Chapter 36

DISEASES TRANSMITTED PRIMARILY FROM ANIMALS TO HUMANS

PATRICK W. KELLEY, MD, DRPH
KENT E. KESTER, MD
CLIFTON A. HAWKES, MD
ARTHUR M. FRIEDLANDER, MD
JULIE PAVLIN, MD, MPH
KELLY T. MCKEE, JR., MD, MPH
WILLIAM R. BYRNE, MD
CHRISTIAN F. OCKENHOUSE MD, PHD
LISA A. PEARSE, MD, MPH
COLONEL DAVID HOOVER, MD

P.W. Kelley; Colonel, Medical Corps, US Army (Retired); Director, Board on Global Health, Institute of Medicine, 500 Fifth Street, NW, Washington, DC 20001; Formerly, Director, Division of Preventive Medicine, Walter Reed Army Institute of Research, Director, DoD Global Emerging Infections System, Silver Spring, MD 20910-7500

K.T. McKee, Jr., Colonel, Medical Corps US Army (Retired); Director of Extramural Clinical Research, US Army Medical Research Institute of Infectious Diseases,1425 Porter Street, Fort Detrick, MD 21702; Formerly, Chief, Medical Operations Division, US Army Medical Research Institute of Infectious Diseases

K.E. Kester, MD, Lieutenant Colonel, Medical Corps, US Army; Chief, Department of Clinical Trials, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Silver Spring, MD 20910-7500

W.R. Byrne, MD, Colonel, Medical Corps US Army; Infectious Disease Officer, Infectious Disease Service, Walter Reed Army Medical Center, Washington, DC 20307-5001; Formerly, Chief, Medical Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702-5011

C.A. Hawkes, MD, Colonel, Medical Corps, US Army; Chief, Infectious Disease Service, Walter Reed Army Medical Center, Washington, DC 20307-5001

C.F. Ockenhouse MD, PhD, Colonel, Medical Corps, US Army; Infectious Disease Officer, Department of Immunology, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Silver Spring, Maryland 20910-7500

A.M. Friedlander, MD, Colonel, Medical Corps, US Army (Retired); Senior Military Scientist, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702-5011; Adjunct Professor of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814-4799

L.A. Pearse, MD, MPH, Major, Medical Corps, US Army; Chief, Mortality Surveillance Division, Armed Forces Institute of Pathology, 1413 Research Boulevard, Building 102, Rockville, MD 20850; Formerly, Chief, Preventive Medicine Service, William Beaumont Army Medical Center, El Paso, TX 79920-5001

J. Pavlin, MD, MPH, Lieutenant Colonel, Medical Corps, US Army; Chief, Department of Field Studies, Walter Reed Army Institute of Research, Silver Spring, MD 20910-7500

D. Hoover, MD, Colonel, Medical Corps, US Army; Infectious Disease Officer, Department of Bacterial Diseases, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Silver Spring, MD 20910-7500

LEPTOSPIROSIS

Introduction and Military Relevance

Leptospirosis is a zoonotic infection in which humans are incidentally infected when they have direct or indirect skin or mucous membrane contact with the contaminated urine of infected wild and domestic animals. Leptospirosis has been well documented in military populations with a history of exposures to mud or various bodies of water in endemic locales.[1,2] It is caused by a spirochete, and transmission can occur in urban, suburban, and rural settings in both tropical and temperate areas. The clinical spectrum ranges from an asymptomatic or influenza-like infection (the most common presentation) through hemorrhagic manifestations, meningismus, jaundice, and renal failure. The more severe end of the spectrum is called Weil's syndrome. Although efforts at prevention can include immunization of domestic animals, the major means of prevention used in the military setting include education, rodent control, and, when appropriate, weekly doxycycline prophylaxis.

Description of the Pathogen

There are more than 200 antigenically distinct serovars or strains of leptospire classified into about 23 groups under the species *Leptospira interrogans*. The distribution of serovars varies around the world. The pathogenicity of serovars varies from animal to animal and even between "identical" serovars from different regions.[3] A newly recognized species, *L fainei*, may also affect humans. A nonpathogenic species, *L biflexa*, also exists. Advances in molecular genetics are leading to a variety of new classification schemes that do not relate to traditional serological groupings.

Leptospires are obligate aerobes. They are flexible, tightly coiled, helicoidal rods with one or both ends usually hooked. Due to its thinness and motility, darkfield microscopy is necessary for optimal visualization even with staining.

Epidemiology

Transmission

Leptospires can live free or in association with human and animal hosts. Animals that survive the acute infection may shed the organisms in their urine for years. In a 1982 survey of 139 small animals trapped at the Jungle Operations Training Center in Panama, 42% had leptospira in their urine (Takafuji ET. 1982. Unpublished data). Even dogs immunized to prevent clinical disease can develop renal shedding.[4] In the environment, *L interrogans* can survive under favorable conditions for as long as 6 months. Tropical, unpolluted, nonsaline, slightly alkaline waters provide an ideal environment.

Although leptospires usually enter the person through breaks in skin or mucous membranes, prolonged immersion in water may facilitate infection through otherwise intact skin. Service members seem to be at particular risk during operations in swamps, streams, ponds, and muddy areas. Leptospirosis has been particularly noteworthy among US forces operating in Panama and Vietnam.[1,2] Other professions that are notably at risk include agriculture and aquaculture, sewer and construction work, animal husbandry, veterinary and slaughterhouse work, mining, and laboratory work. Increasingly, recreational pursuits have been shown to be correlated with risk; this includes care of pets, hunting, fishing, swimming in ponds and other bodies of fresh water, rafting, and playing sports on muddy fields.[5–7] Other possible routes of infection implicated include contaminated drinking water and food preparation surfaces, and, rarely, animal bites. Extremely rare routes of transmission include human-to-human transmission through urine, breast milk, and sex.[8,9]

Geographic Distribution

Leptospirosis can be found in almost every country, though the distribution within a country reflects the variations in host-animal populations and the environment.[3] Cases have been recognized in virtually all states in the United States, though most reports are from Hawaii and the less-arid southern states.[5]

As was clearly evident with US troops training in Panama, risk may increase significantly in the rainy season. Rains or flooding can enhance the flushing of subsurface leptospires into surface waters and draw shedding rodents and other animals to swampy areas.[8] Urban flooding following hurricanes and other heavy rains is also associated with increased risk. The seasonality of reported cases may also reflect the cyclic nature of human agricultural or recreational activity. Though persons in arid areas tend to be at lesser risk of leptospirosis, significant transmission can occur under the right environmental conditions, carrier prevalence,

and human behaviors. For example, the concentration of shedding animals and people around scarce water holes or oases may create opportunities for transmission.

Incidence

The incidence of leptospirosis is grossly underreported because the clinical presentation is often that of a nonspecific influenza-like illness. Most cases are not specifically diagnosed or are misdiagnosed as a more common febrile infection such as dengue. On occasion this can be a fatal mistake. Clinical awareness of the patient's epidemiologic history and a high index of suspicion are essential to making this diagnosis and instituting timely, effective therapy.

Leptospirosis occurs both sporatically and in common-source outbreaks. Probably the highest incidence documented has been in US forces training in Panama. Between 1977 and 1982, close surveillance was conducted on seven US Army units attending the 3-week course at the Jungle Operations Training Center during the fall rainy season. This surveillance yielded 91 confirmed and probable cases, for an annualized incidence estimate of 41,000 per 100,000 person years (Takafuji ET. 1984. Unpublished data) (Figure 36-1). Since these troops in training did not have daily exposure, the real risk was probably even higher. Clearly these attack rates indicate that an intense operational exposure to contaminated environments can have a major impact on not only individual health but also unit capability and mission accomplishment.

Pathogenesis and Clinical Findings

The underlying pathologic effect of acute leptospirosis resembles a vasculitis with damage to the endothelial lining of capillaries coupled with hepatitic and renal tubular dysfunction.[10] Historically most leptospirosis deaths have been due to renal failure, but dialysis has reduced the fatality of this factor. Cardiac effects, to include myocarditis and arrhythmias, are now the leading cause of death in leptospirosis. Hemorrhagic manifestations are also common in severe cases. Jaundice is a major feature of severe leptospirosis and appears to reflect hepatic cell dysfunction more than hemolysis. Survivors generally have no lasting liver or renal dysfunction. Repeated infections with other serovars can occur. Intrauterine infections can cause fetal loss, premature labor, and congenital infection.

The incubation period for leptospirosis is typically 7 to 12 days (range: 2 to 26 days). Very short incubations have been seen in laboratory exposures. Although about 90% of recognized cases present as a mild, self-limited febrile illness, in a prospective serosurvey of soldiers that identified 24 infected persons, only one denied any symptomatology.[2] It is quite possible that if all cases were ascertained, even more than 90% would be recognized as mild.

Mild, anicteric patients often present with the sudden onset of fever that peaks at 38°C to 40°C. Other complaints may include headache, chills, back and joint pain, neck stiffness, and intense myalgia. Even lightly touching the skin over the thighs, calves, and lumbosacral muscles may elicit notable pain. A commonly described finding dur-

Fig. 36-1. A typical occupational exposure: soldiers in the mud at the Jungle Operations Training Center, Panama.
US Army photograph.

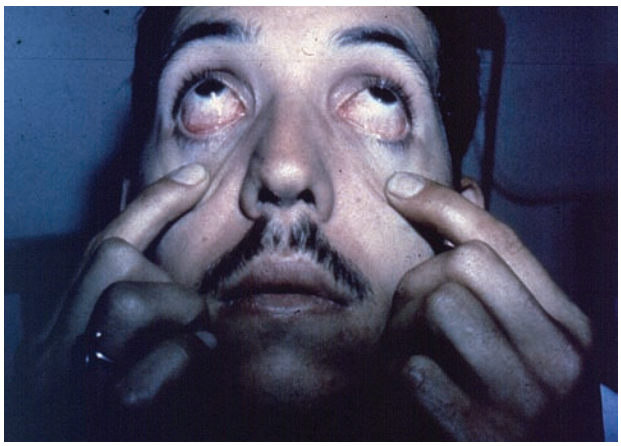

Fig. 36-2. Conjunctival suffusion in a soldier with leptospirosis Photograph: Courtesy of Colonel Ernest Takafuji, Medical Corps, US Army.

ing the first 3 days of illness is conjunctival suffusion, a dilation of the conjunctival vessels without associated signs of inflammation (Figure 36-2). Generalized abdominal pain is sometimes noted and may suggest a "surgical abdomen" or enteric fever. Nausea, vomiting, diarrhea, and constipation may also be reported. Skin manifestations of mild leptospirosis can include a variety of rashes, mainly but not exclusively on the trunk. Meningeal irritation, photophobia, and a variable degree of physiological dysfunction may also noted in mild disease.

Although often not noted by clinicians, classically leptospirosis is described as having an initial leptospiremic phase followed after a 1- to 5-day fairly asymptomatic period by a secondary leptospiuric phase. The leptospiremic phase typically lasts 4 to 7 days and ends about the time antibodies appear. During the leptospiruric phase, fever returns and may be associated with signs of aseptic meningitis[10] (Figure 36-3). The severity of the meningitis is variable. The second phase may last 4 to 30 days or longer. In a review of 150 cases in service members serving in Vietnam, however, only 48% of cases were noted to have this second phase, which usually lasted only 1 day.[1]

In that minority of cases with severe leptospirosis, the initial fever and generalized abnormalities can progress to manifest jaundice, azotemia, hemorrhage, anemia, shock, and altered mental status. As the disease progresses into the second week, hemorrhagic manifestations may be noted in the skin, conjunctiva, and sputum.[10] Deaths due to adrenal hemorrhage have been noted but are rare. Renal failure, acute respiratory distress syndrome, congestive heart failure, and arrhythmias are additional manifestations of severe leptospirosis. Hemorrhagic pneumonitis has been a significant manifestation in infections acquired in Korea, other Far Eastern locales, and Nicaragua. Mild proteinuria is a notable laboratory finding. Vitamin K can correct the prothrombin deficiency that is sometimes seen. Other common laboratory findings include elevated creatinine phosphokinase and amalyse levels, neutrophilia, and thrombocytopenia. During the secondary phase, the cerebrospinal fluid may show a pleocytosis. A variety of chest roentgenogram abnormalities are common and may include pulmonary opacities, pleural effusion, and evidence of myositis or pericardial effusion. Electrocardiographic abnormalities are also noted.

Published case fatality rates vary widely, probably reflecting geographic variation in serovars and in the low proportion of mild cases that are specifically diagnosed. Overall, the true case fatality is

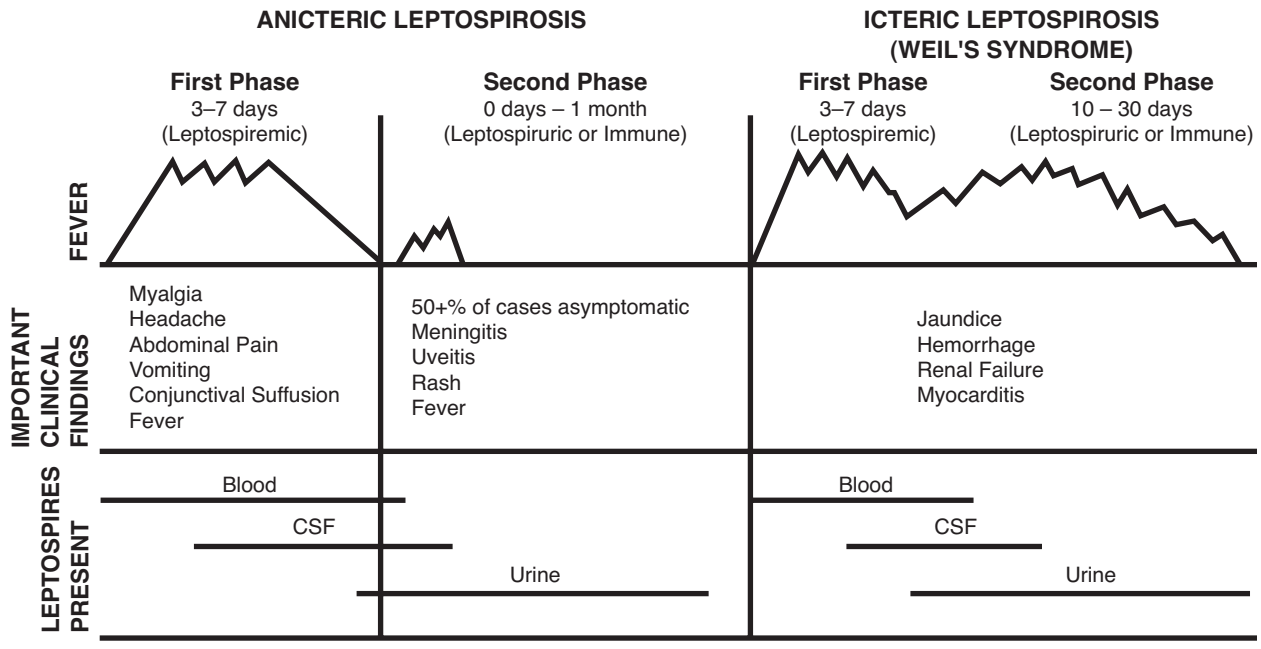

Fig. 36-3. The clinical course of leptospirosis
Reprinted with permission from: Feigin Rd, Anderson DC. Leptospirosis. In: Feigin RD, Cherry JD, eds. *Textbook of Pediatric Infectious Disease*. Vol 2. Philadelphia: W.B. Saunders; 1998: 1534.

probably less than 1.0%, but severe cases may have rates of well over 10%. In survivors, convalescence may extend to several months. In a small percentage of survivors, a variety of delayed ocular complications, including anterior uveal tract inflammation, may become manifest during convalescence or as long as a year later. These and headaches may persist for years.[11]

Diagnostic Approaches

Early diagnosis is important in leptospirosis for antibiotic therapy to be effective and to prevent future cases. Key to diagnosis is a good epidemiologic history, especially when patients present with a febrile illness associated with an abrupt onset, myalgias, and severe headache. The differential diagnosis includes heat injury, influenza, dengue, rickettsioses, typhoid fever, brucellosis, relapsing fever, toxoplasmosis, malaria, yellow fever, septicemia, toxic shock syndrome, Kawasaki syndrome, Hantaan virus infection, and Legionnaires' disease. In the Caribbean region, the relative frequency of dengue has contributed to the missed diagnosis of leptospirosis, sometimes with fatal consequences. In the Balkans, distinguishing between leptospirosis and hantavirus infection has also been difficult.

