disease in the United States and the OIE in 1999 and is distributed worldwide, except for New Zealand. Locations of frequent reports and outbreaks include Europe, Australia, United States, northern Africa, and Southwest Asia. A large outbreak occurred in the Netherlands (2007–2010) with a reported human incidence reaching 14.5 cases per 100,000 people.[159] Out of 426 dairy goat and sheep farms nationwide, animals from 99 dairy farms were reported to be infected with this organism.[160,161]

Incidence and Prevalence. Q fever is enzootic in the United States. Among animals, seroprevalence has been reported from 3.4% among cattle and as high as 41.6% among goats.[162] A 2002 to 2004 study found a

greater than 93% prevalence of Q fever in bulk milk samples from US dairy herds.[163] Similarly, another study found that 92% of US veterinary school dairy herds had positive Q fever specimens in bulk tank milk.[164]

In humans, Q fever is a zoonotic but largely occupationally associated disease. Proof in point: while a published serosurvey conducted among 508 US veterinarians during 2006 detected a 22% seroprevalence,[165] another 2006 study found the total US average annual incidence between 2000 and 2004 was only 0.28 cases per million persons.[166] Moreover, the CDC reported that the 2003 to 2004 National Health and Nutrition Examination Survey listed the seroprevalence level of Q fever antibodies at only 3.1% in US individuals over 20 years old. In 2008, the annual US incidence reached 2.7 cases per million persons (depending on location) with an overall incidence of 0.6 cases per million persons, which is still lower than the 2006 veterinarian rates.[167,168]

Pathogenesis and Clinical Findings

Most animals do not demonstrate signs or symptoms of illness, and the acute form usually remains inapparent. When visible, acute signs include abortion storms, premature births, weak newborns, metritis, and retained placenta. Mortality is rare in animals. In humans, acute infections are also often subclinical but can sometimes include symptoms of high fever, chills, and sweating.[169,170] Other signs and symptoms may include headache, myalgia, pharyngitis, nausea, vomiting, diarrhea, disorientation, coughing, and chest and abdominal pain. Atypical manifestations include granulomatous hepatitis, myocarditis, acute cholecystitis, aseptic meningitis, and acute respiratory distress syndrome.[145,169] Mortality is reported at 1% to 2% in untreated cases.[140]

Chronic Q fever appears to be uncommon and may not develop until years after initial infection. Chronic infection commonly manifests as an endocarditis, usually among patients with preexisting valvular heart disease.[146,169–177] Mortality among individuals with chronic infections has been reported up to 65%.[145]

Diagnostic Approaches

Due to the infectious nature and ease of transmissibility of *C burnetii,* the CDC lists Q fever as a Category B bioterrorism agent, and a Biosafety Level 3 laboratory is required for organism culturing and safe handling. (See also Chapter 15, Veterinary Pathology, for more information about bioterrorism agents and biosafety levels in laboratories.) Organism isolation is a method of diagnostics but is not regularly performed for clinical diagnostics. Other tests include immunohistochemical staining for organism antigen detection in tissue and PCR tests for nucleic acid detection.

Most diagnostic testing is performed using paired serologic testing for IgM and IgG immune responses in animals and man. Testing platforms include IFA, ELISA, and complement fixation. The IFA is most commonly used.

In humans, antibody response occurs against phase I and phase II Q fever antigens, producing phase I and phase II antibodies. In acute infections, antibodies to phase II antigens increase first, followed by a slow but defined increase in antibodies to phase I antigens; overall, however, more phase II antibodies are produced than phase I antibodies. In chronic infections, phase I antibodies tend to be at equal or higher levels than phase II antibodies.

In animals, the antigen antibody response is not as defined as in humans, and seroconversion is not indicative of organism shedding. In fact, because seroconversion often is delayed or even nonexistent and animals may remain seropositive after recovery, antigen detection in placental tissues is a better infection determinant.

Recommendations for Therapy, Prevention, and Control

Animal treatment is limited, although prophylactic treatment with tetracycline or doxycycline reportedly reduces shedding. Currently, no animal vaccine is approved for US use; in fact, preventive vaccine use is not universally well understood. Prior to 2010, two animal vaccines were produced by two different French pharmaceutical companies.[178] One was commercially available in France (Chlamyvax FQ®, Merial, Lyon, France) and the other was commercially available in Slovakia (Coxevac®, CEVA-Phylaxia Veterinary Biologicals Co. Ltd., Budapest, Hungary).[179] At first, neither vaccine was approved by the European Medicines Agency to be widely used throughout the European Union. However, with the advent of the Q fever outbreak in the Netherlands and after much scrutiny and discussion, the European Medicines Agency approved Coxevac® for marketing throughout the European Union in September 2010 under the caveat of exceptional circumstances.[180]

For example, during the 2007 to 2010 outbreak in the Netherlands, vaccination was used as a method of outbreak response and control among goats and sheep in conjunction with other measures.[181,182] Prior to the outbreak, an increase of abortions and stillbirths in dairy goats attributed to *C burnetii* infections had been noted during 2005 and 2006. Likely reasons for these increases are the endemic nature of the disease, the

high shedding rates in birthing tissues and fluids, and the dense concentration of dairy goat farms coupled with the housing systems used and seasonal cleaning methods performed.[183]

The Netherlands government instituted a voluntary animal vaccination control measure in 2008, which became compulsory in 2009 with bulk-milk PCR monitoring. During 2010, all pregnant animals were culled on infected farms, and a nationwide breeding ban for dairy goats and sheep was instituted, combined with the compulsory vaccination. Also, breeding was only allowed on noninfected vaccinated farms. In 2011, annual vaccination of dairy goats and sheep continued as did monitoring for new infections.

Use of these control methods seemed successful. Human Q fever cases peaked in 2009 and sharply dropped in 2010 and 2011, eventually leveling off to preoutbreak levels in 2013. The result of one study during this vaccination period also suggests that vaccination reduces animal shedding of the organism, which, in turn, reduces environmental contamination.[184] Thus, in conjunction with vaccination, instituting more drastic measures that reduce breeding of infected animals (ie, culling and breeding bans) may be required to decrease the proliferation and shedding of the organism into the environment to prevent and control Q fever outbreaks.

Because of the hardiness and resistant nature of the organism to heat, drying, and common disinfectants and the ability for the organism to become aerosolized, environmental cleaning during an outbreak should be conducted cautiously, using proper protective equipment, clothing, and boots. Steps preventing airflow from an infected animal housing area to other areas used by animals or humans also should be implemented. Other means of strengthening biosecurity include restricting access of wild birds and companion animals to stable areas, controlling the source of straw used for bedding, and using quarantine procedures, especially when adding new animals to the herd or flock.[185,186]

ANIMAL DISEASES OF IMPORTANCE IN MILITARY WORKING ANIMALS

In addition to zoonotic disease threats, countless infectious diseases affect only animals. Because a full discussion of all diseases infecting MWAs is beyond this textbook's scope and they are detailed in other texts such as *The Merck Veterinary Manual*,[111,187] just a representative sample of military important diseases affecting MWAs is covered in this chapter's section on animal diseases. In general, these diseases meet one or more of the following criteria: (1) highly transmissible between members of the same species with the potential for explosive outbreaks, especially in kennels; (2) generally severe, potentially fatal, outcomes if left untreated; or (3) no specific treatment (eg, symptomatic only).

Although the risk of infection for many of these diseases can be reduced through regular application of prophylactic measures (eg, annual vaccination, monthly preventative tablets, and antiparasitic topicals), the fact that prophylaxis must be routinely used for many of these diseases speaks to the clinical and military importance of the diseases themselves. Even with routine prophylaxis, these diseases remain a threat to MWAs because of possible reduced effectiveness from missed doses (eg, monthly tablet), inadequate immune response (eg, vaccination), or developed resistance among arthropod vectors (eg, topicals).

Distemper

Introduction and Military Importance

Canine distemper is an important infectious disease affecting a wide range of terrestrial carnivores, including Canidae (ie, wild and domestic dogs); wild Felidae; Mustelidae (eg, ferrets, weasels, minks, skunks, and badgers); Procyonidae (eg, coati, raccoons, and red pandas); and seals.[188–190] The canine distemper virus (CDV) causes serious respiratory, gastrointestinal, and central nervous system diseases.[188] Other manifestations include ocular disease, cutaneous lesions, dental defects, and abortion.[188] Additionally, viral infection of lymphocytes and macrophages can lead to widespread destruction of lymphoid tissues and subsequent immunosuppression.[188,191]

Description of the Pathogen

CDV is in the genus *Morbillivirus*, family Paramyxoviridae, and is a 150- to 250-nm diameter, single-stranded, negative-sense RNA virus closely related to human measles, rinderpest, peste des petits ruminants, and phocine distemper viruses.[188,191] The viral envelope is studded with hemagglutinin glycoproteins that mediate viral attachment to host cells and fusion glycoproteins that allow penetration of host cells and fusion of infected with uninfected cells.[188]

Epidemiology

Transmission. CDV is shed in the respiratory tract secretions of infected animals and, to a lesser extent, from other secretions (eg, urine) within 7 days postinfection. Naïve animals become infected when they inhale aerosolized secretions or come in close contact with infected animals.[189]

Geographical Distribution. Canine distemper is a ubiquitous disease with worldwide distribution.[188,190]

Incidence and Prevalence. The prevalence rate of spontaneous canine distemper in cosmopolitan dogs is greatest between 3- and 6-months old, corresponding with loss of maternal antibodies in weaned puppies.[189]

Pathogenesis and Clinical Findings

CDV gets trapped in the nasal turbinates' mucosa, infects local macrophages, and spreads to the retropharyngeal lymph nodes and tonsils within 24 hours of infection.[190,191] The virus then replicates within local lymphoid tissues. Replication is followed by a primary viremia that rapidly disseminates the virus to lymphoid tissues throughout the body, reaching the thymus, spleen, and systemic lymph nodes within 48 hours of exposure.[188,190]

Clinical signs of fever, lethargy, decreased mentation, and anorexia develop when viremia occurs, approximately 5 days after infection.[188,191] Further disease progression is highly dependent on host immune status, antibody titer against viral glycoproteins, host age, and virus strain and virulence. For example, dogs with adequate humoral and cellular immunity neutralize and clear the virus within 14 days of infection. Dogs with intermediate levels of cellular and humoral immunity may experience infection of mucosal epithelium and brain at the viremic stage.[188] Dogs with a poor ability to mount an immune response develop systemic infection of epithelial tissues, resulting in clinical signs of respiratory and enteric disease, central nervous system infection, and viral secretions.[188,189]

There are four typical clinical presentations. The first, classical canine distemper, is seen in young puppies between 12- to 16-weeks old as passive immunity declines.[188,192] Disease begins with fever and conjunctivitis with rapid progression to respiratory and gastrointestinal signs, including coughing with a variable serous to mucopurulent oculonasal discharge, vomiting, and diarrhea, with other nonspecific signs such as depression and inappetence.[188,191,192] Affected animals may die, fully recover, or progress to the neurologic form of the disease 1 to 4 weeks later in which seizures, paraparesis, myoclonus (ie, tremors, twitches, and "tics"), or ataxia may be observed.[188,192]

A second form of canine distemper is multifocal distemper encephalomyelitis in mature dogs, which occurs when a naïve dog, ages 4- to 8-years old, becomes infected. This rare, chronic disease is not preceded by the classic signs of canine distemper but is characterized by a slow progressive course during which the dog develops pelvic limb weakness, generalized incoordination, and, occasionally, head tremors, with no seizures or personality changes.[193]

The third form, old dog encephalitis, is also considered a rare variant of canine distemper and has an insidious onset with neurological signs such as circling, swaying, and weaving. Compulsive walking with pushing against fixed objects is typical, but paralysis and seizures are not observed. The disease progresses to coma or death after 3 to 4 months.[193]

The fourth clinical presentation of CDV is postvaccinal canine distemper encephalitis, which occurs in young animals 1 to 3 weeks after being vaccinated with attenuated CDV vaccine. This disease has an acute to subacute course of 1 to 5 days with clinical signs resembling the furious form of rabies.[193] (See also Chapter 12, Rabies and Continued Military Concerns, for more information about rabies.)

Diagnostic Approach

A diagnosis of canine distemper is usually based on clinical suspicion. A characteristic history of a 3- to 6-month-old unvaccinated puppy with a compatible illness supports the diagnosis. Abnormal hematological findings typically include absolute lymphopenia caused by severe lymphoid depletion. Regenerative anemia and thrombocytopenia have been found in experimentally infected puppies but are not consistently observed in older or spontaneously infected dogs. The magnitude and type of serum biochemistry changes in acute systemic infections are nonspecific.[189]

Recommendations for Therapy and Control

Treatment is supportive and nonspecific; efforts should be made to prevent spread of infection to susceptible animals. Dogs with upper respiratory infections should be kept in areas that are clean and warm, and the eyes and nasal passages should be kept clear of discharges. Pneumonia is often complicated by secondary bacterial infection, and broad-spectrum antibiotics may be administered with good success. Nebulization and coupage are good adjunct therapies.[189]

When diarrhea is present, food, water, and oral medications should be avoided. Parenteral nutrition may be necessary if diarrhea is protracted. Supplemental fluid therapy is also often required to maintain adequate hydration status.[189]

Although therapy for neurological symptoms can be unrewarding, euthanasia should not be recommended unless the neurological disturbances progress to a point that they are no longer compatible with life. Seizures, myoclonus, and optic neuritis are often irreversible neurological manifestations, but are ones owners may tolerate because the animal can still live productively.[189] MWDs with canine distemper infections require consultation with the local kennel mas-

ter, regional clinical medicine consultant, and the US Military Working Dog Center to determine whether they will remain on active duty or be medically retired. (See also Chapter 4, Medical Evacuation of the Military Working Dog, for additional details on the MWD disposition process.)

Immunity to natural CDV infection is long lasting and the virus' immunologic homogeneity has made disease prevention through vaccination possible. Maternal antibodies acquired in utero and in colostrum block immunity development in puppies from birth until after weaning. A puppy that has not had colostrum is probably protected for 1 to 4 weeks, while maternal antibodies in nursing puppies are probably lost by 12 to 14 weeks of age. The typical vaccination strategy in puppies that have received colostrum for CDV is to vaccinate every 3 to 4 weeks from 6 to 16 weeks of age. Because older vaccinated dogs can still develop distemper, periodic boosters are recommended for this disease, despite long- lived immunity provided by vaccination.[189]

CDV is extremely susceptible to disinfectants. Because infected animals are the primary source of the virus, they should be kept apart from healthy susceptible animals. Dogs usually shed the virus for 1 to 2 weeks following acute systemic illness, although those that develop later neurologic signs without systemic disease may still shed virus.[189]

Parvovirus

Introduction and Military Importance

One of the most common causes of infectious diarrhea in dogs, canine parvovirus type 2 (CPV-2) or parvo primarily affects young dogs (ie, less than 6 months of age).[194] CPV-2 is generally of little significance in the stateside MWD population, primarily because dogs are procured for training after they are 1 year old and have received a full set of vaccinations. However, given the worldwide distribution of CPV-2, its long lifespan in the environment, and lack of control through vaccination in the majority of the world, the likeliness of exposure for the deploying MWD is high. The emergence of a new strain, CPV-2c, also demonstrates a potential increased risk to the MWD population because this variant may cause morbidity and mortality in fully vaccinated, healthy adult canines.[195]

Description of the Pathogen

The causative agent of canine parvovirus enteritis is a parvovirus, of the genus *Parvovirus* in the family Parvoviridae, a nonenveloped single-stranded DNA virus that requires rapidly dividing cells for replication such as intestinal epithelial cells. No virus of the genus *Parvovirus* is known to infect humans. However, humans are affected by different viruses within the family Parvoviridae, the most common being *parvovirus* B19 virus, which causes erythema infectiosum, or fifth disease, in children. Other *Parvovirus* genera of the family Parviviridae are also associated with disease in several different animal species, including feline panleukopenia, porcine parvovirus, minute virus of mice, Aleutian disease virus of mink, and mink enteritis virus.[194,196]

Epidemiology

Transmission. CPV-2 is highly stable in the environment and can persist for many months. The virus is readily spread to dogs via contact with contaminated feces; fomites (eg, veterinary equipment and grooming tools); insects; rodents; and even a dog's hair coat. The virus is also transmissible to cats; CPV-2a and -2b variants readily replicate in the feline intestinal tract and may cause clinical disease, especially in cats concurrently ill with feline panleukopenia virus. Cats shedding virus in their feces also serve as a potential reservoir to infect susceptible dogs.[197]

Geographic Distribution. CPV-2 is distributed worldwide and seems to evolve frequently. It was first isolated and identified in 1978, after emerging in Europe in 1976, spreading, and causing high morbidity and high mortality in global naïve canine populations.[195,198] Differing opinions exist as to the origin of CPV-2 but generally it is thought to have emerged from either the feline panleukopenia virus or an existing wild carnivore parvovirus.[195] Its name (CPV-2) distinguished it from a previously identified, but unrelated, minute virus of canines (CPV-1), a less common and less virulent strain generally causing mild diarrhea and disease.[194,198]

Serologic studies indicate that the original CPV-2 strain circulating in the dog population was replaced by a new variant named CPV-2a around 1980. Interestingly, results indicate that the spread of CPV-2a and replacement of CPV-2 occurred in three years, between 1979 and 1982, similar to that of the original spread (between 1976 to 1978) of CPV-2 but in a population that was considered to be immune.[198] The virus quickly mutated again, and the CPV-2b variant was discovered in circulation in 1984. In 2000, CPV-2c, was discovered in Italy and is now widely distributed in circulation with the 2a and 2b variants.[199]

The most common variant now seen in the United States and Japan is CPV-2b, replacing earlier identified strains. In Europe and the Far East, both CPV-2a and -2b are found in approximately equal incidence.[194] As of 2007, CPV-2c was found to be present in several

US states and has been reported in Europe; in South America in Uruguay; and in Asia, including India, Vietnam, and Japan[199,200]; there were no reports of CPV-2c in Africa or Australia.[200]

Incidence. Although acute CPV-2 enteritis can be seen in dogs of any breed, age, or sex, young, rapidly growing pups aged 6 weeks to 6 months are most susceptible to developing severe disease, especially if they have a concomitant intestinal parasite burden or an intestinal bacterial infection (eg, *Salmonella, Campylobacter, or Clostridium* species).[194] This age span also corresponds to waning maternal antibody levels that create a window of susceptibility, particularly in the absence of a proper vaccination program. Breeds demonstrating an apparently increased risk of developing disease include Rottweilers, Doberman pinschers, Labrador retrievers, American Staffordshire terriers, German shepherd dogs, and Alaskan sled dogs.[194,201] Whether a breed predilection truly exists is somewhat debatable; as of 2007, none was identified for CPV-2c emerging in the United States.[200]

Pathogenesis and Clinical Findings

CPV-2 spreads rapidly between dogs via oronasal exposure to contaminated feces or fomites; the incubation period is generally between 3 days and 1 week.[195] The virus enters the oronasal cavity and begins replicating in the tonsils' regional lymphoid tissues, retropharyngeal lymphoid tissue, and mesenteric lymph nodes.[202] Upon becoming viremic (approximately 3 days following infection), the virus disseminates to systemic and intestinal lymphoid tissues such as Peyer's patches, followed rapidly by infection of the gastrointestinal epithelium.[203]

The most common gross lesions found on necropsy, typical of *Parvovirus* infection, are segmental enteritis, including segmental discoloration (ie, reddening) and roughening of affected serosa; fibrin adhered to serosal surfaces; and intestinal mucosa appearing smooth and glassy from villi loss (Figure 11-5). The small intestinal contents may vary from watery to yellow mucoid to bloody.[195,202] The large intestine is rarely affected.[195] The severity of intestinal lesions relates to the severity of systemic lymphoid tissue lesions and the magnitude and duration of viremia.[203]

Histologically, *Parvovirus* enteritis is characterized by shortened or obliterated villi and necrosis and loss of intestinal crypt epithelium; the presence of lymphoid necrosis helps differentiate parvoviral enteritis from coronaviral enteritis.[202] Intranuclear inclusion bodies within the crypt epithelial cells also may be observed in acute cases.[195,202] Other findings include lymphoid necrosis and bone marrow hypocellularity due to depletion of myeloid cells, erythroid cells, and megakaryocytes. Myocarditis with intranuclear inclusion bodies may be observed in a small number of cases, especially in younger animals.[195]

Some dogs who are naturally infected with CPV-2 may never demonstrate clinical disease signs; however, the typical presentation of a newly infected host is the acute onset of vomiting, followed by foul-smelling bloody diarrhea, anorexia, and subsequent dehydration. Destruction of germinal cells, leading to blunted intestinal villi, impaired absorption ability, and eventually villi death, causes the common clinical sign of diarrhea.[195,202]

Viral infection of the rapidly growing precursor cells in lymphoid organs (eg, thymus, lymph nodes, bone marrow, and spleen) may cause neutropenia, with counts as low as 500 to 2,000 white blood cells per μL, and less pronounced lymphopenia.[195] Secondary bacterial infections may occur subsequent to the disrupted intestinal architecture and impaired local immunity, leading to bacteremia, septicemia, and disseminated intravascular coagulation. Neurologic disease may occur, usually as a result of hemorrhage into the central nervous system during disseminated intravascular coagulation, or from hypoglycemia, sepsis, or acid-base electrolyte imbalances; CPV-2 itself is rarely a sole cause of neurologic disorders.[194] Sudden death (ie, within 24 hours of developing clinical signs) may occur, especially in younger animals.[195]

Pups infected with CPV-2 in utero, or when infected at less than 8 weeks of age, may develop the myocardial form, which commonly causes sudden death, with or

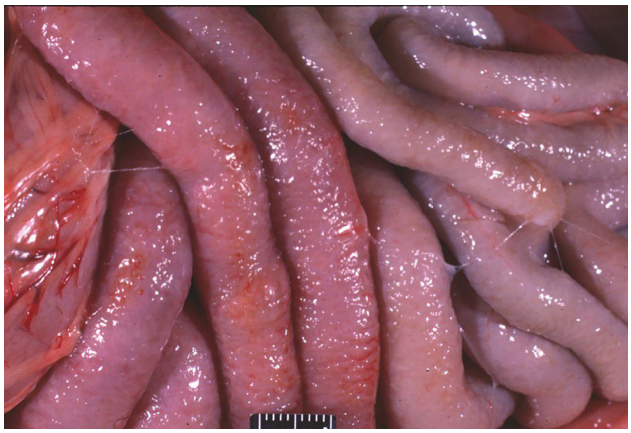

Figure 11-5. Canine intestine: Segmental enteritis as demonstrated by reddened loops of small intestinal loops. Serosa has dark red patches and appears roughened. Note fibrin strands attached to serosal surfaces.
Photo courtesy of Bruce H. Williams, Doctor of Veterinary Medicine, Senior Pathologist, Department of Defense Joint Pathology Center, Silver Spring, Maryland.

without preceding signs of illness, such as dyspnea or GI disease. Puppies that do not die immediately and unexpectedly often develop congestive heart failure and die suddenly weeks to months later.[194]

Diagnostic Approaches

CPV-2 infection should be considered for all puppies presenting with acute, foul-smelling diarrhea. However, because these signs are not specific for CPV-2 infection, other enteric pathogens should also be considered.

A fecal ELISA, commercially available for in-hospital use, is relatively sensitive and specific for detecting CPV-2 infection but is not without critique. Although conducting the patient-side test is simple and rapid, it requires a large amount of viral antigen to produce a clearly visible band and detects approximately only 50% of infected dogs.[195]

Detection failures might stem from the short window of fecal viral shedding (ie, about 10 days, corresponding to days 5–7 of clinical illness) or improper testing procedures, resulting in false-negative results.[195] Subjectivity in the accuracy of reading the results might also lead to false-negatives, especially with low antigen levels.[204] False-positives may occur if the pup has been recently vaccinated with an attenuated live virus vaccine (ie, within the past 5–12 days).[194]

A single serum sample demonstrating a high hemagglutination inhibition titer, collected after a dog has been clinically ill for 3 or more days, is diagnostic for CPV-2 infection because *Parvovirus* causes hemagglutination of erythrocytes. Seroconversion, demonstrated by rising titers in paired sera using HI or virus neutralization tests, can also be used to diagnose *Parvovirus* infection antemortem.[194]

Other diagnostic options include electron microscopy on feces or tissues and conventional or real-time PCR for detecting antigen in feces. Of these testing methods, real-time PCR is sensitive, specific, and more reproducible, quantifying CPV-2 nucleic acid within a few hours and detecting over 90% of infected animals.[195,204] PCR may also be used to distinguish between virulent and vaccine CPV strains.[194]

Postmortem histologic examination of tissues is definitive. *Parvovirus* identification in tissue may be demonstrated by IFA; in situ hybridization may be used on formalin-fixed or wax-embedded tissues.[194]

Recommendations for Therapy and Control

Therapy consists of supportive treatment, such as correcting dehydration and electrolyte imbalances and preventing secondary bacterial infections. Fluid therapy, the mainstay treatment for *Parvovirus* enteritis,[205] should continue as long as vomiting or diarrhea persists. Antiemetics, motility modifiers, and broad-spectrum antibiotics, such as a combination of penicillin and an aminoglycoside, are recommended; antidiarrheal agents are usually contraindicated. Contrary to most GI-treatment advice, nutritional support, via nasogastric tube or per os, is also recommended; clinical disease duration is shortened by maintaining body weight and minimizing hypoglycemia.[194]

Depending on clinical presentation, other therapies include administration of whole blood, plasma, or colloids to correct anemia or hypoproteinemia. Antiendotoxin sera, glucocorticosteroids, and flunixin meglumine may treat early endotoxemia or sepsis. However, neither a recombinant bactericidal-permeability-increase protein, which counteracts endotoxemia, nor recombinant human granulocyte colony-stimulating factor, which treats severe neutropenia, improve clinical outcome.[194] Administering a single dose of immune plasma containing high anti-CPV antibody titers to dogs within 24 hours of onset of clinical CPV enteritis also did not improve hematologic values, length of hospital stay, or cost of treatment.[206]

Although puppies who survive a natural infection have immunity for at least 20 months, and likely for life,[194,195] using commercially prepared attenuated or modified live vaccines is the recommended means of preventing disease. Vaccination invokes both humoral and cell-mediated immune response, making even low levels of maternal antibodies less of an inhibitant.[207]

Currently available CPV-2 vaccines contain either CPV-2 or CPV-2b variant.[207] However, one problem with this prevention method is that puppies are susceptible to CPV-2 infection 2 to 3 weeks before they can be vaccinated.[194,195] This window of susceptibility is further affected by maternal antibody interference for a period of time once vaccination begins. Such interference is the most common cause for vaccine failure.[194,207]

For best results, the initial vaccination series should be administered every 3 to 4 weeks between 8 and 16 weeks of age. This schedule allows the puppy to receive at least one dose of vaccine once the maternally derived antibody levels have waned enough to not interfere with vaccine-induced immunity. The administration of a single, initial dose of a vaccine to any dog greater than 16 weeks of age is considered protective and acceptable. Accepted practice is to booster a year later, followed by every 1 to 3 years. AAHA guidelines recommend any modified live or attenuated vaccine be boostered a minimum of every 3 years based on extensive research.[207]

Vaccinating pups less than 6 weeks of age is not recommended because these pups' immune systems are probably too immature to produce a sufficient immune response.[207] Vaccination effectiveness ranges from 25%

in 6-week-old pups to 95% in 18-week-old pups.[195]

Parvovirus-related disease can occur after vaccination, probably from infection with a wild-type strain, rather than reversion of the vaccine strain. Additional factors contributing to clinical disease after vaccination include infection with variant strains, overwhelming viral dose, and route of exposure.[195]

A small percentage of dogs (an estimated 1 out of 1,000 dogs) are considered "nonresponders," meaning they are genetically incapable of developing an immune response to CPV-2 vaccines. This genetic component may explain why some breeds demonstrated a perceived susceptibility to CPV-2 in the 1980s; when compared to the general population, certain breeds and family lines had a higher prevalence of low or nonresponders.[207]

Serologic testing to determine or monitor for immunity is available and frequently used for CPV-2, especially upon completion of the puppy vaccination series. The "gold standard" tests for antibodies to CPV-2 are virus neutralization and HI, performed by many commercial laboratories. Although most results are reported as titers, some results are reported simply as positive (ie, antibodies present) or negative (ie, no antibody detected). US Department of Agriculture (USDA)-approved in-hospital tests are also available to determine presence or absence of antibodies. With the HI test, a positive result indicates the serum sample has an antibody titer greater than 20; a negative result indicates either the titer is less than acceptable or there are no antibodies.[207]

According to the American Animal Hospital Association, all CPV-2 vaccines currently available on the market provide sustained protection from all CPV variants, including the newest, CPV-2c.[207] However, because confirmed cases of CPV-2c infections in adult dogs with complete vaccination histories have occurred in the United States and Italy,[195,200,208] concern is growing that current vaccines may not actually provide cross-protection against the emerging CPV-2c.