The specific diagnosis of leptospirosis usually requires paired sera to document a 4-fold rise in antibody titer and is thus often retrospective. Commercial enzyme immunosorbent assays or macroagglutination serologic screening tests are typically used. Polymerase chain reaction methods are also available in some laboratories and can help with early diagnosis. The definitive antibody assay is the microagglutination test, but this is found only in reference laboratories and requires that the battery of live leptospiral organisms used reflect the serovars prevalent in the location where the putative exposure occurred.[8] The importance of this is illustrated by the report that 70% of the leptospirosis acquired by US soldiers in Panama could be diagnosed serologically by the microagglutination test only when Panamanian isolates were used as antigen.[12] Presumptive diagnosis can be made when there are compatible clinical findings and either a positive macroagglutination slide test or a microagglutination titer of at least 1 in 100. Many other assays are under development, including rapid dipstick-type tests.

Cultures of blood and cerebrospinal fluid established during the first week or so on specific media are also valuable in confirming the diagnosis, but it may take 6 to 8 weeks for the leptospires to grow.[8] After the first week, successful cultures of urine are possible. Specimens of blood, cerebrospinal fluid, and urine for culture must be innoculated into special culture media such as Tween 80-albumin (EMJH) media. To reduce the effect of inhibitory substances, three serial dilutions should be made to yield specimen to media concentrations of 1:10, 1:100, and 1:1000. The cultures should be incubated in the dark at 28°C to 30°C. If an appropriate leptospiral growth medium is not immediately available, leptospira can survive for a week or longer after collection in a tube of blood anticoagulated with heparin or sodium oxalate. In one study of US soldiers who trained in Panama in which blood and urine cultures were taken within 3 days of fever onset, more than 94% of patient cultures were positive.[2] Culture results are sometimes positive in the face of negative serologies.

Recommendations for Therapy and Control

Most experts feel that antibiotic therapy of leptospirosis is useful if given early in the illness. Doxycycline, 100 mg by mouth twice a day for 7 days, has been shown to be effective in soldiers who acquired leptospirosis in Panama if given within the first 4 days of illness.[13] Penicillin has also been used with success in some studies[14–16] but not in others. Erythromycin, some of the newer penicillins, and cephalosporins may also be effective.[17] Jarisch-Herxheimer reactions (ie, temperature rise, drop in blood pressure, and precipitation or exacerbation of other symptoms and signs) have been reported to occur after antibiotic therapy for leptospirosis. Concern over these reactions, however, does not justify avoiding antibiotics. Nonspecific therapy may be useful to manage pain, fever, mental status changes, vomiting, renal failure, hypotension, and hemorrhage.

Prevention in humans is based on exposure reduction and antibiotic prophylaxis. Exposure reduction requires an understanding of the epidemiology of the infection in a particular setting. Education of service members, commanders, operational planners, and health care personnel on the epidemiology of leptospirosis is important. Although military operational imperatives may require exposure to potentially contaminated soil and water, an awareness of leptopsirosis may strengthen consideration of tactical alternatives that can mitigate exposures. Knowledge of the epidemiology of leptospirosis can also help influence a commander to put some areas off limits for recreational use. Other methods (eg, rodent control in camps, use of protective clothing

and surface disinfectants) have a place in some occupational exposure settings. Simply wearing shoes rather than going barefoot reduces the risk of leptospirosis for individuals walking around fields, farms, bivouac sites, lawns, or other areas where infected wild and domestic animals may urinate. Prevention programs in domesticated animals can include immunization.

Chemoprophylaxis with weekly doxycycline (200 mg) has been extremely effective in preventing troops training in Panama during the high-risk rainy season from getting leptospirosis; such a regimen should only be used for short, high-risk exposures.[2,7]

[Patrick W. Kelley]

HANTAVIRUSES

Introduction and Military Relevance

Infectious febrile syndromes complicated by renal and hematological abnormalities have been described in northern Europe, the Balkans, and throughout the Far East for many decades. Since the 1930s, these geographically distinct but clinically and epidemiologically similar entities have frequently been cited in association with the military and its operations. During World War II, huge outbreaks of these diseases were documented among Japanese and Soviet soldiers encamped along the Amur River valley in the Far East, while more than 1,000 German and Finnish troops were stricken in Lapland.[18,19] Beginning in the 1950s, hemorrhagic nephroso-nephritis became increasingly recognized as a hazard to military camps in central Europe.[20] Despite their widespread recognition across the Eurasian landmass, however, these disorders were unknown to US military physicians until the Korean War, when more than 3,000 United Nations military personnel developed so-called epidemic (or Korean) hemorrhagic fever between 1951 and 1953.[21] Although these diseases were widely assumed to be infectious zoonoses, details of their epidemiology and pathogenesis remained undefined until Lee and colleagues recognized the causative agent of Korean hemorrhagic fever, Hantaan virus, in 1976.[22] Since that time, these disorders, now known collectively as hemorrhagic fever with renal syndrome (HFRS), have been linked to a variety of similar but serologically distinct agents called hantaviruses. As evidenced by repeated severe and occasionally lethal outbreaks among soldiers in Korea[23] and Europe,[24] hantaviruses continue to plague the field operations of US military forces in endemic areas.

Description of the Pathogens

Hantaviruses are negative-stranded RNA viruses sharing morphological and biochemical characteristics of the family *Bunyaviridae*. Taxonomically, they are classified as a separate genus within this large virus family.[25] Each hantavirus has evolved in association with a predominant rodent host in which it establishes a chronic, inapparent infection.[26] As of 1997, more than 25 hantaviruses had been described belonging to four phylogenetic and antigenic groups. About half of the currently recognized hantaviruses have been linked to human disease.[27–29] Hantaviruses of particular interest to military populations include the Seoul, Hantaan, Puumala, Dobrava/Belgrave, and the so-called "American" viruses (eg, Sin Nombre, Andes).

Epidemiology

Transmission and Geographic Distribution

Chronically infected rodents shed large amounts of virus in urine, saliva, and, to a lesser extent, feces.[30] Humans most likely become infected either by contact with these contaminated excreta and secreta through inhalation of small particle aerosols or by percutaneous or mucous membrane contact with infectious materials. Individuals at highest risk include those living or working in rural areas, such as farmers and foresters. Military personnel in the field also represent a high-risk group. Person-to-person spread of hantaviruses associated with HFRS has never been documented.

Hantaviruses have been found on every continent except Antarctica. The epidemiology of each different virus is a function of the ecology, population dynamics, and geographic distribution of its predeominant rodent host. Hantavirus reservoirs tend to be among the most abundant rodents found in a given disease-endemic area. However, infection within rodent populations is not uniform—a characteristic reflected in the microfocal distribution of human disease.[31]

With the exception of Seoul virus, hantaviruses typically cause human disease in rural settings. Hantaan virus, the cause of classic severe ("Far Eastern") HFRS, circulates across China, northern Asia, and the Korean peninsula.[32] Its reservoir, *Apodemus agrarius*, is the ubiquitous striped field mouse. The

etiologic agent of a generally milder disease variant known as nephropathia epidemica, Puumala virus, is associated primarily with the bank vole *Clethrionomys glareolus*. This rodent ranges throughout Scandinavia and the rest of northern Europe south into the Balkan states.[32,33] Dobrava/Belgrade virus is associated epidemiologically with severe HFRS. This agent is carried (perhaps along with Hantaan-like viruses) in central Europe and the Balkans by the yellow-necked field mouse, *Apodemus flavicollis*.[34–36] *Rattus* species serve as reservoirs for Seoul virus. Seoul virus is recognized worldwide and has been linked to comparatively mild human illness, most frequently in eastern Asia, but it can also cause severe HFRS.

Until relatively recently, hantaviruses were thought to be associated exclusively with HFRS and to cause disease largely in Europe and Asia. In late 1993, however, an outbreak of a rapidly progressive respiratory syndrome among inhabitants of the Four Corners region of the southwestern United States was shown to be caused by a novel hantavirus (ultimately called Sin Nombre) associated with deer mice (*Peromyscus maniculatus*).[37–39] As efforts intensified to define the geographical extent of what came to be known as hantavirus pulmonary syndrome (HPS), some cases were identified outside the known distribution of *P maniculatus*. It soon became evident that hantaviruses other than Sin Nombre were probably responsible for this disorder as well.[40,41] As of 1997, more than 150 domestic cases of HPS had been identified, and it became clear that several different hantaviruses could cause the syndrome. With expansion of surveillance to other parts of the Western hemisphere and application of newer molecular diagnostic technologies, numerous related but clearly distinct agents have been identified throughout North, Central, and South America.[28,29,41,42] Some viruses (eg, Sin Nombre, Andes, Laguna Negra, Black Creek Canal, Bayou, Monongahela, Juquitiba, New York-1) have been associated with acute, symptomatic human disease. Other viruses (eg, Prospect Hill, Muleshoe, Cano Delgatido, El Moro Canyon, Rio Segundo, Rio Mamore) have been found only in rodents. With increasing awareness, it is highly likely that additional hantaviruses indigenous to the Americas will be identified.

Incidence

Numerically, HFRS is the most important of the viral hemorrhagic fevers, with tens to hundreds of thousands of cases occurring annually across endemic areas. HFRS is recognized year-round, but the highest incidences coincide with periods of maximal rodent activity: late autumn and early winter (with a smaller peak in the spring) in Asia and central Europe; late summer and early fall in colder areas of western Europe (eg, Scandinavia); and spring in warmer parts of Europe (eg, France, Belgium).[33,43]

Pathogenesis and Clinical Findings

The basic pathological abnormality in human hantaviral infection is vascular endothelial damage, probably due to both direct (cytopathic) and indirect (immunopathologic) virus effects. While pathological changes are found in virtually all organ systems, a characteristic triad of lesions is associated with fatal, classic HFRS: hemorrhagic necrosis of the renal medulla, anterior pituitary gland, and cardiac right atrium.

Classic HFRS seen in the Far East is a complex, multiphasic disorder that poses significant challenges in clinical management[21] (Table 36-1). While HFRS may present as a fulminant, rapidly progressive and fatal hemorrhagic fever, most cases (70% to 80%) are more benign. Asymptomatic infections occur but are probably uncommon. After an incubation period of 2 to 3 weeks (range: 4 to 42 days), onset of illness is usually abrupt and nonspecific, with fever, myalgia, headache, and anorexia most frequently reported. Severe abdominal or lower back pain, facial flushing, periorbital edema, and injection of the conjunctivae, together with axillary and palatal petechiae, are typical early disease features. As the clinical course evolves, hypotension (which may be severe) and oliguria or anuria are seen. With recovery of renal function, massive diuresis occurs. Convalescence is prolonged. Complete recovery often takes months. Leukocytosis, thrombocytopenia, and massive proteinuria are characteristic laboratory features during the acute stages of disease, while anemia and hyposthenuria are generally seen during convalescence. Similar clinical features, though of lesser severity, are seen in most western European HFRS (nephropathia epidemica-like) patients. Mortality in Hantaan or Dobrava/Belgrade infections may approach 15% or more, while deaths due to Puumala virus are uncommon. Severe and occasionally fatal cases of HFRS among animal handlers and scientists have been caused by Seoul virus transmitted from colonized laboratory rats with inapparent infections,[44] and Seoul virus has been epidemiologically linked to chronic renal disease in some areas.[45]

TABLE 36-1

FAR EASTERN HEMORRHAGIC FEVER WITH RENAL SYNDROME: TYPICAL CLINICAL AND LABORATORY CHARACTERISTICS

Stage	Duration	Prominent Clinical Features	Laboratory Findings
Febrile	3-7 d	Fever, malaise, headache, myalgia, back pain, abdominal pain, nausea, vomiting, facial flush, petechiae (face, neck, trunk), conjunctival hemorrhage	WBC: normal or elevated Platelets: decreasing Hematocrit: rising Urine: proteinuria 1+ to 3+
Hypotensive	2 h-3 d	Nausea, vomiting, tachycardia, hypotension, shock, visual blurring, hemorrhagic signs, ±oliguria (late)	WBC: increasing with left shift Platelets: markedly decreasing Bleeding time: increasing, prothrombin time may be prolonged Hematocrit: markedly increasing Urine: proteinuria 4+ hematuria 1+ hyposthenuria BUN and creatinine rising rapidly
Oliguric	3-7 d	Oliguria ± anuria, blood pressure may rise,nausea and vomiting may persist, 1/3 withsevere hemorrhage (epistaxis, cutaneous, gastrointestinal, genitorurinary, central nervous system)	WBC: normalizes Platelets: normalize Hematocrit: normalizes then falls Urine: proteinuria 4+ hematuria 1+ to 4+ BUN and creatinine: markedly increasing Na^+, Ca^{++}: falling; K^+ rising
Diuretic	Days to weeks	Polyuria (up to 3-6 L/d)	BUN and creatinine: normalize electrolytes: may be abnormal (diuresis) Urine: normalizes
Convalescent	Weeks to months	Strength and function regained slowly	Anemia and hyposthenuria may persist for months

NOTE: All phases may not be present in a given patient; phases may "blend" in some individuals
WBC: white blood count, BUN: blood urea nitrogen
Reprinted with permission from Military Medicine, the official journal of the Association of Military Surgeons of the United States (AMSUS): McKee, KT Jr, MacDonald C, LeDuc JW, Peters CJ. Hemorrhagic fever with renal syndrome—a clinical perspective. *Mil Med*. 1985;150:640–647.