Because CPV-2 is one of the most resistant viruses in dogs, contamination of the environment is also of great concern. In fact, CPV-2 persists for months to years if not properly disinfected or exposed to direct sunlight. Recommended disinfection is bleach diluted with water (1:30). This solution should be used on any equipment, bedding, or surfaces that are tolerant to bleach and should remain on the surface for a minimum of 10 minutes. Upon release from isolation, this same concentration can be used to dip puppies to kill any virus contaminating their fur. Items that cannot be exposed to bleach may be steam cleaned.[194]

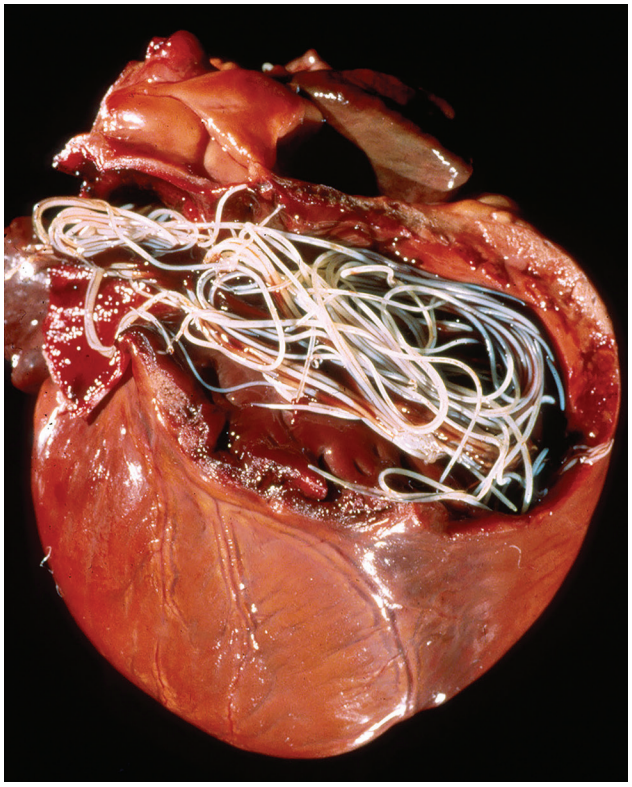

Figure 11-6. Canine heart with heartworms (*Dirofilaria immitis*).
Photo courtesy of Bruce H. Williams, Doctor of Veterinary Medicine, Senior Pathologist, Department of Defense Joint Pathology Center, Silver Spring, Maryland.

Heartworm Disease

Introduction and Military Importance

Heartworm disease, diagnosed worldwide, is endemic in many areas, but as vectors continue to expand their territories, environmental conditions continue to change, and animals continue to move throughout the world, the potential for contracting heartworm steadily increases. Virtually all MWDs are at some risk for exposure and infection. However, the worldwide locations of US military bases and vast number of places where MWDs travel increases their infection risk and potentially contributes to the disease's spread. In fact, a microfilaremic dog can be a reservoir of infection anywhere favorable climatic conditions exist, spreading further disease and creating more endemic areas around the globe.

Description of the Pathogen

Heartworm disease is caused by *Dirofilaria immitis*, a parasitic filaroid nematode.[209–212] The normal definitive host and main reservoir for this pathogen is the domestic dog and some wild canids. Other susceptible

species include cats, ferrets, wild felids, wild mustelids, monkeys, marine mammals, and rodents,[212] but these incidental hosts generally have low-level transient microfilaremias and rarely serve as sources of infection.[209] Humans are aberrant hosts and can develop ocular, pulmonary, or subcutaneous disease syndromes resulting from migration of larvae.[212]

Epidemiology

Transmission and Life Cycle. The developmental cycle of *D immitis* is approximately 7 to 9 months from microfilaria (L1) to adult (L5). The life cycle starts when a mosquito feeds on an infected host and ingests stage 1 larvae (ie, microfilariae). Over the next several weeks, the larvae mature to the L3 stage, then migrate to the mosquito's mouthparts. When the mosquito feeds, the infective L3 enter the host's body via the bite wound, beginning the mammalian portion of their life cycle. The larvae develop from L3 to L5 in the subcutaneous tissue before migrating to pulmonary vasculature, where they sexually mature and reproduce 180 to 210 days postinfection. Generally, adult worms reside in the pulmonary arteries but can move into the right ventricle, right atrium, or vena cava if the worm burden is high (Figure 11-6). Adult worms typically survive for 3 to 5 years in dogs and 1 to 2 years in cats. The microfilariae can survive 1 to 2 years in the bloodstream, where they serve as a reservoir for the disease.[209–211]

Geographic Distribution. *D immitis* can be found in warm climates worldwide. In the United States, it has been found in all 50 states and is locally endemic within areas of the lower 48 states, Hawaii, Puerto Rico, the US Virgin Islands, and Guam. As climates change and the ranges of the vector mosquitoes expand, *D immitis* will continue to thrive, potentially becoming endemic in many more areas.[209]

Incidence and Prevalence. Prevalence of heartworm infection varies greatly and depends on multiple factors, including the animal population (ie, owned vs. stray), the environment or location, and the mosquito population. Prevalence is much higher in stray dogs and cats than owned pets and is also higher in dogs that spend most of their time outdoors. In the United States, surveys show prevalence in dogs to be as low as 0.06% in Nevada and up to 7.4% in Mississippi. The highest rates in dogs are found within 150 miles of the Atlantic Coast from Texas to New Jersey, along the Mississippi River, and in dogs not on heartworm preventative.[212]

Pathogenesis and Clinical Findings

Pathogenesis. Worm numbers, infection duration, host activity level, and host immune response are all important factors that determine the severity of cardiopulmonary pathology in dogs with heartworm infection.[209,211] Long-term infections lead to chronic lesions and scarring via a combination of immune response, worm death, and direct irritation. In fact, live adult worms often cause pathology to pulmonary arterial walls through direct mechanical irritation. This irritation chronologically leads to (*a*) perivascular cuffing with eosinophil infiltration, (*b*) thickened vessel walls, and (*c*) pulmonary hypertension.[211,212] In severe cases, sustained hypertension causes right-sided heart failure. Live worms are also believed to immunosuppress the host.[211]

Unlike live worms, dead worms are known to elicit a strong host immune response. *Wolbachia*, an intracellular Gram-negative Rickettsial bacterium, an endosymbiont of *D immitis*, may help invoke this response in this way: dead worms release the bacteria into the bloodstream; the host then produces antibodies against the *Wolbachia* surface protein.[211,212]

Clinical Findings. Heartworm disease in dogs usually has an insidious onset with slow progression.[212] Patient activity level and lung pathology have a strong correlation with the frequency and severity of clinical signs. The more active the patient is, the more severe the lung pathology will be.[209] Many infected dogs are identified with serologic screening prior to the onset of signs. Common clinical signs of heartworm infection in dogs include coughing, dyspnea, exercise intolerance, unthriftiness, syncope, epistaxis, hemoptysis, cyanosis, and possibly ascites, due to right-sided heart failure as the disease progresses.[211]

Canine heartworm disease is classified as Class I to IV based on physical exam, hematology, urinalysis, and thoracic radiographic findings. Dogs without clinical or radiological signs or laboratory abnormalities are Class I, asymptomatic to mild. Dogs that present with an occasional cough, moderate exercise intolerance, increased lung sounds, loss of condition, possible anemia and proteinuria, and mild to moderate radiographic changes (ie, right ventricular enlargement) are Class II, moderate heartworm disease.[209]

Class III is severe disease. These dogs may present with marked anemia, persistent coughing, weight loss, exercise intolerance, syncope, tachypnea at rest, hemoptysis, ascites, anemia, thrombocytopenia, and proteinuria. Radiographic findings may include right ventricular hypertrophy, diffuse pulmonary densities, and main pulmonary artery enlargement.[209]

Class IV is caval syndrome, which is usually fatal if not treated immediately via surgical extraction of the worms. Dogs with caval syndrome will present with sudden onset of severe lethargy, dyspnea, pale mucous membranes, and weakness, along with hemoglobinuria and hemoglobinemia.[210,211]

Diagnostic Approaches

Antigen testing (ie, ELISA and immunochromatographic tests)—the most sensitive diagnostic method and preferred method for screening asymptomatic dogs—detects a protein secreted mainly by female adult worms.[209] The earliest a dog should be antigen tested is 7 months postinfection.[211] False-negatives, caused by unisex (ie, male) adult worm infection, immature adult worms, low worm burdens, or incorrect testing procedures, may occur. Another limitation of antigen testing is that worm burden levels cannot be determined based on the color intensity of a positive result.[209]

In antigen-positive dogs, microfilaria testing should always be utilized as a complimentary test to validate serologic results and determine if the infective L1 lifestage is present in the dog. This testing has high specificity, but due to variable sensitivity, should not be used as the primary screening test to identify infected dogs. The modified Knotts test and the filtration test are the two types of concentration techniques used to identify microfilaria. The modified Knotts test is preferred because it enables observation of morphology and measurement of body dimensions to differentiate between *D immitis* and other nonpathogenic filaroid species. Microfilaria tests may be negative in infected dogs that are not microfilaremic or that have been on macrolide prophylaxis.[209]

Echocardiography or radiography may provide additional information to support a diagnosis of heartworm disease, but neither should be used as the sole diagnostic test. Also, although echocardiography can provide definitive proof of infection through direct visualization, it is not a preferred method in dogs because it can be inefficient, depending on the location and number of worms.[209]

Radiography provides the most objective method of assessing the severity of heartworm cardiopulmonary disease secondary to heartworm infection in both dogs and cats, according to American Heartworm Society guidelines. Radiography can also be used to monitor regression or progression of disease. Typical radiographic findings include enlarged and tortuous branches of pulmonary arteries, variable degrees of pulmonary parenchymal disease, and potentially right heart enlargement.[210–212]

Recommendations for Therapy and Control

Preadulticide evaluation should include a thorough history, physical exam, antigen test, and thoracic radiography, with additional clinical and laboratory data gathered as necessary based on the patient's clinical status.[209,211] The infection's severity, dog's activity level, and extent of concurrent pulmonary vascular disease are the most important factors influencing the treatment's outcome and the probability of postadulticide thromboembolic complication. Active dogs like MWDs with severe infections displaying radiographic signs of pulmonary arterial obstruction are at highest risk for this complication.[211,212]

Adulticide Therapy. Melarsomine dihydrochloride is the only US-approved adulticidal drug for heartworm treatment and is effective against worms that are over 4 months old.[210,212] The three-dose protocol for melarsomine is recommended by the American Heartworm Society for patients with all classes of disease except caval syndrome and has been demonstrated to be more effective and safer than the two-dose protocol.[209] (Spreading the three adulticide injections over 31 days kills the worms more gradually, reducing worm emboli impact on pulmonary arteries and lungs. This protocol also allows larvae that are too young to be susceptible at first injection to mature, so they can be killed by the second and third injections.)

Melarsomine is administered as an intramuscular injection into the epaxial lumbar muscles. In approximately one-third of dogs, injection site swelling and soreness develops. Strict exercise restriction, the key to minimizing postadulticide pulmonary thromboembolism, should begin at the time of diagnosis and continue for 4 to 6 weeks after melarsomine injections.[209,211,212]

Adjunct Therapy. Multiple adjunct therapies can increase adulticide therapy's safety and efficacy. The patient's clinical condition should always be considered when determining the management protocol to be used for treatment. If the clinical presentation does not demand immediate intervention, a macrocyclic lactone (ie, heartworm preventive) should be administered for 1 to 3 months prior to starting adulticide therapy. Benefits include reduction or elimination of *D immitis* microfilariae, reduction of female worms' mass by destroying their reproductive systems, and stunting growth of immature worms. By collectively reducing antigenic mass in the ways described, macrocyclic lactone therapy also reduces the risk of pulmonary thromboembolism from adulticide therapy.[209]

However, in dogs determined to have high microfilariae counts prior to treatment, macrocyclic lactones may cause anaphylaxis due to rapid death of large numbers of microfilariae. To minimize potential reactions, patients may be pretreated with glucocorticoids and antihistamines. Administering glucocorticoids at diminishing anti-inflammatory doses can control clinical signs of pulmonary thromboembolism, which is especially important in patients suspected to have a high worm burden and thus have more risk for this complication.[209]

Using doxycycline also improves the safety and efficacy of management protocols. Administering doxycycline orally for the initial 4 weeks reduces the *Wolbachia* numbers in all stages of heartworms and the pathogenesis of the host immune response. In addition to its effect on *Wolbachia*, doxycycline is lethal to L3 and L4 larvae and gradually suppresses microfilaremia in dogs with adult infections.[209,212]

Alternative Therapy. In cases where melarsomine therapy is contraindicated or not possible, a protocol using a macrocyclic lactone in combination with doxycycline and daily exercise restriction may be considered. The macrocyclic lactone and doxycycline should be administered monthly, and dogs should be antigen tested every 6 months with continued treatment until there are two consecutive negative antigen tests.[209]

Long-term administration of just macrocyclic lactones for a slow kill is not recommended. This method could take 2 years or longer, during which time pathology could continually worsen.[209]

Caval Syndrome Treatment. In cases of caval syndrome, surgical removal of worms from the right atrium and tricuspid valve's orifice is necessary to prevent death. The procedure of choice for heavily infected and high-risk dogs is insertion of either rigid or flexible alligator forceps introduced through the right external jugular vein.[209–211] All worms that can be identified and reached should be removed. Fluoroscopy can assist with the identification of worms and their locations.

Prevention. Heartworm infection is preventable with appropriate chemoprophylaxis and surveillance.[209,211,212] Oral, topical, and parenteral formulations of macrocyclic lactones are available and are all effective against microfilariae and L3 and L4 larvae. Ivermectin and milbemycin oxime are given orally on a monthly basis. Moxidectin and selamectin are available in topical formulations and are applied monthly. A slow-release formulation of moxidectin-impregnated lipid microspheres is available for parenteral use and provides continuous protection for 6 months.[209]

All of these formulations are safe, and many are also effective against other endo- and ectoparasites. Because MWDs may deploy to endemic regions at any time, chosen preventives should be administered year-round.

Annual antigen testing is recommended for dogs on heartworm preventive to ensure that prophylaxis is maintained.[209,211,212] In cases of missed doses with less than a 6-month gap between doses, dogs should immediately be restarted on prophylaxis, antigen tested 6 months after the initial dose, and then antigen tested annually thereafter. Antigen testing at the time of restarting is optional but not required; results are likely to be negative because not enough time has passed for adult worm development.[212]

Tick-borne Diseases: Ehrlichiosis and Babesiosis

Introduction and Military Importance

Because of the worldwide distribution of tick-borne diseases (TBDs) and the tick's ability to transmit a variety of zoonotic pathogens with each blood meal, TBDs are relevant to the military.

TBDs, more broadly classified as vector-borne diseases, are rapidly emerging and globally distributed.[213,214] In fact, in recent years, a large number of emerging infections and zoonotic diseases are described to be caused by tick-borne pathogens, and more than 800 tick species exist worldwide. These ectoparasitic arthropods feed on mammal, bird, and reptile blood and are extremely vigorous, effective vectors for a large number of pathogens, transmitting viruses, rickettsial agents, alpha-proteobacteria species, spirochetes, and protozoal parasites.[213]

TBDs affecting only animals are relevant to the military because of potential mission failures and inadvertent importation upon redeployment. Canine populations are susceptible to most tick-borne pathogens known to infect mammals, including humans.[122,215–219] MWDs are routinely deployed to many regions of the world where exposure to various TBDs is possible.[216,219–222] If infected MWDs spread these debilitating infections to a larger number of animals, military missions are directly compromised, and casualties are possible.