In contrast to disease caused by hantaviruses across Eurasia, the clinical features of American hantavirus infections center primarily on the cardiorespiratory systems[46] (Table 36-2). HPS is a serious disease with high mortality. After a 3- to 6-day prodromal period of fever, myalgia, and often gastrointestinal symptoms, dyspnea, cough, and hypotension appear and progress rapidly; patients may be in extremis within hours. Renal abnormalities tend to be relatively insignificant. Progressive hypoxemia and pulmonary edema, followed by cardiac arrhythmias and intractable hypotension, challenge survival. Fatality rates approach 50% of cases reported to date, with most deaths occurring within 24 hours of onset of pulmonary decline.[46,47] Among survivors, recovery from acute illness may be rapid, with normalization of most clinical and laboratory parameters. However, lassitude and weakness continue for weeks to months, and pulmonary function abnormalities may persist in some patients.[48] Leukocytosis and thrombocytopenia are common laboratory findings, as is mild proteinuria. Atypical lymphocytes frequently can be found on peripheral blood smears.

Diagnostic Approaches

A high index of suspicion is critical to diagnosing hantavirus infections. Any patient presenting in or returning from a hantavirus-endemic area with fever of abrupt onset, headache, gastrointestinal symptoms, respiratory distress, lower back or abdominal pain, or any combination of these symptoms should be evaluated for possible HFRS or HPS.

TABLE 36-2

HANTAVIRUS PULMONARY SYNDROME: TYPICAL CLINICAL AND LABORATORY FEATURES

Stage	Duration	Prominent Clinical Features	Laboratory Features
Prodrome	3-7 d	Fever, myalgia (especially back, lower extremities), nausea, vomiting, diarrhea, abdominal pain; occasional headache, dizziness	WBC: normal or rising Platelets: normal or falling Hematocrit: normal or rising
Cardiopulmonary	3-16 d	Fever, dyspnea, cough, tachypnea, tachycardia, rapidly progressive respiratory failure, hypotension; cardiogenic/hypotensive shock, cardiac arrhythmias	WBC: rising with left shift and atypical lymphocytes Platelets: falling Hematocrit: rising PTT: rising Mild proteinuria Metabolic acidosis
Convalescent	Weeks to months	Rapid normalization of cardio-respiratory function, diuresis; strength and function regained slowly	Normalization of most parameters, pulmonary function abnormalities may persist

WBC: white blood count, PTT: partial thromboplastin time

An elevated white blood cell count, low platelet count, or proteinuria in this setting should precipitate serological testing for hantavirus infection. The differential diagnosis of HFRS includes rickettsiosis, leptospirosis, meningococcemia, leukemia, hemolytic-uremic syndrome, other viral infections, and poststreptococcal syndromes. HPS can be confused with pneumonic plague; legionellosis; psittacosis; other pneumonic processes of viral, bacterial, or fungal origin; autoimmune disorders, such as thrombotic thrombocytopenic purpura; or pancreatitis accompanied by adult respiratory distress syndrome. Diagnosis is established either by demonstrating hantavirus-specific IgM antibodies in acute serum using enzyme immunoassay or by a 4-fold or greater rise in IgG antibodies using enzyme immunoassay or immunofluorescence.[49] Viral antigens or nucleic acid sequences can be detected in tissue samples by immunohistochemical or molecular amplification techniques.[50] Recovery of hantaviruses from clinical specimens is typically difficult and efforts to cultivate those agents from body fluids are generally unrewarding.

Recommendations for Therapy and Control

Bed rest and early hospitalization in an intensive care environment are critical to successful treatment of hantavirus-infected patients. With HFRS, evacuation from outlying areas should be as atraumatic as possible, to avoid damage to an already compromised microvascular bed. Where facilities exist, patients should be hospitalized locally (ie, "in-country"). Aeromedical evacuation, particularly over long distances and at altitude, should be avoided. Patient isolation is not required. Wide fluctuations in fluid status and the attendant biochemical disturbances are important considerations in patient care and require careful attention. Peritoneal dialysis or hemodialysis is often required in managing metabolic complications.

In HPS, adequate oxygenation and tissue perfusion are the goals of treatment; therefore, mechanical ventilation and use of pressor agents are important adjuncts to general supportive care. Respiratory management should be provided in an intensive care setting. Fluids must be meticulously managed to avoid overhydration and exacerbation of pulmonary edema. As with HFRS, transfer should be avoided if possible; if the patient must be moved, hypoxia and trauma to the fragile vascular beds should be minimized. Isolation of patients with HPS is generally considered unnecessary. In the United States, person-to-person transmission has not been seen, but a single report has suggested interpersonal spread during an outbreak in Argentina.[51] Hantaviruses are sensitive in vitro to the antiviral drug ribavirin. This drug has been shown to reduce morbidity and mortality in Chinese HFRS patients[52]; however, its utility has not been proven elsewhere. In the case of HPS, ribavirin appeared to be ineffective in reducing mortality in an open-label trial.[47] Experience to date is therefore insufficient to rec-

EXHIBIT 36-1

MEASURES TO MINIMIZE EXPOSURE TO RODENTS IN HANTAVIRUS-ENDEMIC AREAS

- Store human and animal food under rodent-proof conditions
- Burn or bury garbage or discard it in rodent-proof containers
- Inspect vacant cabins or other enclosed shelters (to include seasonal latrines) for evidence of rodent infestation before use; do not use such structures until appropriately cleaned and disinfected; regularly inspect occupied buildings for evidence of rodent activity
- Disinfect rodent-contaminated areas by spraying a disinfectant, such as dilute bleach, before cleaning
- Avoid inhalation of dust by wearing approved respirators when cleaning previously unoccupied areas*; mist these areas with water before sweeping or mopping
- Remove dead rodents promptly from the area; wear disposable gloves or plastic bags over the hands when handling dead rodents; place all dead rodents into a plastic bag (preferably a bag containing sufficient disinfectant to wet the carcass) before disposal
- Never attempt to feed, handle, or keep wild or stray animals as pets or mascots
- Do not pitch tents or place sleeping bags near potential rodent shelters (eg, burrows, garbage dumps, woodpiles)
- Sleep above the ground on cots if feasible
- Use bottled water or water from approved sources for drinking, cooking, washing dishes, and brushing teeth
- Launder or dry clean rodent-contaminated clothing and bedding, using rubber or plastic gloves when handling contaminated materials
- Be on the lookout for rodents and their burrows or nests and avoid contact; be aware that exposures may not seem significant in all cases

*If clear evidence of infestation is present, HEPA-filtered respirators, goggles, solvent-resistant gloves, coveralls, and boots should be worn while cleaning.
HEPA: high efficiency particulate air

ommend its unqualified use in HPS.

Elimination or significant reduction of reservoir rodent populations in the field setting is logistically impossible. Exposure to rodent-contaminated brush, dusts, or fomites constitutes a theoretical infection risk to humans, but these have not been documented as routes of hantavirus transmission. General avoidance of rodents, together with their burrows and nesting areas, is useful advice in all endemic areas. Additional measures to control exposure to rodents and their habitats should also be undertaken[53] (Exhibit 36-1).

Hantavirus vaccines produced outside the United States are in use in some endemic areas, but these products are of unproven utility. A candidate vaccinia-vectored recombinant immunogen developed by the US Army is under study.

[Kelly T McKee, Jr.]

TOXOPLASMOSIS

Introduction and Military Relevance

Owing to its widespread distribution, soil reservoir, and association with suboptimal food preparation and inadequate personal sanitation practices, toxoplasmosis has particular relevance for service members deployed in the field and the military physicians who support them. Reports have documented toxoplasmosis outbreaks among deployed US Army troops.[54] *Toxoplasma gondii*, the etiologic agent of toxoplasmosis, was first described in 1908 by Nicolle and Marceaux,[55] with the first case of human disease described 15 years later.[56] The organism is a ubiquitous parasite with a worldwide distribution. While infection of humans is common, most primary infections in immunocompetent individuals are relatively asymptomatic. Greater morbidity and mortality is instead associated with toxoplasmosis affecting immunocompromised patients, especially those with HIV infection, and congenitally infected neonates.[57p500–503] *T gondii* is associated with three major syndromes: lymphadenopathy (usually in immunocompetent hosts), opportunistic infections in immunocompromised

hosts, and congenital disease associated with an acute maternal infection.[58] In addition, ocular toxoplasmosis may occur either as a result of an acute infection or a reactivation of a congenital infection.[59]

Description of the Pathogen

Initially discovered in 1908 in a northern African rodent, the gondi, *Toxoplasma gondii* has been subsequently found in an unusually wide array of animals.[60] It is a crescentic-appearing, obligate intracellular coccidian parasite that can multiply in virtually any cell of its vertebrate hosts.[61] Felines serve as the definitive host, and many other animals function as intermediate hosts.[62] While only one species has been identified, strain variation based on differences in virulence has been characterized.[63] Three distinct life forms have been described: oocyst, tachyzoite, and tissue cyst.

Oocysts

Oocysts are the infective form shed by infected cats in their feces. After release, the oocysts require a maturation step, known as sporulation, to become infective. The time to completion of sporulation may vary widely and depends on the ambient temperature (2 to 3 days at 24°C; 14 to 21 days at 11°C). Sporulation will not occur at temperatures less than 4°C or greater than 37°C. Oocysts may remain viable in moist soil for as long as 18 months, leading to an additional environmental reservoir that may prove difficult to eradicate.[64,65] Oocysts are generally shed for 1 to 3 weeks by primarily infected cats; prolonged shedding may be associated with feline immune system defects.[66,67] Oocyst shedding usually occurs only once in an infected cat's lifetime; however, it may occasionally recur with repeated *Toxoplasma* exposure or infection with the coccidian protozan *Isospora felis*.[61,68] At peak release, an infected cat may shed 20 million oocysts in its feces per day.[67]

Tachyzoites

Tachyzoites represent the rapidly dividing asexual invasive form found in an acute *Toxoplasma* infection. These rapidly dividing organisms, best seen with a Wright-Giemsa stain (Figure 36-4) can infect virtually all mammalian cells except nonnucleated erythrocytes.[69] Spread of tachyzoites into the bloodstream accounts for the disseminated nature of *Toxoplasma* infections. Tissue invasion leads to expanding focal lesions.[62] The tachyzoite form of *Toxoplasma* is susceptible to desiccation, freezing

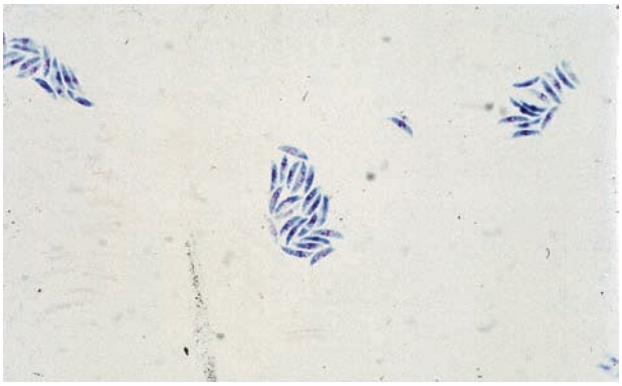

Fig. 36-4. Tachyzoites of *Toxoplasma gondii* from cerebrospinal fluid stained with Wright-Giemsa and photographed at x1,200.
Photograph: Courtesy of Lieutenant Colonel Kent Kester, Walter Reed Army Institute of Research.

followed by thawing, and gastric acid.[69] Unpasteurized milk from infected goats and cattle has been found to harbor viable tachyzoites.[57]

Tissue Cysts

Tissue cysts are the end result of all *T gondii* infections; these are collections of dormant organisms, termed bradyzoites, formed by tachyzoite dissemination (Figure 36-5). They serve as a tissue reservoir for recurrent infection.[60] Bradyzoites tend to reactivate in the context of immune impairment, cre-

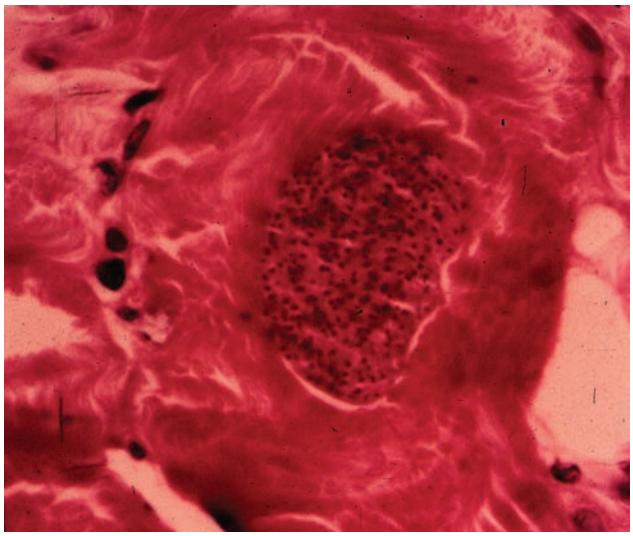

Fig. 36-5. A toxoplasmic cyst in skeletal muscle, stained with hematoxylin-eosin an photographed at x1,200.
Photograph: Courtesy of the Armed Forces Institute of Pathology. AFIP negative 73-7677.

ating a significant hazard in immunocompromised hosts. Cysts may form in any tissue, but they are commonly localized in the cardiac and skeletal muscle and the brain, possibly because of suboptimal immune surveillance in those sites.[61] Encysted organisms persist for the life of the host.[66,70]

The *Toxoplasma* life cycle splits into an exoenteric cycle and an enteric cycle. The exoenteric cycle occurs in most warm-blooded mammals. Tachyzoites invade macrophages and divide within vacuoles. Later, cellular rupture occurs, and the released organisms proceed to infect adjacent cells. Some of these successive infections may lead to the formation of tissue cysts. In the enteric cycle, which occurs only in felines, the liberated parasites enter the cells of the small intestine and develop; released merozoites subsequently infect adjacent cells. As the cycle is repeated, gametogenesis occurs. Fusion of two gametes leads to the formation of a zygote, which then becomes an oocyst.[61]

Epidemiology

Transmission

Infection occurs predominantly via the ingestion of either viable oocysts or tissue cysts. Consuming oocysts is related to contamination of the food or drink with feces from infected cats. Consuming viable tissue cysts is due to a lack of proper food preparation, often in raw or undercooked meat.[60]

While the seropositivity rate of domestic cats ranges between 30% and 80%, pet cats are probably not the direct source of human infection. They tend to be fastidious animals, rarely having fecal material present on their coats long enough for oocysts to sporulate.[67] Transmission from pet cats is more likely from careless handling of litter box contents by their owners. Other members of the family Felidae, such as ocelots and jaguarundis, have also been shown to pass viable *Toxoplasma* oocysts in their feces.[71] Thus, wild felines probably also play a role in transmission of toxoplasmosis.[54] Infected domestic and wild animals, including cattle, pigs, dogs, horses, and rodents, may serve as intermediate hosts and carry viable cysts in their tissues for long periods of time.[70]

Contamination of foodstuffs by cat feces may lead to the presence of infectious oocysts. Additionally, cat feces may contaminate other items, such as soil and drinking water. Ingestion of any of these items may lead to infection with *Toxoplasma*. It has been estimated that the simple act of handling soil contaminated with feces from infected cats may lead to the deposition of 10 to 100 oocysts under the fingernails. Suboptimal personal hygiene may lead to the transfer of the oocysts into the mouth, leading to the development of a primary infection. Various coprophagic insects, including filth flies, cockroaches, and dung beetles, may also serve as transfer agents for infectious oocysts.[60]

Ingestion of viable tissue cysts may be the more common method of infection in the Western world. The large number of bradyzoites found within a tissue cyst makes the probability of infection following cyst ingestion quite high.[60] This mode of infection, which results from the consumption of raw or undercooked meat that contains tissue cysts, likely contributes to the very high *Toxoplasma* seropreva-lences in countries such as France, where less emphasis is placed on thorough cooking of meat. Conversely, the seroprevalence rate in Great Britain, where meat is more likely to be cooked thoroughly, is much lower.[66]

Infection can also be passed from an infected woman to her fetus. Congenital toxoplasmosis occurs as a direct consequence of acute maternal infection during pregnancy. Laboratory accidents have been reported as a cause of acute *Toxoplasma* transmission.[69] Occasionally, organ transplants from seropositive donors are compromised by *Toxoplasma* reactivation in the transplant recipient, often with devastating circumstances.[61,70] Rarely, blood transfusions may serve as a means of transmission, especially if circulating tachyzoites are present. Tachyzoites may survive up to 60 days in citrated blood.[69]

Geographic Distribution

Both animals and humans on five continents have serologic evidence of *Toxoplasma* infection.[72] Higher prevalence is present in areas with warm and humid climates, such as Haiti, Tahiti, or the lowlands of Guatemala, where nearly all people are seropositive.[72,73] This is presumably related to greater oocyst survival in the warm, moist soil characteristic of these areas.[64,65] In addition, areas with higher cat populations also have higher prevalence rates. Dryer and colder environments are associated with lower rates of seroprevalence because oocysts are less likely to survive.[72] Specific area variations are often due to changes in weather and animal populations.

Incidence

Approximately 500,000,000 persons worldwide are estimated to be seropositive for *Toxoplasma* antibodies.[66] Overall, serosurveys suggest that 30% to

40% of the US population is seropositive for *Toxoplasma* by age 50. The majority of these have no symptoms and never come to medical attention.[69] Regional differences in seropositivity have been described, with the highest levels found in the southeastern United States; seroprevalence rates are much lower in the western and northwestern portions of the country. Interestingly, an assessment of seroprevalence data of military recruits showed a significant decrease in overall seropositivity rates as compared with similar, older studies; this is possibly related to a decline in the presence of viable *T gondii* cysts in meat products.[74,75] *Toxoplasma* antibody prevalence rates vary as much in animals as in humans.[73,76]

The incidence of congenital toxoplasmosis varies in the United States, occurring in between 1/1,000 and 1/8,000 live births, leading to 500 to 4,100 congenitally infected infants born each year. Seroprevalence among US women ranges from 3.3% to 30%.[77]

Fig. 36-6. This photograph shows a node in a US soldier who was deployed to the Jungle Operations Training Center in Panama from Fort Bragg, NC, in 1981.
Photograph: Courtesy of Colonel Ernest T. Takafuji, Medical Corps, US Army.

Pathogenesis and Clinical Findings

There are two major patterns of toxoplasmosis in humans: postnatally acquired toxoplasmosis and congenital infection. While both are characterized by dissemination of *Toxoplasma* organisms throughout the body, the manifestations and complications of these two disease variants are quite distinct. Since most human infection with *T gondii* is asymptomatic, it is important to make a clear distinction between infection and disease. The clinical disease of toxoplasmosis may occur at any time during or after parasitemia.[58]

Infection of Postnatal Hosts

In contrast to the congenital form, toxoplasmosis acquired after birth is characterized by a much different course. Most cases remain asymptomatic. The most common clinical manifestation of acquired toxoplasmosis in children and young adults is lymphadenopathy involving single or multiple nodes[58,61] (Figure 36-6). Incubation periods are variable and are likely influenced by the number of viable organisms ingested. The affected nodes are usually in the 1- to 2-cm range and, while tender to the touch, are usually not intrinsically painful. Node suppuration is not a typical finding. Most patients with symptomatic acquired toxoplasmosis present with a syndrome, typified by malaise and fever, similar to that seen in infectious mononucleosis.[58] Other manifestations, such as encephalitis, myocarditis, pericarditis, hepatitis, pneumonitis, and macular papular rash are much less common in immunocompetent individuals.[78] While clinical involvement of the heart, brain, lungs, and liver is rare during an acute acquired infection, *T gondii* can disseminate to these and other organs and may cause clinical disease.[79]

Patients may have localized lymphadenopathy and an elevated number of atypical lymphocytes in their peripheral blood smear; 10% to 20% may have peripheral eosinophilia.[78] A minority of patients have an elevated erythrocyte sedimentation rate.[79] Hepatomegaly and splenomegaly have been described.[78] Heterophile antibodies are negative and hepatic transaminases are usually normal, but severe hepatitis has been reported.[79] Occasionally, the lymphadenopathy may involve retroperitoneal, mesenteric, and mediastinal nodes.[78] During the course of the illness, symptoms and lymphadenopathy may fluctuate. Resolution occurs in most patients after a few months.[58] It has been estimated that as much as 15% of unexplained lymphadenopathy in normal hosts may be caused by toxoplasmosis.[80] Failure to consider toxoplasmosis as a cause of lymphadenopathy may lead to unnecessary lymph node biopsies. McCabe[77] assessed 107 cases of histologically confirmed toxoplasmosis with lymphadenopathy. The majority presented with a solitary node in the head or neck area without other symptoms, but nearly one third had significant malaise. The overall clinical course of these patients was typically benign. Some patients, however, did have serious extranodal disease, including myocarditis, pneumonitis, encephalitis, and fetal transmission. Both brief and prolonged illnesses were described.

Retinochoroiditis may also be seen in immunocompetent adolescents and adults and may be either a late sequela of congenital infection or a manifestation of acute infection.[58,59,76]

Reactivation of latent *Toxoplasma* in immunocompromised patients, including those infected with human immunodeficiency virus, is often associated with serious illness, typically encephalitis. *Toxoplasma* encephalitis in patients with acquired immunodeficiency syndrome remains a significant cause of morbidity and mortality in those infected with human immunodeficiency virus.[67]

Congenital Toxoplasmosis

Congenital toxoplasmosis results from an acute *Toxoplasma* infection of a pregnant woman. Nearly 50% of fetuses exposed become infected. Of these, between 10% and 20% have clinical symptoms of infection at birth and the remainder appear normal.[77] The classical triad of congenital toxoplasmosis consists of retinochoroiditis, hydrocephalus, and intracranial calcifications.[60,81] While the symptoms are often mild, severely affected infants may bear evidence of generalized or central nervous system infection. Other clinical findings of infected infants may include prematurity, in†utero growth retardation, hepatosplenomegaly, jaundice, vasculitis, vascular thrombosis, seizures, and microcephaly.[60,77,81]

Diagnostic Approaches

While the definitive method for the diagnosis of an active *Toxoplasma* infection is mouse inoculation, it is normally only found in research laboratories. Serologic tests are the primary means of diagnosis for *Toxoplasma* infections elsewhere.[80] The gold standard serological test has been the Sabin-Feldman dye test (DT).[82] This assay, which measures specific IgG directed against *T gondii*, is not always readily available because it requires live parasites. The IgG immunofluorescent assay (IFA) is generally in agreement with the DT. Unfortunately, neither the DT or the IFA is very specific for acute infection. IgG is detectable approximately 2 weeks after the onset of infection and peaks within 1 to 2 months. High titers may persist for months in some patients.[80] IgM antibodies, as determined by IFA, usually appear during the first week of infection and peak within a month. IgM titers often return to undetectable levels within a few months of the initial infection. Sulzer[83] examined the immune responses of 32 individuals who became acutely infected with *Toxoplasma* during military jungle training in Panama. Rapid rises of both IgM and IgG antibodies occurred within 2 weeks of infection. Interestingly, IgM did not precede IgG in any of the subjects. Many of the patients had persistently elevated IgM levels for 6 months to 1 year. A newer double sandwich IgM enzyme-linked immunosorbent assay (DS-IgM-ELISA) appears to be even more sensitive and specific for acute *Toxoplasma* infection and can be performed in about 2 hours.[84,85] All other available *Toxoplasma* antibody tests are associated with chronic infection and are not useful for the diagnosis of acute infection.[58] Newborns from mothers who are antibody positive have passively transferred maternal IgG. Demonstration of IgM antibodies in the neonate's circulation is thought to provide a more accurate indication of infection in the newborn because IgM does not cross the placenta.[81]

The diagnostic approach for a patient who may have acute toxoplasmosis with lymphadenopathy would include obtaining an IgM IFA or a DS-IgM-ELISA or both. A positive result on either test suggests an acute infection occurring within the past 3 to 4 months.[86] If negative, however, these tests do not rule out an acute infection, although a negative DS-IgM-ELISA greatly decreases the chance of an acute *Toxoplasma* infection being present.[87] A negative IgG IFA virtually excludes an acute infection.[79] High levels of specific IgG may block the IgM assays, giving a false-negative result. Removal of IgG from the sample may yield a more reliable IgM determination.[88] A 4-fold rise in IgG level by IFA over a 3-week period also provides supportive evidence of an acute infection.[83] Using the polymerase chain reaction, it may be possible to identify those patients with active toxoplasmosis who have equivocal or negative serologic tests.[87,88] While not yet standardized, polymerase chain reaction–based identification offers the possibility of very specific diagnosis based on the detection of circulating *Toxoplasma* DNA. Unfortunately, assays for *Toxoplasma* serology are not generally found in forward-deployed medical units. Thus, the issue of screening febrile military personnel for toxoplasmosis, especially in tropical areas, remains problematic.

Recommendations for Therapy and Control

Therapy

Therapy for toxoplasmosis varies with the type of infection present. Immunocompetent patients with acute acquired toxoplasmosis probably do not require any therapy unless their symptoms are particularly severe or persistent.[61] Immunodeficient patients with acute toxoplasmosis require longer courses of therapy, while acquired immunodefi-

ciency syndrome patients with encephalitis require lifelong suppressive therapy. Immediate treatment of acute maternal infections in pregnant women may decrease the chance of fetal infection. Although treatment will not reverse tissue damage, congenitally infected infants probably benefit from therapy.[58] Active retinochoroiditis should be treated to prevent vision loss. Minimally symptomatic or asymptomatic patients do not require therapy.[69]

The most effective regimen for therapy of toxoplasmosis consists of the synergistic combination of pyrimethamine and sulfadiazine.[69] This combination exerts a growth-inhibiting effect rather than a parasiticidal effect on proliferating *Toxoplasma* tachyzoites.[61] Unfortunately, the cyst form of the organism is resistant to this therapy and persists in tissues. Substitution of the antibiotic clindamycin for sulfadiazine appears not to affect efficacy and toxicity, although the specific effect of clindamycin on *Toxoplasma* is unclear.[89,90] Both regimens appear to reduce symptoms in acute acquired disease. For immunocompetent patients with acute toxoplasmosis, pyrimethamine is first given as a loading dose of 200 mg in two divided doses. Follow-on therapy is given at a dose of 25 to 50 mg/day for 2 to 4 additional weeks.[69] Since pyrimethamine is a folic acid antagonist, hematologic toxicity is commonly seen as a dose-related myelosuppression. Twice weekly blood counts during therapy will help to identify those patients at risk of significant myelotoxicity. Addition of folinic acid (5 to 10 mg with pyrimethamine) will help to prevent or ameliorate the myelosuppression.[61] Sulfadiazine is given along with pyrimethamine, first as a loading dose of 75 mg/kg up to a dose of 4 g, then as 1 to 1.5 g every 6 hours.[69] Maintenance of good urine output is essential to prevent crystalluria and oliguria. Clindamycin is given at a dose of 600 mg every 6 hours, either orally or intravenously.[61]

Control

The most important goal in the prevention of toxoplasmosis is the protection of seronegative pregnant women and patients with immunodeficiencies.[69] These two groups, however, are not usually associated with military deployments. Thus, without an effective vaccine, toxoplasmosis prevention for the military revolves around the prevention of acute infection in immunocompetent individuals, most of whom will not become symptomatic even after infection. Prevention of infection will help them in the future should they ever become immunocompromised. To date, no immunization scheme directed against *Toxoplasma* has proved effective.[61]

The major goal in controlling the spread of toxoplasmosis is the reduction in the number of potentially infectious organisms present. Full cooking of all meat products will effectively prevent transmission from viable tissue cysts. While freezing meat can reduce the infectivity of tissue cysts, it does not completely eliminate the risk of infection.[57] Individuals should also avoid raw eggs and unpasteurized dairy products, especially goat's milk.[61] Bradyzoites within tissue cysts are killed by irradiation of at least 25 rads, temperatures higher than 61°C for at least 4 minutes, or freezing at -20°C for 24 hours followed by thawing.[69] Salting, pickling, and curing of meat also kills tissue cysts.[66]

Ideally, cats should be maintained only as indoor pets to prevent them from acquiring *Toxoplasma* from the environment. In addition, cats should not be fed raw meat, because they may acquire an acute infection from the ingestion of viable tissue cysts.[60] Cat feces should be disposed of daily to prevent sporulation of oocysts. Wastes may be buried, burned, or flushed into a waste collection system that has no communication with the drinking water system. Gloves should be worn whenever handling containers bearing cat wastes. Care should be taken to prevent aerosolization of cat wastes via shaking. Pregnant women should not clean cat litter containers, and they should wear gloves during any encounter with soil (eg, gardening). Strict handwashing before eating should be mandated. Similarly, hand washing is important for all who handle raw meats or soil. Stray cats should be removed from sand-containing structures to prevent their depositing oocyst-containing feces. Open sandboxes or sand piles should be covered when not in use so as not to attract cats. Since agricultural produce may be contaminated with oocysts, it is important that all such items be thoroughly washed with clean water.[57] Additionally, local drinking water supplies may also be contaminated with infectious oocysts.[87] Benenson and colleagues[54] reported an outbreak of acute toxoplasmosis in 39 US soldiers associated with the ingestion of oocyst-contaminated drinking water in the Panamanian jungle. Effective source protection and water purification schemes need to be in place to prevent this type of contamination. Vector control should focus on control of potential transfer vectors, such as flies and cockroaches.[61] Stray cats should be eliminated from encampment sites. There is no need for isolation of patients presenting with acute symptomatic toxoplasmosis, since person-to-person spread is not a part of the parasite's normal life cycle.[57]

Primitive sanitary conditions associated with a mass deployment increase the risk of acquiring toxoplasmosis. Proper personal hygiene, food preparation, and water supplies should effectively prevent most *Toxoplasma* infections from occurring. Consideration of toxoplasmosis in the differential diagnosis of lymphadenopathy syndromes may obviate the need for immediate patient evacuation for lymph node biopsy.