Description of the Pathogens

Two TBDs are of main concern to the military, especially for MWDs: (1) ehrlichiosis and (2) babesiosis. *Ehrlichia canis*, the causative agent for canine monocytotropic ehrlichiosis, is an intracellular organism infecting circulating and marginal lymphocytes and mononuclear cells in the mammalian host (Figure 11-7). These organisms are typically only seen during the acute, febrile stage of infection. If undetected and untreated, they can persist for years in an infected host, potentially causing chronic, cyclic illness and mortality rates upwards of 25%.[223] *Rhipicephalus sanguineus,* the brown dog tick, (Figure 11-8) is one of the few species with a worldwide distribution and is the primary vector in dogs.[224]

Babesia canis, an intracellular, erythrocytic parasite infecting dogs and other canine species, is approximately 4 to 7 μm in length, and is often described as teardrop in shape (Figure 11-9). Like *E canis, B canis* is most commonly transmitted by the brown dog tick, *R sanguineus*, but depending on the subspecies of *E canis* and the geographic location, may also be transmitted by tick genus *Dermacentor* (eg, the American dog tick) or *Haemaphysalis* (eg, the yellow dog tick).[225]

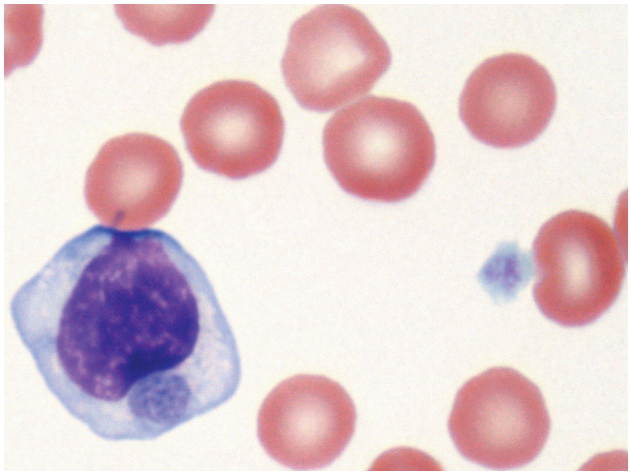

Figure 11-7. *Ehrlichia canis.* Peripheral blood from a dog, Wright Giemsa, 100X objective. A blue, stippled, round morula, consistent with *E canis* or *Ehrlichia chaffeensis*, is adjacent to and indenting the round nucleus of this intermediate lymphocyte.
Photo courtesy of Heather L. Wamsley, Doctor of Veterinary Medicine, Assistant Professor, Clinical Pathology Residency Coordinator, Department of Physiological Sciences, University of Florida, College of Veterinary Medicine, Gainesville, Florida 32608.

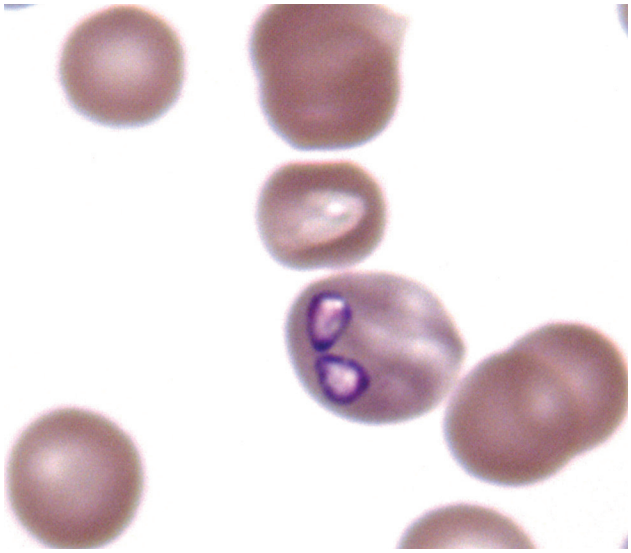

Figure 11-9. *Babesia canis.* Peripheral blood from a dog, Wright Giemsa, 100X objective. Two pyriform, basophilic intraerythrocytic piroplasms consistent with a large *Babesia*, such as *B canis*, are present.
Photo courtesy of Heather L. Wamsley, Doctor of Veterinary Medicine, Assistant Professor, Clinical Pathology Residency Coordinator, Department of Physiological Sciences, University of Florida, College of Veterinary Medicine, Gainesville, Florida 32608.

Figure 11-8. The brown dog tick, *Rhipicephalus sanguineus*, adult male, adult female, and nymph.
Photo courtesy of Katherine Sayler, chapter author.

Epidemiology

Transmission. Transmission for both *E canis* and *B canis* is primarily from infected tick bites but may also occur as a result of blood transfusion or inadvertent inoculation of blood (eg, bite wound or contaminated needle) from an infected carrier animal.[226]

Geographic Distribution. Infections with *E canis* have been reported in all 50 states and regions in Canada, Asia, South America, and Africa. Canine babesiosis also is of worldwide significance. Subspecies of *B canis* can be found in the United States, Africa, Asia, Australia, Europe, and other parts of North and South America.[225]

Incidence and Prevalence. Prevalence varies with tick density. For example, certain areas of the western and southeastern parts of the United States have high seroprevalence, ranging from 1.9% to 7.4%. Although published data is limited, areas in South America and the Caribbean Islands may have a much higher seroprevalence and more pathogenic strains of *E canis*.[227] Seroprevalence of *B canis* is highest in the southern region of the United States, ranging from 3.8% to upwards of 50%; parts of Europe and South Africa have similar rates.[228]

Pathogenesis and Clinical Findings

Erhlichiosis. *E canis* infection is a multisystemic disease potentially involving multiple organs and organ systems. The course of infection may present in three clinical phases: (1) acute, (2) subclinical, and (3) chronic. The acute phase of infection is often seen 2 to 4 weeks after tick inoculation and typically manifests in transient illness that largely goes unrecognized. Many infected dogs recover spontaneously without medical attention.[229]

However, depending on the strain's virulence and the dog's health status, clinical signs and symptoms, including fever, anorexia, lethargy, oculonasal discharge, and petechiation, might be visible. Lymphadenomegaly and splenomegaly also have been observed in about 20% of symptomatic cases. Common laboratory findings for acute stage infection include thrombocytopenia, mild leukopenia, and mild anemia (usually nonregenerative).[229]

If clinical findings go undetected during the acute stage, the infection progresses into the subclinical phase, during which the patient appears clinically healthy, but remains persistently infected, possibly exhibiting thrombocytopenia or mild nonregenerative anemia. Some animals then progress to the chronic phase of infection. During this stage, various clinical findings reemerge in varying degrees of severity among patients: weakness, anorexia, weight loss, fever, pallor lymphadenopathy, hepatomegaly, splenomegaly, retinal lesions, edema, nonseptic polyarthritis, and CNS disease.[229]

The most common hematologic abnormality observed in chronic disease is thrombocytopenia. In severe cases, pancytopenia develops as a result of severe, typically irreversible bone marrow damage. Other common laboratory abnormalities seen with chronic ehrlichiosis include granular lymphocytosis, elevated liver enzymes, and hyperglobulinemia. The prognosis for such chronically infected dogs is grave, with mortality rates of up to 25%.[229]

Babesiosis. The severity of babesiosis varies with the animal's age and strain of *Babesia* involved. For example, although US strains generally cause mild or unapparent disease in nonimmunosuppressed adults and severe disease in puppies or immunocompromised dogs, South African (and possibly South American) strains cause severe disease or death in pups and healthy adult dogs. Clinical signs may include lethargy, anorexia, pale mucous membranes, fever, emesis, amber to brown urine, splenomegaly, icterus, weight loss, rapid respiration, and rapid heart rate.[225]

Infected animals are also usually anemic, primarily resulting from intravascular hemolysis and less commonly from extravascular erythrocyte destruction. A regenerative response (ie, reticulocytosis) is present in most cases. Mild to severe thrombocytopenia is also often present, with no hemorrhaging. Clinical chemistry profiles can be normal, but they may demonstrate bilirubinemia and abnormalities related to anemic hypoxia. Although bilirubinuria is common, prominent hemoglobinuria is rarely noted in US dogs.[225]

Diagnostic Approaches

Microscopic Evaluation. Detecting *E canis* morula in leukocytes is not a reliable means of diagnosis because it is so difficult, even during the acute infection stage. Detection is optimized by performing buffy coat smears of peripheral blood or by evaluating tissue aspirates taken from the spleen or lymph nodes, which typically harbor the organism.[229]

A definitive diagnosis of *B canis* infection is usually made by identifying the organisms in stained blood films. Blood collected from capillaries (typically by performing an ear prick) may have higher concentrations of parasites than blood collected from a large vein, such as the cephalic or jugular vein. However, when parasites are not observed in the blood film, this disease is often confused with autoimmune hemolytic anemia because most animals with babesiosis are Coombs' test positive, and sometimes have autoagglutination.

Serology. Detection of antibodies to *E canis* is the most reliable and frequently used method for confirming a diagnosis. A negative antibody response in animals suspected to be acutely infected should be repeated in 2 to 3 weeks. As with other vector-borne infections, a positive serology could indicate active infection, latent infection, or previous exposure.

Most veterinarians use the in-house ELISA assay (SNAP® 3DX, 4Dx, and 4Dx-Plus) as their go-to qualitative method. If quantification of antibody levels is desired, the IFA test can be used. IFA tests for *E canis* are offered at most commercial diagnostic laboratories. Although this assay is highly sensitive in detecting *E canis* antibodies, false-positive reactions can occur from cross-reactive antibodies and nonspecific binding. Serological diagnosis can also be made for babesiosis using IFA tests, but some cross-reactivity occurs between babesial species, sometimes resulting in false-positive results. High titers suggest current infection, but IFA tests may be negative in acutely infected animals, especially pups.[225]

Polymerase Chain Reaction. Nucleic acid detection is rarely performed in the diagnosis of *E canis* infection but can be used to differentiate between organisms of the genus *Ehrlichia*. PCR testing for *E canis* is available at national and state diagnostic laboratories. Dogs in the acute phase of clinical disease may be PCR-positive, even prior to seroconversion. However, PCR analysis is not reliable in detecting subclinical, seropositive persistently infected carriers or animals in the chronic phase of the disease. Many false-negative results occur from the scarcity of circulating organisms in these animals. Unlike ehrlichiosis diagnosis, PCR analysis is the most reliable and accurate method to diagnose active infection or subclinical carrier animals with babesiosis. This assay can be performed at most commercial laboratories and requires a whole blood sample collected in anticoagulant.[225]

Recommendations for Therapy, Prevention, and Control

E canis must be treated early. Doxycycline is the current antimicrobial drug of choice, with a recommended dose of 5 to 10 mg/kg, twice daily for a period of 28 to 30 days. In dogs with acute or mild chronic illness, clinical signs will usually go into rapid remission within 2 to 3 days after initiating therapy. Dogs with severe chronic disease or those with pancytopenia may not respond to antimicrobial therapy.[229]

The currently recommended therapy for infection with *B canis* is Imidocarb dipropionate along with supportive care (eg, transfusions and fluid therapy). In treated dogs, Imidocarb dipropionate eliminates the infectivity of feeding ticks and provides prophylactic activity for up to 6 weeks following a single injection. However, antibabesial drugs are potentially dangerous, causing neuromuscular signs and liver or kidney damage. Treated and untreated dogs may also remain carriers even after clinical signs have resolved.[218]

Tick-borne disease surveillance is a prevention and control technique that provides period- or point-prevalence rate information. Past studies demonstrate the value and need for such zoonotic and infectious disease surveillance to the military.[222,226–230] In fact, in order to effectively decrease disease exposure risk and occupational and environmental hazards, medical and military planners must know the risks for contracting tick-borne pathogens in each and every deployment. This information must then be disseminated to service members so that other preventative measures can be appropriately implemented and tick-borne disease symptoms can be recognized sooner for more immediate medical intervention. The proper use of protective clothing and application of acaracides and repellants are important measures for all service members and MWDs that engage in activities where the risk of tick exposure is high. In addition, frequent examinations for embedded ticks should be conducted after at-risk civilians and service members receive instructions regarding proper techniques for tick removal. Surveillance of tick-borne pathogens—combined with aforementioned preventive measures, treatment regimes, and other disseminated information—best ensures continued health and well-being of deployed MWDs, handlers, and other service members.

Staphylococcus Aureus

Introduction and Military Importance

Although many infectious diseases are reported in marine mammals, *Staphylococcus aureus* is currently the most significant single pathogen for the cetaceans within the US Navy Marine Mammal Program (MMP) population[231] (see also Chapter 7, Marine Mammal Program), and it is an important cause of pneumonia within managed dolphin populations.[232] Outside of the MMP population, *S aureus* has been isolated not only from animals with pneumonia, but also from animals with septicemia, embolic nephritis, cerebral abscesses, and cutaneous lesions.[233] Within the MMP population, *S aureus* has been isolated from animals with overwhelming sepsis, acute septicemia with renal and hepatic involvement, bacterial bronchopneumonia, fulminating bacterial pneumonia, fibrinous pleuritis, mastitis, placentitis, abortion, vaginitis, abscesses, and skin infections. Additionally, rare methicillin-resistant *S aureus* or MRSA infections have been identified within the MMP population (MMP, unpublished data, 2011).

Description of the Pathogen

S aureus is a facultative anaerobic, Gram-positive cocci that occurs singly, in pairs, and tetrads, and is typically an opportunistic pathogen, with infection resulting from bacteria invading a breach in the integrity of the immune system or integument. Along with *Streptococcus* and *Pneumococcus*, *S aureus* is classified as a pyogenic cocci.[234]

Evidence also suggests that *S aureus* is part of the normal microbial flora in marine mammals[235] and has been isolated from healthy dolphins in both managed and wild populations.[236] A 2-year study of managed healthy dolphins in coastal open seawater found that in 20% of the animals (ie, 11 out of 55) *S aureus* was isolated from the blowhole.[237] Additionally, *S aureus* was cultured from tongue and oropharynx samples from healthy animals within the MMP population (MMP, unpublished data, 2011).

Epidemiology

Transmission. Transmission is suspected to be through the respiratory tract, mucocutaneous surfaces, and broken skin. While air exchange in humans is 20% per breath, consisting mainly of air in the upper airway, dolphins take short and deep breaths, with an exchange of 75 to 90% of air in one-third of a second, enabling deep lung exposure to airborne threats at the marine surface. This large volume exchange increases the risk of respiratory infections.[238] The risk of deep lung infections also is increased because dolphins have no turbinates or nasal hairs to filter inhaled foreign material and pathogens.[238]

Anecdotal evidence suggests that a preexisting skin infection may lead to systemic infection. Within the MMP population, several cases reveal a route of

infection through skin lesions with more systemic clinical signs appearing later (MMP, unpublished data, 2011).