[Kent E. Kester]

Q FEVER

Introduction and Military Relevance

Q Fever is a zoonotic disease caused by *Coxiella burnetii,* a rickettsia-like organism of low virulence but remarkable infectivity. There is a sporelike form of the organism that is extremely resistant to heat, pressure, and desiccation, allowing it to induce infection by airborne dissemination at sites miles distant from an infected source. The sporelike form may also persist in the environment for weeks or months under harsh conditions and subsequently cause infection after indirect exposure. The acute clinical disease associated with Q fever infection in humans is usually benign but temporarily incapacitating.

Since description of the disease in Australia in 1937, thousands of cases involving military personnel of many countries have been reported.[91] Many US soldiers in Italy during World War II were affected, with five confirmed outbreaks.[92–95] In one of these outbreaks, approximately 1,700 cases occurred at an airbase in southern Italy as a result of sheep and goats herded in pastures nearby.[94]

Hundreds of cases of atypical pneumonia consistent with Q fever were also observed during World War II in German soldiers in Serbia, southern Yugoslavia, Italy, Crimea, Greece, Ukraine, and Corsica,[91] usually in the apparent absence of disease in the indigenous population.

Q fever has been identified even in service members stationed or training in their home countries close to sheep or goats, particularly parturient animals[91]; outbreaks have been described among Swiss soldiers, Greek soldiers, and British airmen on the Isle of Man. Outbreaks have also been described in deployed British and Swedish troops stationed in Cyprus, US airmen in Libya, and French soldiers in Algeria.[91,96,97] Among US military personnel in the Persian Gulf War, one case of meningoencephalitis associated with acute Q fever was reported, with the onset of symptoms 2 weeks after the individual's return from the Persian Gulf.[98]

Because the infectivity of *C burnetii* is at least equivalent to that of anthrax and tularemia,[99] Q fever has been evaluated as a potential biological warfare agent by the United States, but stocks and munitions (except for strains necessary for vaccine research) were destroyed between May 1971 and May 1972, in accordance with the executive order signed by President Richard Nixon.[100]

Description of the Pathogen

C burnetii is classified in the family *Rickettsiaceae*, but it is not included in the genus *Rickettsia* and therefore is not a true rickettsia. It is not closely related to any other bacterial species when comparative 16s ribosomal RNA analysis is performed[101]; the genus *Coxiella* has only one species.

C burnetii must occupy an intracellular environment to grow or reproduce, similar to true rickettsia, but the sporelike form can survive extracellularly and transmit infection by itself.[102] The sporelike form may also be seen in human tissue.[103] *C burnetii* in the host occupies the phagolysosome of eukaryotic cells, which is usually a very destructive, acidic environment with numerous digestive enzymes.

Phase variation has been described with *C burnetii*.[104] Phase I, the virulent form associated with natural infection, has a smooth lipopolysaccharide (LPS) component of the outer membrane; phase II, an avirulent form resulting from serial laboratory passage of the organism in eggs or cell culture, has a rough, incomplete LPS.

Epidemiology

Transmission

Human infection with *C burnetii* is usually the result of inhaling infected aerosols, although tickborne disease does occur, as do cases resulting from ingesting unpasteurized milk from infected animals. *C burnetii* is extremely infectious; under experimental conditions, a single organism is capable of producing infection and disease in humans.[105]

The primary reservoirs for human infection are livestock animals, such as sheep, cattle, and goats, particularly parturient females. *C burnetii* also infects a large number of other species of wild and domesticated animals, including cats, dogs, pigs, camels, birds, and poultry. Numerous species of ticks harbor the infection, and transovarial trans-

mission of the organism can maintain the infection in the wild.

During gestation in infected animals, the proliferation of *C burnetii* in the placenta facilitates aerosolization of large numbers of the pathogen during parturition. Unpasteurized eggs from infected poultry may also transmit the infection, as can unpasteurized milk. *C burnetii* is shed in the urine and feces of infected animals, in addition to being present in the blood and tissues. Humans who work with animal products or in animal husbandry, especially abattoir workers or animal handlers and veterinary personnel who assist during parturition (eg, calving, lambing), are thus at increased risk for acquiring Q fever.

Survival of the sporelike form of *C burnetii* as an aerosol or on inanimate surfaces, such as straw, hay, or clothing, allows for transmission to individuals who are not in direct contact with infected animals. Examples include service members sleeping in barns previously occupied by infected animals[93]; laundry workers handling infected clothing[106]; coworkers of an individual with an infected parturient cat at home[107]; and residents of an urban community living along a road used by farm vehicles.[108] Investigations of outbreaks of Q fever frequently report a significant proportion of patients who have no direct contact with animals and cases among people who live or work miles from an infected source. Human-to-human transmission has been reported, but it is a very rare event.[109]

Geographical Distribution

The distribution of *C burnetii* is worldwide,[110] and the host range is very diverse, including a large number of mammalian species and arthropods. Q fever has been identified in most countries where an attempt has been made to identify evidence of infection in humans or animals, except New Zealand. Outbreaks of fever are infrequently reported, however, and the disease may be endemic in areas where cases are rarely or never reported.

In the United States, the epidemiology of Q fever is variable. Isolated but regularly occurring cases have been observed in areas with endemic foci in cattle.[111] Clusters of cases have been described in areas with infected dairy herds.[112] A small outbreak in Maine associated with exposure to a parturient cat has been described,[113] similar to an outbreak in Nova Scotia, Canada.[114] Between 1981 and 1992, five outbreaks of Q fever in the United States were reported from five different states.[115–119] Four of these outbreaks occurred in research facilities using sheep.

Incidence

Although reported outbreaks of Q fever in the United States have been relatively uncommon in recent years,[120,121] underreporting undoubtedly occurs. For example, although the first two cases of Q fever were reported from two adjacent rural counties in Michigan in 1984, a study published 4 years later showed that 15% of the general population surveyed in those two counties and 43% of goat owners were seropositive.[122]

Pathogenesis and Clinical Findings

After an individual inhales an infected aerosol, ingests infected milk, or suffers a tick bite, *C burnetii* organisms are phagocytized, predominately by unstimulated macrophages. There is little host reaction at the portal of entry, either in the lung, skin, or alimentary tract. After phagocytosis, conditions within the phagolysosome trigger growth and multiplication of *C burnetii*, with little initial damage to the host cell. Eventually the phagolysosome and cytoplasm become engorged with *C burnetii* organisms, and the host cell lyses. Dissemination of the pathogen occurs as the organisms circulate freely in the plasma, are carried on the surface of cells, and are carried by circulating macrophages.

In animals, infection with *C burnetii* frequently lasts for the life of the animal in a dormant state. The acute infection is usually inapparent in animals, except for an increased rate of spontaneous abortion in some species.

In humans, after multiplication and dissemination of the organism, polyclonal antibody production represents the initial immune response to *C burnetii*, but control of the infection by the host eventually requires the development of specific cell-mediated immunity, with killing by activated macrophages and natural killer cells. This process may result in a granulomatous reaction without the scarring and tissue reaction observed with true granulomata.

The incubation period for Q fever in humans varies from 10 to 40 days, and the infection may be manifested by, in order of frequency, asymptomatic seroconversion, acute illness, or chronic disease. In epidemiologic surveys, most seropositive individuals do not recall having the illness. The tendency for *C burnetii* to produce asymptomatic seroconversion, particularly when the infecting inoculum is low, has been documented in several publications. In outbreaks, the incidence of asymptomatic seroconversion is usually about 50%.[105,107,123] These observations underscore the value of an epidemiologic investi-

gation when even a single case of acute Q fever is recognized.

There is no characteristic illness for acute Q fever,[124,125] and manifestations may vary considerably among locations where the disease is acquired. The onset of Q fever may be abrupt or insidious, with fever, chills (including frank rigors), and headache being the most common symptoms (Table 36-3). The headache is usually described as severe, throbbing, and frontal or retro-orbital. Diaphoresis, malaise, fatigue, and anorexia are very common. Myalgias are also a frequent complaint, while arthralgias are relatively unusual. Cough tends to appear later in the illness than some of the other more common symptoms and may not be a prominent complaint.[126] Chest pain occurs in a minority of patients and may be pleuritic or a vague substernal discomfort. A weight loss of more than 7 kg is relatively common, particularly when the initial illness is prolonged. Although nonspecific evanescent skin eruptions have been reported, there is no characteristic rash.

TABLE 36-3

FREQUENCY OF SYMPTOMS IN ACUTE Q FEVER (%)

Symptoms	%
Fever	80-100
Chills, rigors	75-100
Headache, retro-orbital pain	50-100
Malaise	50-100
Diaphoresis	40-100
Myalgias	45-85
Weakness, fatigue	40-85
Weight loss ($\geq$ 7kg)	50-80
Cough	50-60
Chest pain	25-50
Anorexia	35-45
Neurological	10-35
Sore throat	5-35
Nausea, vomiting	15-20
Arthralgias	10-20
Diarrhea	5-20
Neck stiffness	5-7

Data sources: Robbins FC, Ragan CA. Q fever in the Mediterranean area: Report of its occurrence in allied troops, I: Clinical features of the disease. *Am J Hyg.* 1946;44:6–22; Feinstein M, Yesner R, Marks JL. Epidemics of Q fever among troops returning from Italy in the spring of 1945, I: Clinical aspects of the epidemic at Camp Patrick Henry, Virginia. *Am J Hyg.* 1946;44:72–87; Langley JM, Marrie TJ, Covert A, Waag DM, Williams JC. Poker players' pneumonia: An urban outbreak of Q fever following exposure to a parturient cat. *N Engl J Med.* 1988;319:354–356; Raoult D, Marrie TJ. State-of-the-art clinical lecture: Q fever. *Clin Infect Dis.* 1995;20:489–496; Tissot Dupont H, Raoult D, Brouqui P. Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. *Am J Med.* 1992;93:427–434; Smith DL, Ayres JG, Blair I, et al. A large Q fever outbreak in the West Midlands: Clinical aspects. *Respir Med.* 1993;87:509–516; and Derrick EH. The course of infection with *Coxiella burnetii. Med J Aust.* 1973;1:1051–1057.

For unknown reasons, cigarette smokers are more likely than nonsmokers to develop symptomatic infection with Q fever.[126] The temperature tends to fluctuate, with peaks of 39.5°C to 40.5°C (103°F-105°F), and in approximately one fourth of the cases is biphasic; in two thirds of patients with acute disease, the febrile period lasts 13 days or less.[127] Neurological symptoms are not uncommon and have been observed in up to 23% of acute cases reported in a recent outbreak.[126]

Physical findings in acute Q fever are also relatively nonspecific. Rales are probably the most commonly observed physical finding; evidence of pleural effusion (including friction rub) and consolidation may also be noted but not in the majority of infections. Although hepatomegaly, splenomegaly, jaundice, pharyngeal injection, and hepatic and splenic tenderness have all been reported, they are relatively unusual in acute infection.

Reports of abnormalities on chest x-ray examination vary with locale but can probably be identified in 50% to 60% of patients. An abnormal chest radiograph may be seen in the absence of pulmonary symptoms, while a normal chest radiograph may be observed in a patient with pulmonary symptoms.[128]

Laboratory abnormalities associated with acute Q fever most commonly involve tests of liver function, and patients may present with a clinical and laboratory picture consistent with acute hepatitis. In 50% to 75% of patients, 2- to 3-fold elevations of the aspartate aminiotransferase or alanine aminotransferase (AST or ALT) or both are observed, while elevations of either the alkaline phosphatase or total bilirubin or both are observed in only 10% to 15%.[126] The white blood count is usually normal; the erythrocyte sedimentation rate is elevated in approximately one third of patients.[97] Mild anemia or thrombocytopenia may also be observed.

The case fatality rate of acute Q fever is low, even without treatment, and chronic disease, usually manifested by endocarditis, probably develops in less than 1% of acute infections. Q fever endocarditis usually occurs on heart valves with preexisting abnormalities. Those with prosthetic heart valves

or other vascular prostheses are also at increased risk of infection.[124] Granulomatous hepatitis also occurs as a chronic complication of Q fever, more commonly after ingestion of unpasteurized infected milk.[125] A syndrome of protracted fatigue following acute Q fever has been described.[129–131]

Diagnostic Approaches

Diagnosis of Q fever is usually accomplished by serological testing. A number of methods have been used,[132] the most common being antibody detection by indirect fluorescent antibody or enzyme-linked immunosorbent assay (ELISA). Significant antibody titers are usually not identifiable until 2 to 3 weeks into the illness; convalescent antibody titers, 2 to 3 months after onset, almost always demonstrate a typical 4-fold rise. After infection, significant antibody titers may persist for years.

In general, antibodies to the phase II organism are identified earlier in the illness, during the first few months after infection, followed by a decline in antibody to the phase II organism and a rise in antibody to the phase I organism. Antibodies of the IgM type are usually observed within the first 6 to 12 months after infection, with persistence of IgG antibodies afterward. Of the methods currently used for the diagnosis of Q fever, the ELISA is the most sensitive and easiest to perform; the utility of the ELISA for epidemiologic screening and diagnosis of Q fever has been confirmed.[133,134] The sensitivity of this test in the convalescent phase of illness or in the first 1 to 2 years after infection approaches 100%.[134] Polymerase chain reaction may also be useful in the future for the diagnosis of Q fever[135–137] but remains to be validated in acute clinical cases.

Recommendations for Therapy and Control

Antibiotic treatment of acute Q fever shortens the course of the disease and is effective in preventing disease when administered during the incubation period.[105] Tetracyclines remain the mainstay of therapy for acute disease.[138] Macrolide antibiotics, such as erythromycin and azithromycin, are also effective.[139,140] Quinolones, chloramphenicol, and trimethoprim-sulfamethoxazole have also been used to treat Q fever,[138] but experience with these antibiotics is very limited.

Q fever in humans can be prevented by immunization with a formalin-killed whole cell vaccine. Although a very effective Q fever vaccine is licensed in Australia (Q-Vax, CSL, Victoria, Australia), all Q fever vaccines used in the United States are currently investigational. Existing vaccines are generally well tolerated after subcutaneous injection, although individuals already immune to Q fever may develop severe local reactions at the site of injection.[141,142] These reactions can be avoided by prior screening with an intradermal skin test to detect presensitized or immune individuals.[143]

Control of Q fever requires, most importantly, recognition of the disease (which may be difficult considering the nonspecific clinical findings) and awareness of the potential risk of infection in appropriate settings. Areas such as cow or sheep sheds that are or have been occupied by animals that may harbor the infection should be avoided. Considering the large number of mammalian species that may carry the infection, close contact with animals, both domestic and wild, outside of the United States should be avoided, especially if birthing is occurring. Disposal of animal products of conception should be accomplished as soon as possible. Unpasteurized or uncooked animal products should never be consumed. In spite of an innate resistance to heat, *C burnetii* organisms are inactivated by pasteurization at 62.7°C (145°F) for 30 minutes, 71.6°C (161°F) for 15 seconds, or boiling.[57p407–411] Dead animals and animal products should also be handled with care, because Q fever may be transmitted by contact with these materials.