Geographic Distribution. *S aureus* occurs throughout the world on skin and mucocutaneous surfaces of terrestrial animals and birds.[239] Because this organism is ubiquitous, evidence of the bacteria in dolphin blowhole swabs or other samples does not establish pathogenicity. In fact, *S aureus* has been found in several MMP dolphin blowhole samples with no indication of concurrent disease (MMP, unpublished data, 2011), providing further evidence that this organism may be part of the normal dolphin microbiota.

Incidence and Prevalence. Within the MMP population, *S aureus* infections resulted in 15 significant clinical cases in the last 20 years and accounted for 5.2% of total bacterial isolates (MMP, unpublished data, 2011). Of the dolphins necropsied from 1980 to 2010, 50% were diagnosed with pneumonia, and 19% of these cases were caused by *S aureus*.[240] Again, while incidence has been tracked, the actual prevalence is difficult to determine because the organism is ubiquitous and often considered normal flora. Additionally, within the marine mammal veterinary field, determining antemortem infection is further complicated by the presence of commensal and environmental microorganisms.[231]

Pathogenesis and Clinical Findings

As a prey species, dolphins may be stoic, often not exhibiting expected clinical signs until significant disease is present. Because initial clinical signs of systemic disease are often subtle and nonspecific, the animal care staff often serves as the most significant source of history, observing minor changes in behavior and alerting the veterinary team about potential disease indicators. Within the MMP population, such observed early clinical signs included reluctance to perform trained behaviors, blepherospasm, abnormal odor from the blowhole, increased blowhole discharge, lethargy, and partial to complete anorexia (MMP, unpublished data, 2011).

As disease progressed, observed clinical signs included abnormal respiratory character, tachypnea, shallow breaths, dull eyes, anorexia, skin desquamation, lethargy, foul breath odor, disorientation, mucohemorrhagic vaginal discharge, abortion, difficulty maintaining buoyancy, dyspnea, and acute death. In one case, frothy purulent discharge from the external auditory meatus was observed when the dolphin was out of the water. Clinical pathology findings include leukocytosis with a mature or left-shift neutrophillia or severe leukcopenia, low iron, low alkaline phosphatase, and increased erythrocyte sedimentation rate, an indication of inflammation (MMP, unpublished data, 2011).

The highest risk infections within the MMP (and other managed dolphin populations) are usually associated with septicemia and pneumonia.[231] In managed populations other than the MMP, early clinical signs for systemic infections include pyrexia, lethargy, and inappetence.[241] More progressive clinical signs include acute onset of vomiting, anorexia, diarrhea, lethargy, and significant leucopenia. Potential sequelae of *S aureus* infection are not limited to superficial necrotizing enteritis,[242] necrotizing suppurative bronchopneumonia, pyelonephritis, pyogranulomatous myocarditis, fibrinous suppurative epicarditis, osteomyelitis, leptomeningitis, abscesses within lymph nodes and skeletal muscle,[243] suppurative nephritis, pneumonia, myositis, and encephalitis.[232]

S aureus skin infections can present with clinical signs that are similar to other superficial infections, such as eruptive or ulcerative skin lesions, abscesses, refusal to perform trained behaviors, and a history of exposure dermatitis. Infections occur following exposure dermatitis, lacerations, tooth rake wounds, or any other insult to the integrity of the integument. Clinical pathology findings are similar to systemic infections but usually less dramatic (MMP, unpublished data, 2011).

Diagnostic Approaches

Initial diagnostics include, as appropriate, a full physical exam, complete blood count, chemistry panel, and full body ultrasound. Ultrasound findings may reveal ascities, pleural effusion, focal to multifocal areas of consolidation in the lung field, increased comet tail artifacts, irregularities noted on the pleural surface, and hypoechoic foci on the pleural surface (MMP, unpublished data, 2011). If respiratory involvement is suspected, evaluation of the blowhole and its sinus cytology is useful. At the MMP, *S aureus* has been cultured from blood, milk, ear exudates, feces, abdominal masses, abscessed lymph nodes, lung abscesses, and blowhole swabs (MMP, unpublished data, 2011).

Other diagnostic modalities employed may include bronchoscopy, ultrasound-guided biopsies and aspirates, radiography, and computed topography. *S aureus* has been isolated on necropsy samples from the lung, pleural fluid, blood, liver, spleen, kidney, stomach, duodenum, jejunum, adrenal gland, lymph nodes, oropharynx, blowhole, and placenta (MMP, unpublished data, 2011).

Recommendations for Therapy and Prevention

Depending on disease severity and organism sensitivity patterns, therapeutic regiments used at the MMP usually include oral, intravenous, or intramuscular

antibiotics. Antifungals are often used concurrently because fungal overgrowth in the gastrointestinal and respiratory tracts is likely during long-term antibiotics regiments. Supportive care is also an essential part of therapy and often includes fluid support, gastrointestinal protectants, pain medication, and appetite stimulants.

Again, because marine mammals are often stoic, clinical signs alone are not useful when determining treatment length. Clinical pathology and other diagnostic tools, including culture, cytology, and ultrasound monitoring, better formulate disease resolution and therapy length.

The preventive medicine program established by veterinary personnel within the MMP is the cornerstone to limiting severe infection occurrences, including those caused by *S aureus*. This program consists of five main components: (1) routine physical exams, (2) sanitation and nutrition oversight, (3) record keeping, (4) animal care education, and (5) deployment support.

Although daily medical checks, routine comprehensive physical exams, routine animal morphometric analysis, detailed record keeping, regular deworming, high quality diet, environmental monitoring and maintenance, and mental and social enrichment when training or when deployed all play a role in maintaining a healthy population, aggressive topical treatment of any break in integument integrity is also essential to prevent severe systemic *S aureus* infections. The MMP's multimodal approach to animal health and preventive medicine also facilitates early detection of disease, which impedes development of severe systemic *S aureus* infections.

TRANSBOUNDARY ANIMAL DISEASES OF MILITARY IMPORTANCE

The Food and Agriculture Organization of the United Nations defines transboundary animal diseases (TADs) as "those that are of significant economic, trade and/or food security importance for a considerable number of countries; which can easily spread to other countries and reach epidemic proportions; and where control/management, including exclusion, requires cooperation between several countries."[244(p6)] Although many TADs are categorized as foreign animal diseases in the United States, they are often endemic in developing countries where US service members deploy. Eradication of endemic diseases requires a coordinated, multinational response that far exceeds DoD abilities. However, during redeployments, military veterinarians work to prevent accidental transfer and importation of TADs into the United States caused by movement of infected animals, introduction of contaminated feed or garbage to livestock, exposure to fomites, and contact with infected humans. An overview of some common TADs and what military veterinarians do to control them at home and abroad follows.

New and Old World Screwworm

Introduction and Military Importance

Several types of larval dipterans can infest the subcutaneous skin or organ tissues of domestic animals and humans, leading to a condition known as myiasis.[245] The New World Screwworm (NWS), *Cochliomyia hominivorax* (Coquerel), and the Old World Screwworm (OWS), *Chrysomya bezziana* (Villeneuve), are obligate parasites of mammals during the larval stages of their life cycle, targeting sites of fresh epidermal wounds and mucous membranes. Aggressive larvae feeding and additional oviposit by subsequent female flies can cause traumatic myiasis, secondary infections, disfigurement, and, if left untreated, host death.[246–250]

Generally, NWS flies are found in the Western Hemisphere while the OWS flies are found in the Eastern Hemisphere.[248] However, with the increasing ease of global movement of humans, animals, and cargo via ships and aircraft, cases of *C hominivorax* transferring to regions or resurfacing in countries where the flies had previously been eradicated have been documented.[249,251–254] For example, in 1988, *C hominivorax* was discovered in Libya, immediately threatening neighboring countries on the African continent until eradication occurred in 1991.[255] Additionally, imported cases of *C hominivorax* were reported in screwworm-free nations such as the United States, Mexico, the United Kingdom, and Australia.[249,251,252]

Military personnel and working and companion animals stationed in areas where the two flies remain endemic are at risk for infestation and possible translocation to the United States. Programs used by the US Army Veterinary Corps to prevent *C hominivorax* entry from military installations in Central America (eg, Panama) and the Caribbean (eg, Cuba) should be applied in countries harboring *C bezziana*, especially in those with high troop populations and movement (eg, Iraq and Afghanistan).[251,256,257] Physicians and other human health care professionals should also be aware of potential screwworm infestation in wounded soldiers.[258–260]

Description of the Pathogen

The adult *C hominivorax* is 8 to 10 mm long and has a blue to blue-green metallic color, with three dark longitudinal stripes on its thorax's dorsal surface. *C*

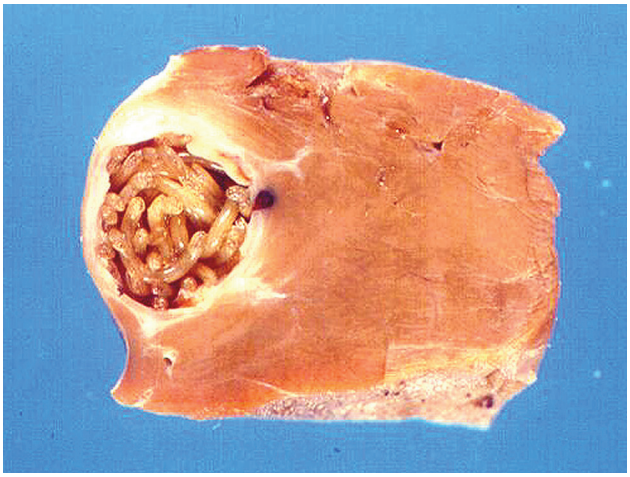

Figure 11-10. Bovine: New World screwworm (*Cochliomyia hominivorax).*
Photo courtesy of Bruce H. Williams, Doctor of Veterinary Medicine, Senior Pathologist, Department of Defense Joint Pathology Center, Silver Spring, Maryland.

bezziana is similar in size and color but has two horizontal thoracic bands, and its squamae have more fine hairs. As third instar larvae, both screwworm species have prominent spiny rings around the body, giving them a "wood screw" appearance, and their dorsal tracheal trunks are darkly pigmented (Figure 11-10). In the NWS, the pigmentation extends from the ninth to the twelfth segment in the NWS; in the OWS, only the twelfth segment is pigmented.[249,261]

Epidemiology

Transmission. The NWS and OWS flies prefer fresh uncontaminated cutaneous wounds, including insect bites, and mucous membranes of warm-blooded mammals.[248,262] Adult gravid female flies oviposit up to 400 eggs in overlapping rows, arranged in a shingle-like pattern on the wound's dry margin.[263] After 12 to 21 hours, the larva hatch and creep into the wound, burrowing deep into the flesh. The larva then feed on fluids and live tissue using their hook-like mouthparts and pass through three larvae stages or instars. Odor emitted from the infested and infected wound attracts other gravid females who contribute additional eggs.[264,265]

After approximately 5 to 7 days, grown larvae emerge from the wound, fall to the ground, and burrow into the soil to pupate. The pupal period varies dramatically from 1 week to 2 months. The life cycle length is also divergent, depending on ambient temperatures. In tropical climates, the life cycle is completed in 18 to 21 days; in more temperate conditions, 24 days; and during cooler weather, 60 days.[249]

Geographic Distribution. Before initiation of eradication programs using the sterile insect technique, *C hominivorax* extended from the southern United States southward to Uruguay, northern Chile, and Argentina. At present, the following areas are considered screwworm-free: the United States, the Virgin Islands, Mexico, Curacao, Puerto Rico, Guatemala, Belize, El Salvador, Honduras, Nicaragua, and Costa Rica. However, *C bezziana* is distributed throughout much of Africa, the Middle East, the Indian subcontinent, and Southeast Asia.[249,266]

Prevalence. Although the NWS and OWS are included on the World Health Organization Global Early Warning System for major animal diseases and zoonoses,[267] infestations continue to be underreported, particularly for *C bezziana*. In endemic areas up to 100% of livestock neonates can become infected, typically through their umbilicus.[248]

Pathogenesis and Clinical Findings

The pathogenesis and clinical findings are the same for both *C hominivorax* and *C bezziana*: suppurative, malodorous enlarged wounds with extensive tissue destruction, an active maggot population, and a serosanguinous discharge. In addition to the major wound, smaller openings in the skin posterior to the main wound may be present. For concealed or pocket-type wounds, animals may also be febrile, uncomfortable, and anorexic. In livestock, the patient might separate from the rest of the herd and exhibit weight loss and reduced lactation.[248,262,268]

Diagnostic Approaches

Larvae should be mechanically removed from the deepest part of the infested animal's wound and fixed in 80% ethanol or isopropyl alcohol for identification under a stereomicroscope at a certified reference laboratory.[249,268] Recently, cuticular hydrocarbon analysis, mitochondrial DNA analysis, random amplified polymorphic DNA PCR, and PCR-restriction fragment length polymorphism also have been explored as identification and geographical distribution mapping options.[264,269,270] Serological tests have not been standardized yet, but remain a promising diagnostic tool for determining exposure.[271]

Recommendations for Therapy and Control

In companion animals, the affected animal's wound is debrided of necrotic tissue, irrigated with saline or an antiseptic solution, and accessible larvae are manually extracted on successive days until the wound heals. Oral and topical flea and tick control,

nonsteroidal anti-inflammatory drugs, analgesics, and antibiotics may be indicated. Ivermectin injections and pyrethrin or pyrethroid sprays also work well.[272] In domestic livestock, effective wound management as described above, in conjunction with the administration of organophosphate insecticides (eg, coumaphos, dichlofenthion, and fenchlorphos); carbamat; and pyrethrtoid compounds as dips or sprays not only successfully clears newly hatched larvae and immature forms, but also repels adult gravid female flies.[249]

In endemic areas, wound prevention, time management, and direct observation of animals are crucial preventative and control measures. Animal husbandry practices such as castrating, dehorning, docking, and ear tagging should be modified, avoided, or done during seasons when screwworm flies are at low levels, as should planning newborn animal arrivals, if feasible.[248,249,251] Prophylactic measures may include organophosphate dips and sprays, ivermectin and doramectin injections, and topical applications of insect growth regulators such as dicyclanil.[273]

Because vaccines and other associated biologicals have not been developed to prevent screwworm infestation,[248,249] sterile insect technique is the only proven method for successfully eradicating the screwworm from infested areas.[274–277] The sterile insect technique is comprised of the following consecutive steps: (*a*) Male pupae are irradiated with gamma rays rendering emerging males infertile[278]; (*b*) a higher number of sterile males than exists in the local screwworm population is released via aerial dispersion into the endemic area; (*c*) the sterile males mate with female screwworms flies, and (*d*) unfertilized eggs are oviposited. Male screwworm flies mate several times while female screwworms mate only once, a physiological feature contributing to the technique's overall success.[279] This procedure, in concert with topical insecticide application, decontamination of animal conveyances, aggressive and committed government regulatory support, and public information campaigns, is vital to eradication programs.[249,280]

For example, animals should be rigorously inspected before and after shipment using consistent quarantine procedures. Within 5 days prior to shipment, MWAs and companion animals returning to the United States from screwworm endemic areas need a valid health certificate specifically stating that the animal in question has been inspected and found to be free of screwworms. Additionally, transport vehicles, other conveyances, and holding equipment should be treated with insecticides to kill any adult or immature screwworms.[248,249,251,280]

African and Classical Swine Fever

Introduction and Military Importance

African swine fever (ASF) and classical swine fever (CSF), also known as hog cholera, are highly contagious, viral pig diseases. Both have devastating impacts on a country's pig industry and can lead to trade restrictions.

ASF and CSF are reportable diseases not only in the United States, but also to the OIE.[47] According to the Food and Agriculture Organization of the United Nations, ASF is also considered a transboundary disease because of the contagious nature of the disease, high mortality rate of some strains, potential for very rapid spread across national borders, and substantial effect on national economies.[281]

ASF and CSF viruses are easily introduced into an area via imported infected pork products; pigs that eat unprocessed garbage containing infected tissue; and fomites such as vehicles, equipment, and personnel.[211] DoD personnel—military and civilian—contribute to disease transmission by transporting infected pig products or acting as fomites for the viruses during movements between countries (eg, foreign humanitarian assistance or veterinary stability operations missions) and upon redeployment. While ASF and CSF are highly contagious diseases in swine, humans are not susceptible to either virus.[47]

Description of the Pathogen

ASF virus, an enveloped DNA virus, is the only member of the genus *Asfivirus,* family Asfarviridae,[47,211,282] and the only arthropod-borne DNA virus. Although different strains have varying virulence, there is only one recognized serotype.[47,249,282]

CSF virus is an enveloped RNA virus and a member of the genus *Pestivirus,* family Flaviviridae. Only one serotype has been identified, although there are many strains. CSF virus is closely related to the ruminant pestiviruses responsible for bovine viral diarrhea/mucosal disease complex and border disease of sheep and goats. Ruminant pestiviruses can infect pigs, producing congenital infections similar to those seen with CSF.[47,211]

Epidemiology

Transmission. ASF virus is transmitted oronasally by contact with infected animals, infected body fluids and tissues, especially blood, and via the soft tick *Ornithodoros moubata*.[47,211] Viral transmission in ticks occurs transovarially, transstadially, and sexually,

which means infected tick colonies can maintain the virus for years.[47,282] *O moubata* lives in warthog burrows, infecting newborn hogs shortly after birth.[47,211]

CSF virus is spread orally or oronasally by direct or indirect contact with infected tissue, blood, secretions, and excretions including semen.[47] The virus is also transmitted transplacentally.[47,249] Mechanical transmission by birds, insects, and other wild or domestic animals may occur. In studies, airborne transmission up to 1 km has been reported.[47]

Most ASF and CSF infections are introduced through the practice of feeding garbage containing unprocessed infected pig products. The viruses can also be transmitted by contaminated vehicles, equipment, and clothing.[47, 211]

Geographic Distribution. Although ASF has never been reported in the United States, it is endemic in most of sub-Saharan Africa and the islands of Sardinia and Madagascar, with the highest incidence occurring just north of the equator.[47,211,282] During the 1970s, ASF was confirmed in the Caribbean and South America but has now been eradicated from these countries. The disease also has been eradicated from most of Europe, with confirmations only in Armenia, Azerbaijan, the Republic of Georgia, and Russia.[47,282]

CSF can be found worldwide. Although the prevalence of the disease on the African continent is unknown,[283] it is endemic in Asia, with a higher prevalence in East and Southeast Asia, India, and China as well as the island of Madagascar; Mauritius; the Caribbean islands of Cuba, Haiti, and the Dominican Republic; Central America, with the exception of Belize and Panama; and South America, with the exception of Chile and Uruguay.[47,211,283]

The disease is endemic in southern Mexico where there is a large number of backyard pig populations.[283] While vaccination is used in southern Mexico to control CSF, Central Mexico is considered a control zone where vaccination is not practiced, and northern Mexico is CSF-free. CSF also has been eradicated from Australia, New Zealand, the United States (the last case was reported in 1976), and Canada. CSF has been eradicated from domestic herds in most of Western and Central Europe as well, but the virus remains endemic in the wild boar population in certain parts of Europe.[47,211]

Host and Reservoir Species. ASF produces clinical disease in domestic swine, feral pigs, and European wild boars.[47,211] The virus infects warthogs, bush pigs, forest hogs, and the peccary, but these species are asymptomatic. The first three species are considered reservoirs for the virus in Africa, and the peccary is a potential reservoir in the Americas. In Africa, the disease is maintained by transmission between the warthog and *Ornithodoros* species.[47,211,282] Unlike ASF virus, domestic pigs and wild boars are the only reservoirs for CSF virus; there is no vectorborne component.[47]

Pathogenesis and Clinical Findings

ASF's incubation period varies from less than 5 days after tick exposure to 5 to 19 days following direct contact with infected pigs.[47,211,249] The more virulent strains produce peracute or acute disease in less than 1 week. Less virulent strains spread more slowly through herds, often lingering for several weeks, causing chronic disease that produces milder symptoms easily confused with other diseases (eg, emaciation, stunting, swollen joints, and respiratory problems).[47,282]

With more virulent strains, mortality rates may be as high as 100%; death occurs within 7 to 10 days from onset of clinical signs, especially in young animals. Although sudden death may be the first sign of ASF infection in herds,[47] other characteristic clinical signs of peracute and acute disease include high fever, inappetence, recumbency, and cyanotic skin blotching or petechial hemorrhages on the skin, especially the ears, tails, legs (Figure 11-11), and abdomen.[47,211,249] Epistaxis and hematochezia also may be observed. Pregnant animals frequently abort. At necropsy, hemorrhagic lesions are observed in the spleen, kidneys, heart, and lymph nodes (Figure 11-12). Animals that recover from acute or chronic ASF are persistently infected and serve as carriers.[47,49]

CSF's incubation period ranges from 5 to 15 days. With low virulence strains, the only observable signs may be poor reproductive performance, decreased litter

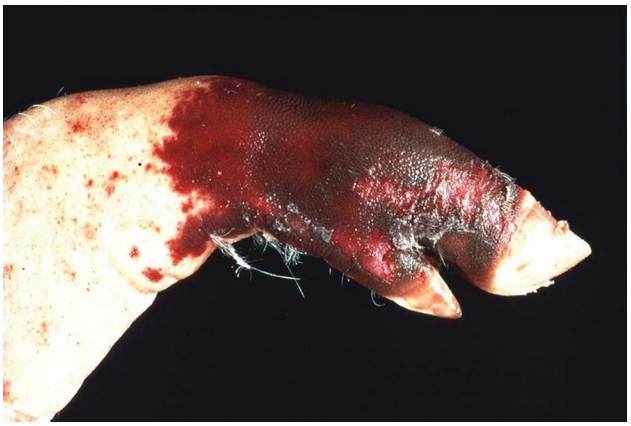

Figure 11-11. Swine: Cyanotic discoloration of the skin seen with African and Classic Swine Fevers.
Photo courtesy of Dr Jens Teifke, Institute of Molecular Biology, Friederich-Loeffler Institute, Federal Research Institute for Animal Health, Riems, Germany.

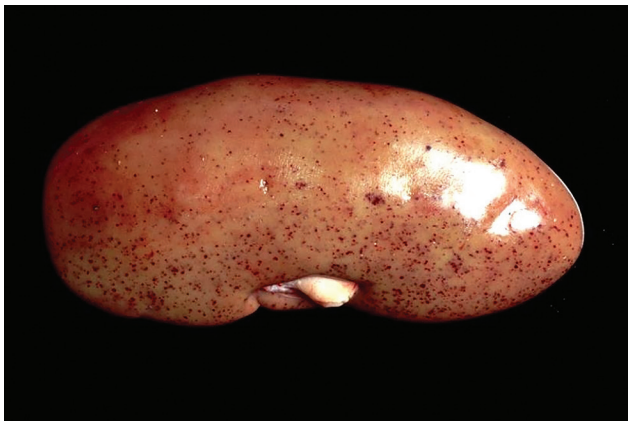

Figure 11-12. Swine: Swollen kidney with petechial hemorrhages seen in acute disease with African and Classic Swine Fevers.
Photo courtesy of Dr Jens Teifke, Institute of Molecular Biology, Friederich-Loeffler Institute, Federal Research Institute for Animal Health, Riems, Germany.

size, stillbirths, and piglets born with neurological deficits. Piglets appearing asymptomatic at birth are viremic and invariably develop clinical disease within a few months, with 100% mortality occurring within the first year.[47,211,284,285]

Acute CSF is usually associated with highly virulent strains, with corresponding high morbidity and mortality.[47] Clinical signs include fever, anorexia, weakness, constipation followed by diarrhea, and incoordination progressing to hind limb paralysis.[47,211] Convulsions may be observed during the terminal stages of the disease. Death occurs within 2 to 3 weeks following onset of clinical signs.[47,211]

Petechial hemorrhages and cyanotic discolorations may also develop on the ventral abdomen, medial thighs, ears, and tail. At necropsy, petechial and ecchymotic hemorrhages are seen on the lymph nodes, kidneys, spleen, bladder, and larynx. Nonsuppurative encephalitis with vascular cuffing may also be present.[47,211]

The subacute form of CSF is typically seen in older pigs.[47] Although subacute morbidity and mortality rates are lower than the acute form, detection is more difficult. Fever may be the only symptom.[285]

The chronic form of CSF occurs with less virulent strains or in partially immune herds. Affected pigs appear to recover within a few weeks, but symptoms recur, wax, and wane for several weeks to a few months, with progressive loss in condition and eventual death.[47,211]

Morbidity is lower with chronic CSF infections (ie, only a few animals in the herd may be affected) but results in 100% mortality. At necropsy, necrotic foci or button ulcers are observed in the mucosae of the larynx and epiglottis and in the intestinal mucosae near the ileocecal junction. In young, growing pigs that survive more than 30 days, bone lesions may be seen at the ribs' costochondral junctions and on long bones' growth plates.[47,211]

ASF and CSF are clinically indistinguishable.[211] Differential diagnoses include acute porcine reproductive and respiratory syndrome, porcine dermatitis and nephropathy syndrome, erysipelas, other septicemias, warfarin, and heavy metal toxicoses.[47] When congenital infections are observed, infections with ruminant pestiviruses also must be ruled out in breeding pigs. As with other viral infections, concurrent bacterial infection (eg, porcine dermatitis and neuropathy syndrome, salmonellosis, and erysipelas) can mask and delay the diagnosis of underlying ASF virus and CSF virus infections.[249]

Diagnostic Approaches

To confirm ASF, laboratory diagnostics are necessary and include virus isolation, fluorescent antibody tests, and PCR. Virus isolation, particularly the hemadsorption test, is the preferred diagnostic methodology because a positive result is a definitive diagnosis. During this procedure, blood, spleen, lymph node, tonsil, or kidney tissue is inoculated into pig leucocytes (ie, monocytes or macrophages) or bone marrow cultures. A positive result is obtained when pig erythrocytes adhere to pig monocytes or macrophages infected with ASF virus (ie, hemadsorption).[47,249]

PCR is the most sensitive test for detecting the virus in persistently infected animals or when virus isolation cannot be performed. In endemic areas, serology using serum or tissue fluids also is utilized. A combination of the ELISA and indirect fluorescent antibody test or immunoblotting test is used to detect subacute or chronic disease. The ELISA is also the prescribed test for international trade.

The laboratory diagnostics necessary to confirm CSF have inherent pros and cons. Virus isolation, the "gold standard," is labor intensive and takes 3 to 7 days to obtain results.[211,284] The virus neutralization test, the most sensitive and specific test for antibodies, can cross-react with ruminant pestivirus infections in pigs. Direct IFA and ELISA are rapid tests, but these antigen detectors require skilled personnel and specific samples. In general, tissue samples from the tonsils, distal ileum, spleen, lymph nodes, and kidney are the preferred submissions for CSF virus antigen testing. However, tonsils are the best tissue to sample for testing during acute disease because they are infected first. During subacute or chronic disease, the distal ileum may be the only tissue to yield a positive result.[47,211,249]

Because CSF virus antibodies do not develop until 2 to 3 weeks after being infected but persist for life,[47] serology is more appropriate for testing sows with poor reproductive performance or who are producing piglets with neurological deficits and for surveillance in wild boar and feral pig populations.[47,211,284] Congenitally affected pigs, though viremic, are usually negative on serology.

The two prescribed tests for detecting CSF in international trade are the neutralizing peroxidase-linked assay (NPLA) and the fluorescent antibody virus neutralization test (FAVN).[249] Either test will differentiate CSF from ruminant pestivirus infections in breeding animals.[47]

Since hemadsorption does not occur in cells infected with CSF, the hemadabsorption test is used to distinguish ASF from CSF. Reverse-transcriptase PCR can also differentiate the two diseases. Based on overall testing advantages and disadvantages, the three tests used most commonly for ASF and CSF are the NPLA, FAVN, and ELISA.[47,211,249]

Recommendations for Therapy and Control

No treatment or vaccine is available for ASF, so preventing ASF virus introduction is critical. Control methods include eliminating importation of infected pork and pig products, safeguarding movement of carrier animals, providing adequate biosecurity, and banning use of unprocessed garbage containing infected pig blood or tissue as feed.[47,211] (If pigs must be fed trash, it should be cooked for 30 minutes at a temperature of at least 70°C to prevent ASF.[47]) Effective tick control with acaricides also should be implemented in *O moubata* endemic areas.

Because the virus can survive for extended periods of time on fomites, strict quarantine, sanitation, and disinfection must be enforced as well. Effective disinfectants include sodium hypochlorite and some iodine and quaternary ammonium compounds. Rapid diagnosis, slaughter of infected and contact animals, and proper disposal of carcasses are also essential to successful eradication programs.

Animals diagnosed with CSF should not be treated. All symptomatic and contact animals must be slaughtered and the carcasses properly disposed of. All animals on affected premises and neighboring premises within a 500-m radius should be depopulated. Similar to control and prevention measures for ASF, strict quarantine, movement control, and thorough cleaning and disinfecting of infected premises should be enforced.

A live attenuated vaccine is available and used in endemic areas.[211,284] While vaccination does control clinical disease, it allows the CSF virus to continue to circulate subclinically. Vaccination is, therefore, not recommended if the goal is eradication because it is not possible to differentiate between vaccinates and field-infected animals. The European Union (EU) controls CSF by stamping out or depopulation. However, central and eastern European countries do vaccinate during an outbreak.