If contamination with *C burnetii* has occurred, disinfection of areas or articles soiled by infected blood, tissue, or other animal products is recommended with 0.05% hypochlorite, 5% peroxide, or a 1:100 solution of Lysol.[57] Although complete inactivation even with these measures is not certain, both 70% ethanol and a 5% solution of N-alkyl dimethyl benzyl and ethylbenzal ammonium chlorides have been shown to completely inactivate the organism after 30 minutes of exposure.[144]

[William R. Byrne]

VIRAL HEMORRHAGIC FEVERS

Introduction and Military Relevance

The hemorrhagic fevers are serious, widely distributed human infections caused by viruses from several different taxonomic families (Table 36-4). As a group, these diseases share many clinical, pathophysiologic, and epidemiologic features. Most are zoonoses; human infections occur as a result of incursion into the cycles of transmission established between each virus and its host or reservoir in nature.

TABLE 36-4

VIRAL HEMORRHAGIC FEVERS OF HUMANS

Family/Virus	Disease	Geographic Distribution	Natural Transmission
Arenaviridae			
Lassa	Lassa Fever	Rural west Africa	Rodent to human*
Junin	Argentine HF	Rural Argentina	Rodent to human*
Machupo	Bolivian HF	Rural Bolivia	Rodent to human*
Guanarito	Venezuelan HF	Rural Venezuela	Rodent to human*
Sabiá		Rural(?) Brazil	Unknown*
Bunyaviridae			
Hantaan	HFRS	Rural Asia, Far East, central Europe/Balkans(?)	Rodent to human
Puumala	HFRS	Rural western Europe, central Europe/Balkans	Rodent to human
Dobrava/Belgrade	HFRS	Central Europe/Balkans	Rodent to human
Seoul	HFRS	Worldwide	Rodent to human
Sin Nombre†	HPS	Americas	Rodent to human
Crimean-Congo Hemorrhagic Fever	CCHF	Rural Africa, west Asia, central Europe	Tick bite; inhalation or contact with blood of infected mammals*
Rift Valley Fever	RVF	Sub-Saharan Africa, Egypt	Mosquito bite; inhalation or contact with blood of infected mammals*
Filoviridae			
Ebola-Sudan	Ebola HF	Sudan	Unknown*
Ebola-Zaire	Ebola HF	Zaire	Unknown*
Ebola-Ivory Coast	Ebola HF	Ivory Coast	Unknown*
Marburg	Marburg HF	Sub-Saharan Africa	Unknown*
Flaviviridae			
Yellow fever	Yellow fever	Africa, South America	Mosquito bite
Dengue	Dengue HF	Tropical/subtropical regions worldwide	Mosquito bite
Omsk	Omsk HF	Northern Asia	Tick bite; contact with infected muskrats*
KFD	KFD	Rural India	Tick bite*

*The threat of severe nosocomial or laboratory-acquired infection or both is particularly high
†Other hantaviruses endemic to the Americas and associated with hantavirus pulmonary syndrome have also been described and characterized but are not included in this table
HF: hemorrhagic fever, HFRS: hemorrhagic fever with renal syndrome, HPS: hantavirus pulmonary syndrome, RVF: Rift Valley fever, KFD: Kayasanur Forest disease

Viral hemorrhagic fever should be considered in the differential diagnosis of febrile illnesses among military personnel living or working in disease-endemic areas. Military personnel tend to be among those at highest risk for acquiring viral hemorrhagic fevers because field operations and warfare are often conducted under adverse environmental conditions in remote regions where exposure to vectors or reservoir hosts for the etiologic agents is likely. The impact of dramatic, highly lethal infections on the morale and welfare of forces cannot be overestimated. Therapeutic interventions are time-critical for infected individuals, and rapid implementation of surveillance and control strategies may be essential to avoiding either panic, epidemic catastrophe, or both. Clinical management of these diseases is

demanding and resource-intensive. Explosive nosocomial outbreaks are a well-documented consequence of unwitting introduction of viral hemorrhagic fevers into routine clinical care settings. Personnel at both clinical and research laboratories are at particularly high risk of infection. No licensed vaccines are currently available for the viral hemorrhagic fevers discussed in this chapter, but safe and effective immunogens have been or are being developed for use in high-risk populations (Table 36-5).

Many viral hemorrhagic fevers are efficiently spread by aerosol transmission from infected reservoirs. This fact, coupled with the recognized high morbidity and mortality associated with naturally acquired human disease and the stability of the etiologic agents under adverse environmental conditions, has generated considerable interest in their potential for use as biological warfare agents. Resources are available for advice and specific guidance from the US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Md, and the Special Pathogens Branch of the National Center for Infectious Diseases at the Centers for Disease Control and Prevention, Atlanta, Ga.

In this chapter, hemorrhagic fevers caused by arenaviruses and filoviruses, together with Crimean-Congo hemorrhagic fever, are considered; elsewhere in this volume are discussions of hemorrhagic fevers caused by mosquito-borne flaviviruses (dengue, yellow fever), tick-borne flaviviruses (Omsk hemorrhagic fever, Kayasanur Forest disease), and other members of the *Bunyaviridae* (hemorrhagic fever with renal syndrome, hantavirus pulmonary syndrome, Rift Valley fever).

Arenavirus Hemorrhagic Fevers

Description of the Pathogens

The family *Arenaviridae* contains segmented, single-stranded RNA viruses. Each human pathogenic arenavirus is associated with a specific rodent species in which it establishes a chronic, persistent infection. Infected rodents shed large quantities of virus in secretions and excreta; humans become infected through inhalation of virus-containing aerosols and via mucous membrane or percutaneous inoculation of virus through contact with contaminated surfaces. Arenaviruses have been classified into 2 groups: the Old World complex (eg, lymphocytic choriomeningitis virus, Lassa virus), and the New World (or Tacaribe virus) complex. Lassa is the only Old World complex virus that causes hemorrhagic fever. Within the New World complex, four viruses are recognized causes of hemorrhagic fever

TABLE 36-5

THERAPEUTIC AND PREVENTIVE MEASURES SPECIFIC FOR SELECTED VIRAL HEMORRHAGIC FEVERS

Disease	Therapy	Prevention
Arenaviruses		
Lassa fever	Supportive, ribavirin	Rodent control, avoidance of reservoir
Argentine HF	Supportive, immune plasma, ribavirin(?)	Candid #1 Junin vaccine (IND)
Bolivian HF	Supportive, ribavirin(?)	Rodent control, Candid #1 vaccine(?)
Venezuelan HF	Supportive	Avoidance of reservoir
Sabiá HF	Supportive, ribavirin(?)	Unknown
Filoviruses		
Ebola HF	Supportive	Unknown
Marburg HF	Supportive	Unknown
Bunyaviruses		
Crimean-Congo HF	Supportive, ribavirin	Tick control, avoidance of slaughtered animals

Note: (?) indicates anecdotal experience or strong theoretical justification for use based upon in vitro findings or experimental models or both
HF: hemorrhagic fever, IND: investigational new drug

in humans: Junin (which causes Argentine hemorrhagic fever), Machupo (Bolivian hemorrhagic fever), Guanarito (Venezuelan hemorrhagic fever), and Sabiá (no specific disease designation).

Lassa Fever

Transmission. The sole reservoir for Lassa virus is *Mastomys natalensis*, the multimammate rat. This peri-domestic rodent is an important source of protein for people in disease-endemic areas, and it regularly contaminates houses and living areas with infectious urine and respiratory secretions.[145,146] Most cases of Lassa fever apparently are acquired through contact with rodents or their excreta, but human-to-human spread also occurs. Clustering of cases and seropositives is frequently found, and transmission of disease through contact with febrile patients and via sexual intercourse during incubation and convalescence has been seen. Nosocomial outbreaks are uncommon but are well documented.[147,148]

Geographic Distribution and Incidence. Lassa fever occurs in the West African nations of Sierra Leone, Liberia, Nigeria, Guinea, and probably in Senegal, Ivory Coast, Upper Volta, and Mali. Infection with Lassa virus is common in all ages and both sexes; estimates range from thousands to hundreds of thousands of incident cases annually.[149] In Sierra Leone, as many as 16% of adult medical admissions to hospitals, 47% of adult febrile admissions, 20% of pediatric febrile admissions, and 30% of adult medical deaths may be due to this disease.[150,151] Mortality in pregnant women is particularly high, reaching 30% or more during the third trimester.[152]

Pathogenesis and Clinical Findings. Lassa fever is a disease of insidious onset. Fever, malaise, and headache appear gradually, usually 8 to 14 days after exposure to the virus. As illness progresses, sore throat, myalgias, arthralgias, epigastric or retrosternal pain, vomiting, and dry cough appear. Physical findings include conjunctivitis, abdominal and muscle tenderness, lymphadenopathy, hypotension, and relative bradycardia. About 40% of patients develop a painful, purulent pharyngitis that is sometimes associated with vesicles or ulcers. A progressively "toxic" appearance is typically accompanied by edema of the face and neck. There is no characteristic skin rash, and neither cutaneous hemorrhage nor jaundice is evident early in the disease. Severe hypotension and bleeding from the nose and mouth, gastrointestinal tract, or vagina are associated with a fatal outcome; these complications occur in the minority of patients, however (15% to 20%). Pericardial rubs occur in about 20% of cases. Acute encephalitis has been described in many hospitalized patients, manifesting as seizures, dystonia, and neuropsychiatric changes.[153] More severe evidence of central nervous system involvement (stupor, coma, focal neurological signs) signifies a poor prognosis. Recovery may take a month or more. Convalescence may be complicated by ataxia, orchitis, uveitis, pericarditis, pleural effusion, or ascites. Eighth nerve deafness occurs in 25% to 30% of survivors and may be permanent; Lassa fever is considered to be among the most important causes of deafness in West Africa.[154]

Clinical laboratory findings in Lassa fever are generally unremarkable. White blood cell counts may be depressed early, but as the disease progresses they frequently are normal or elevated. Platelet counts are normal or elevated but platelet function is abnormal.[155] Proteinuria is inconsistent. High serum viremia ($> 10^{3.6}$ $TCID_{50}/mL$) and aspartate aminotransferase (AST > 150 IU/L) are each associated with a poor prognosis (50% and 73% mortality, respectively). If both factors coexist, mortality is 80%.[156,157]

Diagnostic Approaches. Diagnosis of Lassa fever is made by demonstration of a 4-fold rise in virus-specific antibodies, virus isolation, antigen detection by enzyme immunoassay, or demonstration of virus-specific IgM or high-titered IgG in the setting of a compatible clinical illness. The differential diagnosis includes malaria, typhoid fever, and other febrile illnesses common to West Africa. A single case-control study from Sierra Leone suggested that triads of either pharyngitis, retrosternal pain, and proteinuria or pharyngitis, retrosternal pain, and vomiting were useful to discriminate Lassa fever from other conditions.[150]

Recommendations for Therapy and Control. The specific therapy for Lassa fever is the antiviral drug ribavirin. Administration of ribavirin by both parenteral and oral routes has been shown to be effective in reducing mortality, but the parenteral route is more effective.[157] Optimal benefit is derived within the first 6 days of illness. Although of unproven benefit, prophylactic administration of oral ribavirin represents a prudent approach to management of a significant exposure to Lassa virus (eg, a needlestick). There is no vaccine presently available for Lassa fever; prevention consists of control and avoidance of reservoir rodents. Control of nosocomial spread has been accomplished in endemic areas through use of barrier precautions and care to avoid cross-contamination of needles and other devices. Clinical and diagnostic laboratory samples

are highly hazardous and should be manipulated only by trained personnel in controlled, high-level biocontainment facilities.

South American Hemorrhagic Fevers

Argentine hemorrhagic fever (AHF), Bolivian hemorrhagic fever (BHF), Venezuelan hemorrhagic fever (VHF), and hemorrhagic fever caused by Sabiá virus often are referred to collectively as the South American hemorrhagic fevers. The hemorrhagic fevers they cause are clinically similar, but each disease has its own specific transmission patterns and geographic distribution.

AHF occurs in a progressively expanding endemic zone that includes portions of Buenos Aires, Cordoba, Santa Fe, and La Pampa provinces of north-central Argentina.[158] AHF is a seasonal disease, with a prominent peak in autumn that coincides with the summer grain harvests and the period of maximum population density for the Junin virus reservoir, *Calomys musculinus*, the field mouse. Disease is associated with inhalation of contaminated aerosols generated by mechanical grain harvesters and with exposure to linear habitats frequented by *C musculinus*, such as fencerows and railroad beds. Disease incidence before the introduction of the Junin virus vaccine was 200 to 1,000 cases per year. Although all ages and both sexes are affected, most cases are seen in male agricultural workers who are 20 to 60 years old.

Like AHF, the preponderance of BHF occurs in conjunction with peaks of agricultural activity. Although most BHF is thought to occur after exposure to aerosolized rodent urine, clusters of cases in Cochabamba in 1971 and near Magdelena in 1994 provide strong suggestive evidence for person-to-person spread of Machupo virus.[159,160] BHF is found naturally only in the Beni Department of northern Bolivia, although the range of the reservoir for Machupo virus, *C callosus*, also includes parts of Argentina, Paraguay, and Brazil. *C callosus* differs in habit from that of its Argentine congener in that it also has peri-domestic affinities. Between 2,000 and 3,000 cases of BHF have been recorded since the disease was recognized in 1959; many of these were associated with a series of devastating epidemics that occurred between 1962 and 1964 in and around the village of San Joachin.[161] Effective rodent control and surveillance programs that arose from these outbreaks eliminated epidemic BHF, and only sporadic cases have occurred since. Currently, cases number in the low double digits annually. Most cases of BHF occur in men from rural areas, but community and family outbreaks have involved all ages and both sexes.

VHF was first recognized in 1989 following an outbreak of hemorrhagic fever in Guanarito, Portuguesa State, Venezuela.[162] The reservoir for Guanarito virus is thought to be the cane rat, *Zygodontomys brevicauda*. The cotton rat, *Sigmodon alstoni*, cocirculates in the area and carries a closely related virus (putatively named Pirital virus) whose disease potential remains undefined at this time.[163] As is the case with AHF and BHF, both children and adults are susceptible to infection with VHF virus, although the highest risk tends to be among adult male farm workers. A seasonal peak in disease has been noted from November to January. As of 1997, fewer than 150 cases of this disease had been documented from the endemic area, most of them between 1989 and 1991.