Methods to prevent introduction of CSF into a country or region are similar to those for ASF. Additional methods include decreasing the pig density in high-risk epidemic areas, minimizing live animal markets, and monitoring the disease in the wild boar and feral pig population.[285] In CSF-free countries, periodic surveillance sampling of domestic herds is recommended to monitor for reintroduction of the disease.[47]

Some methods to prevent introduction of CSF virus into a country or region are similar to those for ASF virus. CSF virus is readily inactivated or destroyed by heat, drying, ultraviolet light, and most detergents, including sodium hypochlorite and phenolic compounds.[47] The virus is destroyed by cooking to a temperature of 65.5°C for 30 minutes. However, CSF virus can survive for weeks in refrigerated meats and bodily secretions and for years in the frozen state. Smoking or salt curing pork and pig products also does not destroy or inactivate CSF virus because these proteinaceous environments protect it.[47,211]

Additional CSF virus control methods that differ from ASF virus measures include decreasing the pig density in high-risk epidemic areas, minimizing live animal markets, and monitoring the disease in the wild boar and feral pig population.[285] Because some countries import European wild boar for the purposes of hunting, such vigilance is necessary.[283] In CSF-free countries, periodic surveillance sampling of domestic herds is also recommended to monitor disease reintroduction.[47]

Because the impact these viruses pose on the swine industry and trade is tremendous, certain control measures are also mandated. For example, during a CSF outbreak in the Netherlands in the late 1990s, over 8 million pigs had to be slaughtered, costing $2.3 billion.[286] Now the EU mandates that live pigs and fresh pig products may only be imported from countries or regions where no cases of CSF or vaccination against CSF has occurred within the past 12 months.[284] The United States will only import pigs and pig products from countries declared CSF-free by the OIE. Furthermore, if any EU nation has a CSF outbreak, the United States will prohibit imports of all pigs and pig products from all EU nations and will not resume trade with the EU until 6 months have passed since slaughter of the last infected herd.[286]

To honor these mandates, returning DoD personnel who have been to ASF and CSF endemic areas should neither smuggle in illegal pig products or inadvertently introduce the viruses via their clothing or equipment if they have been in contact with wild or domestic pigs in these areas.

Foot and Mouth Disease

Introduction and Military Importance

Although foot and mouth disease (FMD) affects cloven-hoofed animals, military personnel are still concerned about this severe, highly contagious disease because inadvertent spread can result in serious socio-economic and trade consequences similar to those caused by swine viruses. FMD is an OIE-listed disease based on its proven ability to spread internationally with serious socio-economic consequences and the potential for significant trade and travel restrictions.[281]

In general, FMD is endemic in developing countries already prone to food insecurities; spread of the disease only intensifies subsistence problems because surviving animals are less efficient for work and meat and milk production. In FMD-free countries, which are often developed, introduction of FMD would result in export bans, which, in turn, would lead to long-term, significant economic consequences. Because of these concerns, military personnel worry about FMD being used as a bioterrorist weapon.[287] According to a Government Accountability Office report, just one case of FMD in the United States would cause international trading partners to prohibit exports of US live animals and animal products, resulting in losses of up to $6 to 10 billion a year until the United States regained disease-free status.[288]

The USDA has identified key pathways through which this highly contagious disease might enter the United States, including on imported live animals or in animal products; on the shoes of, or in packages carried by, international passengers; in international mail; and in garbage from international carriers.[288] The USDA recommends that travelers from countries with FMD avoid contact with susceptible animals for 5 days after entry into the United States.[289] These recommendations are important for service members and their families to follow, given their frequent travel to FMD-endemic countries. In addition, service members and their families should heed recommendations protecting foreign regions and countries when deployed or stationed overseas. For example, during the 2010 to 2011 FMD outbreak in the Republic of Korea, the US 8th Army advised personnel to avoid travel to certain areas and implemented numerous other precautions such as vehicle spray points.[290]

Description of the Pathogen

FMD is caused by a virus from the genus *Aphthovirus* of the Picornaviridae family. There are seven immunologically distinct serotypes (A, O, C, SAT1, SAT2, SAT3, and Asia1) and over 60 strains within these serotypes.[47,291] All cloven-hoofed animals, including cattle, pigs, sheep, goats, buffalo, deer, antelope, wild pigs, elephants, and giraffe can become infected with FMD virus (FMDV). Old World camels appear to be resistant to FMD, while alpacas and llamas are only mildly susceptible, so neither likely plays an epidemiologic role.[291] Cattle are usually the most important maintenance hosts, but certain strains are found mostly in pigs, sheep, or goats.[47,291] Wildlife, other than African buffalo, have not been shown to be maintenance hosts.[291]

FMDV is preserved by refrigeration and freezing but becomes progressively inactivated starting at 50°C and is quickly inactivated at a pH less than 6.0 or greater than 9.0. FMDV typically remains viable in the environment for less than 3 months, although it can survive up to 6 months in very cold climates. Organic materials can also protect the virus from sunlight and drying and interfere with disinfectant effectiveness, prolonging environmental viability.[47,291]

FMDV also survives in lymph nodes and bone marrow at a neutral pH, and especially when chilled or frozen, but is destroyed in muscle at a pH less than 6.0, which occurs with rigor mortis.[47,291] The virus can survive high temperature, short-time pasteurization of milk and milk products but is inactivated by ultra-high temperature pasteurization.[291]

Epidemiology

Transmission. FMDV is easily transmitted via numerous pathways, including direct contact between infected and susceptible animals, indirect contact via contaminated fomites, inhalation of aerosols, artificial insemination with contaminated semen, ingestion of contaminated milk by calves, and consumption of untreated contaminated meat products by pigs. FMDV is found in all secretions and excretions of acutely infected animals up to 4 days before clinical symptoms are observed. Studies estimate airborne FMDV can travel up to 60 km over land and 300 km over sea.[291]

The FMDV transmission process is further complicated by the asymptomatic carrier state and human exposure to the virus. Some animals with either natural or vaccine-induced immunity enter into this state if exposed again to FMDV and harbor the virus in the oropharynx for over 28 days.[291] Approximately 15% to 20% of cattle become carriers upon reexposure,

and most will remain carriers for less than 6 months. Domestic buffalo, sheep, and goats are only carriers for a few months; however, African buffalo may remain in the state for up to 5 years.[47,287,291] Humans can harbor the virus in the respiratory tract for up to 48 hours.[47]

Geographic Distribution. Anywhere cloven-hoofed animals exist, a risk also exists for FMDV. However, it is only endemic in parts of Asia, Africa, the Middle East, and South America. Serotype O, the most common worldwide, was responsible for the Pan-Asian epidemic that started around 1990.[47]

In general, serotypes A and O are found in Africa, Asia, and South America; serotypes SAT1, 2, and 3 are currently only in Africa; and Asia1 is only found in Asia. Given these parameters, Afghanistan is of particular worry because serotypes A, O, and Asia1 are all endemic.[292]

Serotype C is extremely rare now. North America, New Zealand, Australia, Greenland, Iceland, and most of Europe are FMD-free.[47,292,293]

Incidence. Although the 2001 FMD serotype O outbreak in the United Kingdom was the single largest FMD epidemic recorded in history, numerous other countries that were previously FMD-free have recently experienced serotype O outbreaks, including Japan, in 2000; France and the Netherlands, in 2001; and the Republic of Korea, in 2002.[292] Since 2010, ongoing serotype O outbreaks have emerged in the Republic of Korea, Japan, China, North Korea, Russia, Hong Kong, and Mongolia.[294]

In fact, according to the OIE disease outbreak maps, 43 countries reported FMD outbreaks in the year 2011 alone, with Iran and Turkey each reporting over 1,000 outbreaks. However, because not all countries reported data, including many of the countries known to have endemic FMD, the number of actual outbreaks should be significantly higher. (FMD is underreported for numerous reasons, ranging from trade implications at the country level to risk of having animals culled at the individual and community levels.) Despite these relatively high numbers, according to the OIE, as of 2011, 65 countries are considered FMD-free without vaccination, while Uruguay is considered FMD-free with vaccination. Fifteen more countries have FMD-free zones with or without vaccination.[293]

Pathogenesis and Clinical Findings

Depending on virus dose and route of infection, the incubation period for FMDV ranges from 2 to 14 days.[47,291] Morbidity can reach 100%, with severity of signs varying by strain, exposure dose, age, breed, species, and level of immunity. Young animal mortality can be over 20%, with myocarditis as the main cause of death. In adult animals, mortality is low (1%–5%), with recovery in approximately 2 weeks in uncomplicated cases.[291]

While cattle, pigs, and small ruminants all can be infected with FMD, their clinical signs differ somewhat. Cattle show the most severe signs, exhibiting pyrexia, anorexia, shivering, and reduced milk production for about 2 to 3 days before vesicles appear. These vesicles are 2 to 10 mm in diameter, appearing on buccal and nasal mucous membranes, between claws and coronary bands, and on mammary glands. Because the vesicles are painful, cattle may smack lips, grind teeth, drool, show lameness, or stamp and kick their feet. After about 24 hours, the vesicles rupture, leaving ulcers or erosions (Figure 11-13) leading to complications such as superinfected lesions, mastitis with permanently impaired milk production, hoof deformation, myocarditis, abortion, permanent weight loss, and loss of heat control. The ulcers and erosions also may become covered in a fibrinous coat and appear like a dry, necrotic lesion.[47,291]

Pigs develop pyrexia, anorexia, and then, vesicles. The vesicles can occur on the snout (Figure 11-14) but are more severe on the feet. Vesicles may cause claw horn detachment, resulting in such severe pain that infected pigs might crawl rather than walk. In addition, ulcers and erosions in the pig's oral cavity tend to have a fibrinous coat, appearing like the cattle's dry, necrotic lesions. Small ruminants tend to not show

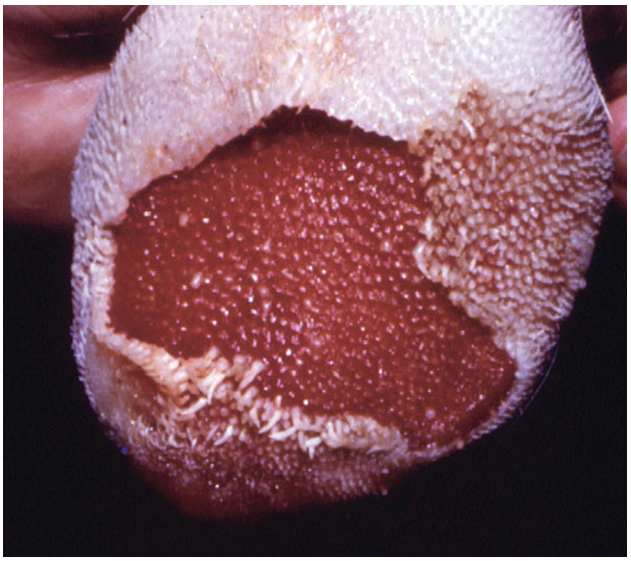

Figure 11-13. Ulceration of a bovine tongue following rupture of vesicles caused by the foot and mouth disease virus. Photo courtesy of Bruce H. Williams, Doctor of Veterinary Medicine, Senior Pathologist, Department of Defense Joint Pathology Center, Silver Spring, Maryland.

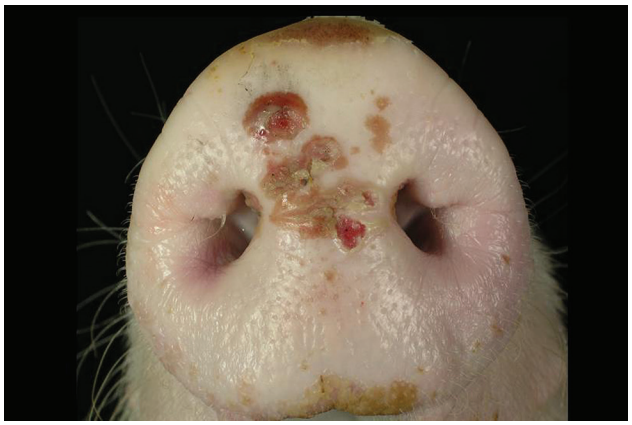

Figure 11-14. Vesicles on a pig snout caused by the foot and mouth disease virus.
Photo courtesy of Bruce H. Williams, Doctor of Veterinary Medicine; Senior Pathologist, Department of Defense Joint Pathology Center, Silver Spring, Maryland.

as many clinical signs as cattle and pigs, but infected sheep and goats may display signs of pyrexia, mild lameness, mild oral lesions, and agalactia.[295]

Diagnostic Approaches

FMD is clinically indistinguishable from vesicular stomatitis, swine vesicular disease, and vesicular exanthema of swine and has a long list of other differential diagnoses, making field diagnosis extremely challenging.[291] Diagnosis can be made in the laboratory via virus isolation, detection of viral antigens or nucleic acids, and serology.[47] The best samples for laboratory testing include tissue and fluid from an unruptured or recently ruptured vesicle and esophageal-pharyngeal fluid collected with a probang cup. Any laboratory testing for FMD virus should meet OIE requirements for Containment Group 4 pathogens and numerous precautions need to be taken prior to sending suspect FMD material within and between countries. The recent availability of commercial pen-side tests may help to mitigate these restrictions.[291]

Recommendations for Therapy, Prevention, and Control

Because FMD impacts not only animal health and productivity, but also trade restrictions and the economy, FMD must be prevented from entering countries or regions that are currently virus-free, and disease outbreaks in endemic areas must be controlled. Prevention occurs via both sanitary and medical prophylaxis. Because FMD is often introduced into a country via contaminated feed or infected animals, border control, surveillance, and quarantine are important for protecting FMD-free zones.[47,291]

In the event of an FMD outbreak, quarantine and movement restrictions, euthanasia of infected and contact animals, and cleaning and disinfection of premises and equipment are necessary for control. Carcasses, bedding, and contaminated animal products should be incinerated, buried, rendered, or disposed of via other appropriate techniques. Premises and all contacted material, including vehicles, equipment, and clothing, should be cleaned and disinfected, removing as much organic material as possible from fomites and surfaces prior to disinfection.