Sabiá virus has been recovered only once from a human; under natural conditions it was acquired near Sao Paulo, Brazil, in 1990.[164] Its reservoir and mode of transmission to humans are unknown. Two subsequent human infections with Sabiá virus occurred following laboratory accidents, emphasizing the importance of confining work with this and related human-pathogenic arenaviruses to biosafety level 3 or 4 facilities.

Pathogenesis and Clinical Findings. The South American hemorrhagic fevers are clinically similar. The incubation period is generally 1 to 2 weeks. Patients experience the gradual development of fever, malaise, myalgia, and anorexia. Headache, dizziness, backache, epigastric pain, vomiting, and diarrhea follow shortly afterward, accompanied by flushing of the face and chest, conjunctival injection, and orthostatic hypotension. Cutaneous petechiae are frequent, appearing most commonly in the axillae and palate. Congestion and bleeding of the gums is frequently seen, and the appearance of hemorrhage along the gingival margin at the point of tooth insertion is a characteristic finding. Neurological changes are virtually universal, with tremors (most commonly of the tongue and hands), lethargy, depressed deep tendon reflexes, and hyperaesthesia being most frequently noted. In most cases, improvement begins after 7 to 10 days, and patients go on to a prolonged recovery that may be accompanied by weakness, autonomic instability, and alopecia. About one third will progress to more severe illness, however, with evolution along one of three fairly distinctive patterns: a hemorrhagic diathesis with mucous membrane and puncture wound bleeding; a progressive neurological deterioration

with convulsions, delirium, obtundation, and coma; or a mixed hemorrhagic-neurologic syndrome with shock. Untreated, mortality may exceed 25%.

Thrombocytopenia (platelets < 100,000/mm^3) and leukopenia (white blood cell count < 4,000/mm^3) are almost universal. Proteinuria with or without microscopic hematuria is freqently seen. In AHF, serum alpha interferon levels may be exceedingly high (1,000-16,000 IU/mL); higher levels correlate with poorer prognoses.

Diagnostic Approaches. Diagnosis of South American arenavirus infections can be accomplished by serology (eg, indirect immunofluorescence, enzyme immunoassay, neutralization tests), antigen detection (eg, antigen detection enzyme immunoassay), or virus isolation. Clinical samples should be considered infectious and handled under biosafety level 4 conditions until treated with radiation or chemicals to inactivate the virus. The differential diagnosis includes lymphocytic choriomeningitis, hantavirus diseases, typhoid fever, and a host of other viral and bacterial diseases endemic to rural South America.

Recommendations for Therapy and Control. Convalescent immune plasma has been shown to be effective in treating AHF when administered within 8 days of disease onset.[165] Effectiveness of plasma therapy correlates with quantity (titer) of neutralizing antibody delivered.[166] A neurological syndrome of uncertain etiology is associated with immune plasma therapy in about 10% of treated survivors. This condition typically begins 4 to 6 weeks after treatment and is characterized by fever, headache, ataxia, and intention tremor but is usually benign. Intravenous ribavirin has shown promise in preliminary studies for therapy of AHF, but controlled trials have not been conducted. The drug has been successful in limited numbers of patients with Machupo and Sabiá virus infections as well, but additional experience is needed to document efficacy.

Rodent avoidance and control constitute the principal nonmedical modalities for preventing South American hemorrhagic fevers. A live-attenuated Junin virus vaccine codeveloped by the US Army and the Argentine government was shown in controlled trials[167] to be 95% efficacious in preventing AHF. This vaccine, called Candid #1, has been administered to more than 160,000 persons in the AHF-endemic area; its use has been associated with a dramatic decrease in disease incidence. Candid #1 successfully protected experimental animals against BHF but not VHF; it has not yet been assessed in humans for these diseases.

Filovirus Hemorrhagic Fevers

The *Filoviridae* are morphologically unique RNA viruses for which neither the reservoirs nor the modes of natural infection to humans are known. Although biochemically similar, the filoviruses are serologically distinctive. They have been classified into the Marburg and the Ebola groups. The Ebola group has three subtypes: Ebola-Sudan, Ebola-Zaire, and Ebola-Reston. A probable fourth Ebola strain recovered from a human infected in the Ivory Coast is incompletely classified at present. All but Ebola-Reston have been documented as causing hemorrhagic fevers in humans.

Epidemiology

The epidemiology of filoviruses is marked by the occurrence of dramatic, explosive outbreaks, typically with high mortality. Despite repeated and extensive efforts, no natural reservoir for any filovirus has been identified. Person-to-person spread of filoviruses has been documented repeatedly in association with parenteral or mucous membrane exposure to contaminated body fluids; health care workers regularly contribute substantially to morbidity and mortality statistics during epidemics.[168] Aerosol transmission is unproved in human infection, but airborne transmission of both Marburg and Ebola has been demonstrated experimentally, and there are indications that droplets or aerosols played a role in the spread of Ebola-Reston among quarantined cynomolgous macaques.

Marburg virus was the cause of simultaneous clusters of viral hemorrhagic fever in 1967 among individuals in Marburg, West Germany, and Belgrade, Yugoslavia, who were connected directly or indirectly with African green monkeys shipped from Uganda.[169] Marburg virus has since been recognized usually in the context of individual infections, both with and without secondary transmission.

Ebola-Zaire and Ebola-Sudan, in contrast, have been associated with repeated large epidemics in sub-Saharan Africa; the former more frequently and recently (1995 and 1996-1997).[170] In each of these outbreaks, case-fatality rates have been high (50% to 90%). Ebola-Ivory Coast virus has been seen only sporadically and has infected both humans and chimpanzees. Ebola-Reston has caused widespread morbidity and mortality of greater than 75% among colonies of cynomolgous macaques exported from the Philippines.[171] Several humans have been infected with Ebola-Reston virus in association with primate epizootics, but, to date, none have become ill.

Pathogenesis and Clinical Findings

Human filovirus infections are severe, debilitating, and frequently fatal. The experience to date with Asian filoviruses (eg, Ebola-Reston) suggests avirulence for humans; but that is based on very limited information. The incubation period for African filoviruses is generally 3 to 8 days but may be longer in secondarily acquired infections. Disease onset is abrupt, with frontal headache, fever, chills, myalgias, anorexia, and extreme malaise. Nausea, vomiting, and diarrhea are common early in the disease course, and conjunctivitis, pharyngitis, and oral ulcerations may be seen. Patients typically are prostrate and apathetic. A maculopapular rash appears on the trunk and back around the fifth day of illness and desquamates in survivors. Petechiae and oozing from venipunctures are frequent, and bleeding from mucous membranes of the gastrointestinal and genitourinary tracts and of the nasopharynx is commonly observed. Death due to intractable shock occurs between 6 and 16 days following disease onset. Infections in pregnancy are particularly severe, and fetal wastage is universal. The catabolic toll of these infections is huge; patients are severely wasted, and convalescence for survivors is prolonged.

Diagnostic Approaches

Platelet and leukocyte counts are depressed early, and a left shift is common. As disease progresses, neutrophilia appears. Serum transaminases are increased (AST > ALT), but bilirubin is generally normal or only slightly elevated. Viremia is present from early in the disease course and may persist for weeks (especially in semen and the anterior chamber of the eye) in survivors. Diagnosis of human filovirus infection is made by serology (enzyme immunoassay), antigen detection (antigen-capture enzyme immunoassay in serum, immunohistochemistry in tissues), visualization of virus particles in tissues by electron microscopy, and virus isolation. Initially developed immunofluorescent antibody tests are not reliable for serological diagnosis. Body fluid and tissue samples are highly infectious, and specimens should be handled only by trained personnel at the highest possible levels of biocontainment. The differential diagnosis includes a wide variety of diseases that occur in sub-Saharan Africa, including malaria, typhoid fever, and rickettsial diseases, as well as other viral hemorrhagic fevers.

Recommendations for Therapy and Control

There is no specific treatment available for filovirus diseases. Supportive care (eg, fluids, pressors, management of shock and hemorrhage) is most important. Exclusion of other, treatable conditions must always be considered. In the absence of insight regarding natural hosts or vectors of filoviruses, control measures are limited to interruption of person-to-person spread through early case identification, quarantine, decontamination, and barrier nursing precautions. The risk of nosocomial infection is high. There is no vaccine currently available for any filovirus.

Crimean-Congo Hemorrhagic Fever

Description of the Pathogen

Crimean-Congo hemorrhagic fever (CCHF) virus is a member of the *Nairovirus* genus in the large *Bunyaviridae* family. This RNA virus is maintained in nature via complex relationships between its arthropod vectors (multiple species of ixodid [hard] ticks) and nonhuman vertebrate hosts. The virus was initially identified in 1947 as the cause of Crimean hemorrhagic fever, but it was not until 1969 that its identity with an agent recovered from a febrile patient in the Belgian Congo in 1956 was recognized and the name revised to reflect the linkage.[172]

Epidemiology

Ticks presumably serve as both vectors and reservoirs for CCHF. Both transstadial and transovarial transmission of virus have been documented in several ixodid species. These ticks feed on a wide variety of domestic and wild animals, including birds. The predominant vertebrate reservoirs for the virus include large herbivores and wild hares; it is likely that vertebrate amplification in these hosts contributes substantively to natural sustainment and spread of the virus. Human CCHF occurs following the bite of an infected tick; through exposure to blood, tissues, or excreta of infected vertebrate reservoirs; or nosocomially.

The CCHF-endemic area is widespread, covering portions of three continents; infections have been recognized in eastern and central Europe, the Middle East, northern Asia, and throughout Africa. Since most CCHF occurs in rural and often remote regions, accurate incidence and prevalence data is scanty. Illness-to-infection ratios approach 50% in

some countries, but infected ticks or seropositive animals or both have been recovered from other areas where human infections are unreported. The reasons for such wide variability in disease expression are unknown. Shepherds, farmers, abbatoir workers, and others engaged in outdoor activities in rural areas (eg, hunters, boy scouts) constitute the majority of those infected through natural exposure. The risk of nosocomial infection is high; explosive outbreaks have been repeatedly documented among hospital staff engaged in the surgical or medical management of unsuspected CCHF cases.[173,174]

Pathogenesis and Clinical Findings

Onset of CCHF is abrupt. After a 2- to 7-day incubation period, patients suddenly develop high fever, chills, myalgias, severe headache, weakness, epigastric pain, nausea, and vomiting. Flushing of the face and chest, palatal petechiae, and conjunctival injection are frequently seen. Between 3 and 5 days later, a remission of several hours duration occurs in one half to two thirds of patients. Subsequently, a second phase of the illness, the "hemorrhagic" phase, ensues and is characterized by the appearance of petechiae, epistaxes, bradycardia, profound hypotension, and pulmonary edema. In severe cases, uncontrolled hemorrhage from puncture wounds and mucosal surfaces occurs. The second stage may last 3 to 10 days. For survivors, recovery is prolonged.

Thrombocytopenia, leukopenia, and elevated serum enzyme levels appear early in the disease course and carry prognostic significance. In South Africa, leukocytosis, platelets levels of less than 20,000/mm^3, marked transaminase elevations, or profound coagulation abnormalities each was more than 90% predictive of a fatal outcome if seen during the initial 5 days of illness.[175]

Diagnostic Approaches

Diagnosis of CCHF is made by serology (eg, indirect immunofluorescence, enzyme immunoassay), antigen detection, or virus isolation. Virus is readily recovered from body fluids and tissues during acute illness. These materials constitute a substantial hazard for health care workers and laboratory personnel, so patients should be managed in isolation and diagnostic samples handled only under biosafety level 4 containment levels. The differential diagnosis of CCHF includes typhoid fever, malaria, and other bacterial or rickettsial diseases. Other viral hemorrhagic fevers should also be considered, particularly those caused by hantaviruses (in eastern and central Europe and Asia) and filoviruses (in Africa).

Recommendations for Therapy and Control

CCHF virus is sensitive in vitro to ribavirin, and the drug has become an important adjunct to intensive supportive care for CCHF in South Africa and perhaps elsewhere.[176] Use of personal protective measures (eg, repellents containing DEET for the skin, permethrin treatment of clothing [see chapter 22, Personal Protection Measures Against Arthropods]) to discourage tick bites is the most effective means available to prevent natural infection. In endemic areas, awareness of the disease in ungulates and hares and avoidance of blood and fluids from these animals as they are slaughtered or marketed should be emphasized. Postexposure prophylaxis with ribavirin is probably indicated after high-risk exposures (eg, needlesticks, resuscitation), though its efficacy in this setting is unproven. A formalin-inactivated mouse brain CCHF vaccine has been produced and used in Bulgaria and the former Soviet Union, but its safety and efficacy are also unproved.

[Kelly T. McKee, Jr.]

RABIES

Introduction and Military Relevance

Rabies is an acute encephalomyelitis caused by a number of closely related rhabdoviruses. It is transmissible, primarily by direct contact, through the secretions of infected animals. The possibility of contact with such animals during the course of field training or deployment and the worldwide occurrence of rabies make it a disease of military importance. Recent deployments to Africa, the Caribbean region, and Latin America, where animal rabies is highly endemic, underscores the significant risk deployed military personnel may face. Military physicians must have a firm understanding of the principles of rabies prevention and management. Despite advances in preexposure and postexposure prophylaxis, rabies as an infectious disease remains almost always fatal.

Rabies ("rage" or "madness" in Latin) was first described in dogs by Democritus circa 500 BC.[177]

Since that time, it has become one of the most feared infectious diseases known. Treatment involved cauterization with a hot iron until 1885. In that year, Louis Pasteur introduced the first effective rabies vaccine, which was prepared from a virus strain isolated from the brain of a rabid cow in 1882 and subsequently passed through rabbits. Today rabies still represents a significant health threat. Although it usually can be prevented with timely immunization, once infection is established, recovery is extremely rare.

The threat of rabies has been a longstanding concern for the US military. Outbreaks of skunk-transmitted disease were reported by the US Army as a source of rabies among horses and soldiers in the western frontier in the 1870s.[178] After World War II, US service members were redeploying from many parts of the world. At the time, there was no reliable data on the distribution of rabies in the United States. The US Army Veterinary Corps, the only source of information, found that rabies was rampant in all the theaters of war except parts of the Pacific, but it was enzootic in the Philippines, Taiwan, and Japan.[179] The global rabies problem in the late 1940s received various degrees of attention, depending on where the US Army veterinarians were posted. In the Philippines, they attempted to control rabies with the Kelser chloroform-treated vaccine, with little success.[180] General MacArthur's staff gave a high priority to the control of rabies, first by educating the civilian population and then by animal control through reduction in numbers and vaccination.

The postwar rabies problem in the United States also caused great concern. This led to a national rabies program, which included animal control through education, elimination of stray animals, preexposure vaccination of pet dogs, and preexposure prophylaxis and postexposure treatment for humans. Since World War II, the Centers for Disease Control has been active in promoting rabies control. But until there are major advances in the primary prevention of rabies among animals and in the control of human disease, it will remain a significant challenge to the military medical community.