The decision to vaccinate as medical prophylaxis must be carefully considered from scientific, economic, political, and societal perspectives because consequences vary, based on choices made. For example, live attenuated vaccines can revert to virulence, making it difficult to differentiate natural infection from vaccination. Two types of inactivated vaccines are available—(1) a commercially produced standard potency vaccine, providing 6 months of immunity for use in endemic regions, and (2) a high-potency vaccine for use in outbreak situations[47,291]—but vaccination against one serotype does not provide protection against other serotypes; even variation within serotype strains causes vaccination failure.[292]

Further, if vaccination is used to control an outbreak and vaccinated animals were not culled, a country may not be declared FMD-free until after 1 year with no evidence of infection because vaccinated animals may become carriers. However, if vaccination is not used, a country may be declared FMD-free after only 3 months with no evidence of infection.[291]

Service members and US government agencies can initiate several control measures to keep the FMD virus out of the United States. Similar to civilians clearing US customs, service members must declare agricultural products and disclose whether they were on farms or in contact with animals while overseas. Because contaminated items will not be allowed into the United States, all personnel's clothing, gear, and equipment (eg, tents, weapons, and vehicles) should be thoroughly cleaned and arrive at US Customs free of any soil, manure, or debris.[295]

The USDA also works to ensure military units or groups returning stateside (eg, animal disease eradication missions) do not harbor infectious agents such as FMD virus. With advanced notification (7 or 30 days, depending on small-scale or large-scale operations) the USDA can determine if extra cleaning facilities are available at the first port of entry, should anything be deemed contaminated and inadmissible when first checked.[295]

Because humans can harbor FMDV in the respiratory tract for up to 48 hours, personal protective equipment needs to be used when disinfecting items utilized in endemic areas. A 3- to 5-day personal quarantine should also be implemented if exposure is suspected.[291]

Highly Pathogenic Avian Influenza

Introduction and Military Importance

While highly pathogenic avian influenza (HPAI) has had little to no direct impact on the US military, it remains a significant threat and is in the top 20 diseases of military importance[296] because of its potential impact on service member health and military readiness, as evidenced by the 1918 to 1919 influenza pandemic. Although most influenza viruses typically kill the very young or old, the 1918 virus resulted in significant morbidity and mortality rates among young adults of military age.[297] Influenza attack rates exceeding 70% were reported among US and Australian naval warships, and an estimated 1.1% of the entire US Navy force died of influenza-pneumonia during the pandemic.[298] Additionally, militaries may have played an important role in the transmission and global spread of the 1918 to 1919 pandemic virus.[299]

As a result of its military importance and potential global impact, the US military maintains an extensive global influenza surveillance system. During the 2009 influenza pandemic, this system not only detected the initial virus, but also contributed a seed virus for the vaccine.[300] This surveillance network also detected the 2009 pandemic virus in US service members stationed abroad prior to initial detection in the local population, again suggestive of the military's potential role in facilitating global virus spread (Armed Forces Health Surveillance Center quarterly surveillance reports, unpublished data, 2010).

Description of the Pathogen

Influenza viruses are single strand RNA viruses within the *Orthomyxoviridae* family and belong to one of three genera: influenzavirus A, B, or C. Influenza A viruses are further subtyped according to 16 hemagglutinin and nine neuraminidase surface proteins. Birds, able to be infected with all known subtypes of influenza A viruses, are considered to be the primary virus reservoirs, but other species including cats, dogs, horses, and pigs, and humans can also be infected.[301]

The classification between high and low pathogenic avian influenza (AI) refers to the virus' ability to cause illness in domestic poultry. While any influenza A subtype can theoretically become highly pathogenic, to date, all HPAI viruses have been H5 and H7 subtypes. Since 2004, the majority of HPAI outbreaks are of the H5N1 subtype.[302]

Epidemiology

Transmission. Among birds, HPAI is primarily transmitted through direct contact and through fecal contamination of feed, water, and other fomites (*eg,* clothing, shoes, and farm equipment). Fecal-oral transmission is especially important as virus persist in water for at least 30 days at 0°C. While movement of infected waterfowl is believed to be the primary means of global transmission, movement of infected domestic poultry or poultry products may also be an important exposure route. Airborne transmission is also possible among birds living in close proximity but is of lesser importance than direct contact or fecal-oral transmission.[303]

Swine, and on rare occasions, humans may also serve as sources of avian infection.[304,305] While the majority of human cases result from direct contact with poultry or poultry products, human-to-human transmission of HPAI has occurred on rare occasions but is generally limited and inefficient without sustained transmission. Swine may become infected with H5N1, HPAI, and other AI viruses, with the potential to serve as an intermediate host between avians and humans. Canine and feline infection with H5N1 has been reported, primarily through consumption of infected meat; however, their role as infection sources for avians and humans is unclear.[306]

Geographic Distribution. All countries are theoretically at risk for HPAI introduction because wild birds, especially waterfowl, which serve as the natural reservoir for AI viruses, may travel large distances during seasonal migrations. Additionally, any low pathogenic AI (LPAI) virus can theoretically mutate and become highly pathogenic through genetic drift and shift. In practice, though, HPAI, specifically H5N1, is only considered endemic in southeastern Asia and northern Africa, specifically Egypt, where the majority of outbreaks among domestic poultry are reported. Occasional outbreaks have also been reported from Australia, the Americas, Europe, the Middle East, and other regions in Africa and Asia. Since 2008, no human case of H5N1 has been reported outside of southeastern Asia and northern Africa.[302]

Incidence. Since 1955, when AI viruses were discovered, over two dozen HPAI epidemics have occurred globally.[302,303] The most recent H5N1 outbreak (ongoing since 1997 when it was first reported) affected 1.4 million domestic chickens in China. Since then, it has affected dozens of countries and hundreds of millions

of domestic birds. In 2011, 15 countries officially reported outbreaks of H5N1 among domestic birds to the OIE. In addition to the ongoing H5N1 epidemic, several smaller and better-controlled HPAI epidemics involving H5N2, H7N3, and H7N7 viruses have occurred in recent years.[302]

Estimating the true incidence of HPAI is difficult for multiple reasons. Initial detection of HPAI within domestic poultry and duck populations often triggers mandatory culling and depopulation of infected flocks on home farms and potentially neighboring farms as well. Because depopulation often destroys hundreds of thousands of bird carcasses, individual testing is generally not feasible. Mandatory flock destruction may also cause underreporting: poultry owners may be unwilling to report or test ill birds fearing the possibility of losing their flock and investments.

Estimating the incidence of HPAI infection among wild waterfowl and birds is even more problematic because these species may be asymptomatic and thus not be sampled or tested. HPAI prevalence in wild birds also varies by species, season, and geography. For example, only 0.82% of 3,000 fecal, cloacal, and nasal swabs from wild birds, domestic poultry, and swine in Uganda were influenza PCR-positive, and none were HPAI-positive.[307] Conversely, among 728 fecal samples from wild birds in Mongolia, 14 samples (1.9%) were positive for HPAI viruses. Routine surveillance has also detected HPAI viruses along flyways in Europe and elsewhere in Asia.[308]

Pathogenesis and Clinical Findings

HPAI refers to a particular viral strain's virulence in chickens only. Wild birds, especially waterfowl, may show little to no overt signs of clinical illness, despite being infected with an HPAI virus. The incubation period is generally between 1 to 7 days; however, the OIE recognizes a 21-day period.[309]

Morbidity and mortality vary by strain and infective dose but may approach 100% in HPAI infections. In fact, the sudden death of large numbers of birds without clinical signs or gross lesions is a common finding in HPAI infections. In those birds that do show signs, the majority will die within 72 hours or less of the onset of clinical signs, which are not specific, and with the exception of depression, vary by viral strain. These signs include decreased feed and water consumption, decreased egg production, ruffled feathers, and watery diarrhea. Respiratory disease is reported to be less common in HPAI infections than LPAI infections but may be present. In broiler flocks, clinical signs may be less obvious, and birds may also exhibit neurologic symptoms such as ataxia, paralysis, and torticollis.[309]

While LPAI infections in domestic poultry may also be asymptomatic, when present, the most common clinical signs of LPAI are respiratory related and include difficulty breathing, swollen sinuses, and nasal discharge. Decreases in feed and water consumption are common, and ill birds may huddle together or near heaters. Laying thin-shelled and misshapen eggs and producing fewer eggs (ie, a 5%–30% production drop) are also common signs, especially among turkeys.[248]

In most other infections, edema and cyanosis of the feet, head, and legs (Figure 11-15), as well as splenomegaly, are commonly reported. Splenomegaly is generally more pronounced with later deaths. Renomegaly with urate accumulation of the ureters is another repeated finding. Pulmonary congestion, edema, and hemorrhage are frequently reported in chickens, guinea fowl, and turkeys but less so in Chukars, pheasants, and quail.[310,311]

Histopathologically, common lesions are encephalitis, myocarditis, pancreatitis with acinar necrosis, myositis, and edema and inflammation of the comb. Neuronal necrosis is generally diffuse but particularly involves Purkinje neurons that also may contain intranuclear, eosinophilic inclusion bodies that stain positive for influenza nucleoprotein. Cardiovascular lesions include endothelial cell hypertrophy and multifocal lymphocytic myocarditis with necrosis. Lymphocyte depletion and necrosis in primary (eg, thymus) and secondary (eg, spleen and cecal tonsil) lymphoid tissues and mild to moderate interstitial pneumonia have also been reported.[310,311]

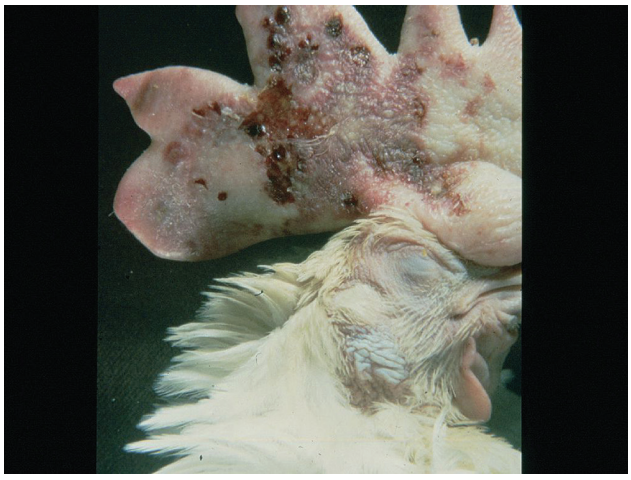

Figure 11-15. Poultry: Cyanosis, edema, and hemorrhage of the comb.
Photo courtesy of Bruce H. Williams, Doctor of Veterinary Medicine, Senior Pathologist, Department of Defense Joint Pathology Center, Silver Spring, Maryland.

Diagnostics Approaches

A presumptive field diagnosis may be made in affected flocks with high mortality and the aforementioned clinical signs. In live birds, oropharyngeal and cloacal swabs are the preferred samples, although fresh feces are an acceptable alternative. Preferred or alternative samples plus samples from the trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver, and heart should be collected. All samples should be shipped on dry ice in phosphate-buffered saline and tested as soon as possible. When immediate testing is not possible, samples may be stored at minus 4°C for up to 4 days.[249]

Samples can be screened for AI viruses via agar gel immunodiffusion or AGID, ELISA, or hemagglutination inhibition. Recently, reverse transcriptase PCR has frequently been used to detect AI virus because it provides results in 3 hours. One such tool used within the Veterinary Corps is the Joint Biological Agent Identification and Diagnosis System or JBAIDS, a ruggedized PCR platform.[307] Using this platform, deployed veterinary personnel can detect the AI matrix gene and identify H5 and H7 genes in field samples. However, while reverse transcriptase PCR and rapid antigen capture immunoassays may be used to detect Type A influenza antigen, additional testing is required to definitively diagnose HPAI versus LPAI.

Virus pathotype may be determined by sequencing the hemagglutinin cleavage site and comparing this to known amino acid sequences of HPAI viruses or through in vivo pathogenicity testing in chicks. Viruses are considered highly pathogenic if mortality in eight, 4- to 8-week-old, intravenously infected chickens is at least 75% within 10 days postinoculation; or the intravenous pathogenicity index in ten, 4- to 8-week-old chicks is greater than 1.2.[249]

To calculate the intravenous pathogenicity index, each bird is examined once daily for 10 days and scored as normal (0), sick (1), severely sick (2), or dead (3). The index is the mean score per bird per observation over the 10-day period. All isolates that meet either of the above definitions or have sequences similar to other known HPAI viruses are classified as HPAI. H5 and H7 isolates that are nonvirulent and lack similar hemagglutinin cleavage-site amino acid sequences to known HPAI viruses are classified as LPAI. All nonvirulent, non-H5, and non-H7 isolates are similarly classified as LPAI viruses.[249]

Recommendations for Therapy and Control

Detection of any HPAI virus in domestic poultry requires OIE notification through the host nation's chief veterinary officer. All H5 and H7 viruses, regardless of virulence, are also notifiable diseases because these viruses mutate into more virulent forms.

Control of HPAI is generally directed at prevention of initial infection and subsequent spread. Although several antivirals, including adamantanes and neuraminidase inhibitors, are available for use in humans, they are not approved for use in birds. Treatment of infected birds is symptomatic but is not recommended. Instead, the USDA recommends this rapid, five-step reaction when responding to HPAI outbreaks: (1) **Quarantine***:* restrict movement of poultry and poultry-moving equipment into and out of the control area; (2) **Eradicate***:* humanely euthanize all infected flocks; (3) **Monitor disease within the region***:* implement a broad area of testing to identify other infected farms and subsequent spread of disease; (4) **Disinfect farms**: clean and disinfect equipment and premises (most detergents and disinfectants kill AI); and (5) **Test**: confirm that the poultry farm is AI virus-free prior to restocking.[312]

Conventional and recombinant vaccines are available for several influenza viruses, including HPAI types. Most routine vaccinations are directed towards circulating LPAI strains; however, they have been used to prevent spread during past HPAI outbreaks and may be used in highly endemic HPAI countries or regions.[249]

Vaccination can reduce clinical signs and mortality in subsequent infections, decrease viral shedding, and increase the infective dose required for transmission; however, effectiveness varies by species and, more importantly, may not consistently prevent infection. Vaccination is also limited by three other factors: (1) the need to match the vaccine virus with the circulating wild type virus, which is inhibited by viral shift and drift; (2) the need for manual injection of vaccines into individual birds; and (3) the difficulty in identifying infected birds within vaccinated flocks.

Although vaccination may help control disease spread, effective prevention relies more on implementation of proper biosecurity measures. All live birds imported into the United States must spend 30 days in an approved USDA quarantine facility where they are tested for HPAI. Returning US origin birds may be home quarantined, and birds arriving from Canada are exempt.[312]

Transportation of equipment between farms is discouraged; however, when necessary, all equipment should be thoroughly cleaned and disinfected prior to transport. In endemic regions, all equipment should similarly be cleaned and disinfected prior to redeployment.

Visitor access to farms should also be restricted, and all personnel should change into clean clothing and disinfect their shoes prior to entering hen houses.

Clothing should be removed and shoes disinfected again upon exiting the hen house to further prevent transmission.

Finally, personnel should be familiar with the clinical signs of AI and report all sick or dead birds immediately, especially in HPAI-endemic regions or countries. Personnel should also wash their hands and avoid touching their mouths and noses after handling raw products and use recommended cooking times and temperatures (ie, 165°C for 15 seconds) to inactivate AI viruses in all raw meat, eggs, and egg products.[312]

SUMMARY

Infectious diseases have long posed a threat to military health and will likely continue to do so for the foreseeable future. Although naturally occurring smallpox and rinderpest have been eradicated, countless other diseases cause concerns for both human and animal military components. Eliminating these diseases within reservoir animal populations will not only protect MWAs but will also protect humans from zoonotic diseases. While military veterinarians may not be able to directly eradicate all diseases, they can work to control them within military-associated populations. By maintaining awareness of military-relevant diseases, veterinarians and other health and military professionals can implement preventative measures to reduce disease transmission and administer timely therapies when such diseases are diagnosed, thus maintaining the overall health of military and civilian populations.

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