Description of the Pathogen

Rabies is caused by a number of different strains of highly neurotropic, single-stranded RNA viruses. Most of those that infect vertebrates and invertebrates are bullet-shaped and belong to the genus *Lyssavirus* and the genus *Vesiculovirus* in the family *Rhabdoviridae*. The lyssaviruses include the classic rabies virus, which has been isolated from terrestrial mammals (eg, dogs, cats) and bats. There are, in addition, a number of rabies-related viruses that on rare occasions may cause human infection; examples include the Lagos bat virus (isolated from bats and cats), the Mokola virus (shrews and dogs), the European bat virus, and the Duvenhage virus prototype strains (bats). These viruses differ antigenically from the classic rabies viruses, engendering concern that current vaccines may not protect against them.

Despite its lethal effect on living tissue, the rabies virus survives very poorly in the environment. It is rapidly inactivated by desiccation, ultraviolet and x-ray irradiation, sunlight, trypsin, B-propiolactone, ether, and detergents.[181] It is, however, stable for many years when frozen at -70°C or freeze-dried and held at 0°C to 4°C.

Epidemiology

Transmission

Rabies viruses are transmitted largely by the bite of an infected animal; however, nonbite exposures have also been reported in which the victim was licked by an animal suspected of being rabid.[182,183] Airborne transmission has been confirmed in two settings: in caves inhabited by bats (from aerosolized bat urine) and in the laboratory.[184] Bats found in private homes may also pose a threat for rabies transmission. A fatal case of human rabies reported in 1995 involving a child illustrates this concern. A bat had been found in the child's bedroom approximately 3 weeks prior to symptoms, but there had been no evidence of a bite. The bat brain was later found to be positive for rabies by direct fluorescent antibody and nucleotide sequence analysis. This case suggests that even apparently limited contact with bats or other animals infected with rabies virus may be associated with transmission.[185] Rabies transmission has also been documented to occur via corneal transplants from infected donors.[186] The risk for transmission of rabies from a patient to family members or healthcare workers is extremely low; human-to-human transmission has been documented only in corneal transplant cases.

The rabies virus can be found in wild and domestic animals, primarily carnivores such as dogs, wolves, domestic cats, foxes, skunks, raccoons, ferrets, jackals, and mongooses. It is also found in bats but rarely in rabbits, rodents, or opossums. The relative importance of each animal species as a vector

in the spread of this disease varies according to geographical location. In highly industrialized countries such as the United States, Canada, and many European countries, canine rabies has been effectively curtailed through canine vaccination. Dogs, therefore, account for less than 5% of the cases of animal rabies. The most common rabid domestic animal is now the cat. In the United States until 1989, the most commonly rabid animal was the skunk. A marked transition in animal rabies has occurred with the recent emergence of raccoons as the most common rabies reservoir and coyotes as the newest mammalian reservoir of a canine variant in Texas.[187] The ultimate reservoirs of variants indigenous to the United States are racoons, skunks, foxes, multiple species of insectivorous bats, and, most recently, coyotes, but in some developing countries (eg, parts of Asia, Africa, and Latin America) where canine rabies has not been adequately controlled, dogs still account for more than 90% of reported cases in animals, posing a substantially higher risk of transmission. Animals are usually infective (shedding virus) at least 3 to 5 days (and in some animals, such as Ethiopian dogs, up to 14 days) before clinical signs of rabies appear. As a general rule, suspected rabid animals should be observed for at least 10 days after the bite. Since the preshedding incubation period in dogs may extend for several months, rabies-free areas such as Hawaii require a period of animal quarantine before entry. Rabid animals continue to shed virus during the course of the disease.

Geographic Distribution

The worldwide incidence of human rabies is not known, but it has been reported from every continent except Australia (the two Australian cases reported in 1987 and 1990 were contracted in other countries)[188] and the Antarctic. The rabies-related viruses appear to be limited to Africa and Europe, but these are areas to which American military personnel are frequently deployed.

Incidence

The World Health Organization estimates that 35,000 rabies deaths occur each year.[189] More than 99% of the cases have occurred in those areas where canine rabies is still endemic, especially China and India. In contrast, only 1 of 9 rabies cases believed acquired in the United States between 1980 and 1993 was canine-associated, while 10 of 11 cases acquired outside the United States were canine-associated. Six of the nine rabies cases acquired in the United States were attributable to bats.[190–194]

The incidence of human rabies in the United States has recently increased, with rabies variants associated with bats predominating among indigenously acquired cases. Between January 1990 and June 1997, there were 24 human cases of rabies diagnosed in the United States, compared to only 10 cases diagnosed during the preceding decade. Among these 24 cases, 19 were acquired in the United States, with 17 (89%) attributable to bats. Five of those cases were caused by canine variants characteristic of those occurring in Mexico, Haiti, India, and Nepal.

Pathogenesis and Clinical Findings

The pathogenesis of rabies virus infection is not entirely understood. While it is highly neurotropic and the infection is largely restricted to nervous tissue, the first site of attachment is believed to be the plasma membrane of muscle cells.[195]

The incubation period following bite exposure is usually between 20 and 90 days; however, a wide range of incubation periods has been reported, varying from as short as 4 days to as long as 19 years.[196] Most cases have incubation periods of less than 1 year. Bites on the head generally have shorter incubation periods (25 to 48 days) than those involving the extremities (46 to 78 days).[197]

The prodrome associated with rabies infection is very nonspecific; symptoms include malaise, fatigue, headache, anorexia, fever, cough, chills, sore throat, abdominal pain, nausea, vomiting, and diarrhea. Prodomal sensations at the bite site are sometimes reported. This may last for 2 to 10 days, followed by the acute onset of neurologic symptoms that herald central nervous system involvement. This stage of the disease usually presents in either one of two ways—a "furious" form or "paralytic" form—and lasts for 2 to 7 days.

Furious rabies is the more common presentation and is characterized by hyperactivity, disorientation, hallucinations, bizarre behavior, and autonomic instability with hyperthermia, hypertension, and hypersalivation. Over half of these patients will experience pharyngeal, laryngeal, and diaphragmatic spasms when attempting to drink liquids. This causes choking, gagging, and aspiration and results in hydrophobia. Similarly, aerophobia may be present since blowing air in the face may also precipitate these spasms. Numerous potential complications may develop and require specific treatment. Some of the more serious ones include

hypoxia, cardiac arrhythmias, hypotension, and cerebral edema.

The paralytic form of rabies occurs in approximately 20% of patients and is characterized by either a generalized and symmetric paralysis or an ascending paralysis. In contrast to furious rabies, hyperactivity is absent, but mental status changes are common. They usually progress from agitation to confusion and disorientation and finally to coma.

Diagnostic Approaches

For the patient presenting with clinical signs and symptoms of hydrophobia and hyperactivity, especially with known exposure to a rabid animal, rabies should be the leading diagnosis. Aside from this clinical scenario, it is very difficult to distinguish rabies from other forms of viral encephalitis. It is important, however, to consider other causes, particularly treatable ones such as herpes simplex encephalitis. Post-vaccinal encephalomyelitis following immunization with Semple type (nerve tissue-derived) rabies vaccine should also be considered. Other central nervous system processes may also mimic rabies, including seizure disorders, cerebrovascular accidents, intracranial mass lesions, and atropine poisoning. Hysterical reactions to animal bites (pseudohydrophobia) are sometimes seen in patients who are extremely fearful of developing rabies. Paralytic rabies may be confused with poliomyelitis, Guillain-Barre syndrome, and transverse myelitis.

Human rabies is very difficult to diagnose early in the course of the illness because of the nonspecific prodrome. A good history, especially of exposure, and a high index of suspicion are very important. Alterations in the hematologic profile and blood chemistries are mild, nonspecific, and not helpful diagnostically. Cerebrospinal fluid (CSF) examination is likely to demonstrate a mononuclear pleocytosis (60% to 85%), but CSF glucose and protein are usually normal. Electroencephalography may show generalized slowing or paroxysmal bursts of spike potentials. Computerized tomography and magnetic resonance imaging may show diffuse enhancement, but such findings are not specific for rabies encephalitis. Furthermore, these imaging techniques may be normal even in patients with advanced rabies encephalitis.

Definitive laboratory diagnosis is by detection of the rabies antigen or antibody or isolation of the virus. The most reliable test early in the disease requires a nuchal skin biopsy with immunofluorescent staining for rabies antibody. Procedurally, a 6- to 8-mm full-thickness wedge or punch biopsy specimen is taken from the nape of the neck above the hairline. It should be placed in a vial with a piece of moist filter paper and stored at -70°C until shipped; it should not be placed in formalin. This test is more sensitive than corneal impression smears for viral antigen and has about 50% positivity during the first week of illness.[198] Corneal impression tests, however, should still be done on every suspected case of human rabies, because the test may be positive before the neutralizing antibodies appear in serum.[199] This test involves the immunofluorescent antibody staining of epithelial cells, which are obtained by pressing a clean glass microscope slide firmly against the cornea and rocking gently to increase the number of cells that stick to the slide.[200] The sensitivity of this test varies anywhere from 31%[201] to 41%[202] and is greatly affected by the number of corneal epithelial cells on a slide.

The role and timing of brain biopsy in the diagnosis of rabies encephalitis is not clearly defined. Brain biopsy in the past has offered the best prospect for identifying virus by fluorescent antibody tests (sensitivity 99%, specificity 100%) or the presence of the characteristic cytoplasmic inclusions called Negri bodies (sensitivity 90%, specificity greater than 99%),[203] but the regions of the brain (eg, hippocampus, horn of Ammon, cerebellum, brain stem) where the virus is most commonly found are not easily accessible for biopsy. Specimens obtained from other areas of the brain may be normal, so a negative biopsy does not rule out rabies.

The standard test for rabies-neutralizing antibody is the rapid fluorescent focus inhibition test, which is positive in 50% of patients by the 8th day of clinical illness and in almost all patients by the end of the second week (sensitivity 95% to 100%, specificity 100%).[204]

Viral isolation has been a disappointing tool for identifying rabies infection. Rabies virus has been isolated ante mortem from a variety of human body fluids and tissues, including saliva, urine, tracheal secretions, CSF, and brain tissue, but the yield is very low. Postmortem, rabies virus has been isolated from skin at the site of the bite and from tissue from the pericardium, adrenal gland, pancreas, liver, and bladder. Nested polymerase chain reaction is a two-step amplification method that has been successful in detecting the rabies virus. The sensitivity of this technique is reported to be 8 picograms of rabies virus–specific RNA, with no false-positive results.[205] In addition, decomposition of specimens has minimal effect on nested polymerase chain reaction detection of rabies viral RNA.

Unfortunately, these specific diagnostic tests will rarely be available in the field setting, so clinical awareness is essential.

Recommendations for Therapy and Control

Therapy

Wounds should be thoroughly and vigorously washed immediately with a 20% soap solution, followed by debridement when appropriate. This simple maneuver alone reduces the risk of rabies by up to 90%.[206,207] Sutures should be avoided whenever possible and occlusive dressings or topical ointments should not be used. If sutures are used, they should be placed after local infiltration with human rabies immunoglobulin (HRIG) and should fit snugly but not too tightly. Antimicrobial prophylaxis should be considered for a moderate-to-severe injury that has occurred within 8 hours, especially if there is possible bone or joint penetration and for wounds involving the hands. The antibiotic of choice is the combination of amoxicillin/clavulanic acid 250-500 mg given three times a day for 3 to 5 days. This will kill most bacteria associated with bite wounds, which include *Pasteurella multocida*, *Staphylococcus aureus*, and anaerobic organisms. For those allergic to penicillin, a combination of clindamycin and ciprofloxacin should provide comparable antimicrobial coverage. In addition, the patient's tetanus immunization status should be addressed.

Antiviral agents, such as adenosine arabinoside and isoprinosine, have been ineffective for treatment of clinical rabies. Treatment with human leukocyte interferon (peripherally and intrathecally) has been unsuccessful.[208] HRIG also has been shown to be ineffective, and there is concern that it may exacerbate the disease process.

Management is largely supportive, with intensive respiratory and cardiovascular monitoring and support. In the field, the air-evacuation process must be started as soon as this diagnosis is suspected. Steroids, which are often administered for cerebral edema (one of the possible complications of rabies), should be avoided because they have been shown to increase rabies mortality in experimentally infected animals and to decrease the immune response to vaccines.

Control

Infection Control. Infection control procedures for patients with known or suspected rabies infection should include standard, contact, and droplet precautions.[209] Standard precautions apply to blood, all body fluids, secretions, excretions (except for sweat), nonintact skin, and mucous membranes. Handwashing and the use of gloves, nonsterile gowns, masks, and eye protection or face shields are extremely important when patient-care activities are likely to generate splashes or sprays. And in accordance with contact precautions, the patient should be placed in a private room and movement of the patient from the room should be limited to essential purposes only. Droplet precautions require the use of a mask whenever working within 3 feet of the patient, even if splashes or sprays are not likely. Special air handling and ventilation are not necessary.

For contacts of patients with proven rabies, exposures for which prophylaxis may be recommended include: (*a*) bites, with penetration of skin by teeth, (*b*) exposure to the patient's saliva or other potentially infectious material in direct contact with mucous membranes or broken skin, and (*c*) scalpel nicks or needle sticks if the instrument was in contact with the patient's CSF, nervous tissue, ocular tissue, or internal organs.[210]

Prevention. Rabies prevention includes a variety of components: vaccination of animals, minimization of exposure to potentially infected animals, proper management of animals that bite humans, preexposure prophylaxis for selected high-risk persons, and postexposure prophylaxis that includes wound management, passive immunization, and active immunization when indicated.

Animal Measures. In the United States, local governments are responsible for initiating and maintaining effective programs to ensure vaccination of all dogs and cats and to remove strays and unwanted animals. This has resulted in a decline of laboratory-confirmed rabies cases in dogs from 6,949 in 1947 to 130 in 1993. Preexposure vaccination is very important in the control of rabies in these animals. There are at least 28 rabies vaccines (inactivated) for animals marketed in the United States and recommended by the National Association of State Public Health Veterinarians.[211]

Livestock, as a rule, are not vaccinated, but immunization should be considered for animals that might have frequent contact with humans in areas where rabies is epizootic in wild animals. Rabies control in wildlife through oral vaccination (via aerial drops of bait containing the vaccine) has been successful in controlling rabies in red foxes in Europe and Canada.[212,213] Similar programs in the United States have targeted raccoons, coyotes and foxes. Population reduction of wildlife rabies res-

ervoirs is not a recommended or cost-effective method for rabies control.[214] This is especially true with bats, so human and domestic animal contact with bats should be minimized. Bats should be physically excluded from houses and surrounding structures by sealing potential entrances.[211] In addition, bats should never be handled unless appropriate safety precautions are taken, especially by untrained and unvaccinated persons. Bats should never be kept as pets.

Other measures to limit exposure include the removal of all stray dogs and cats from the community. This is especially important when military personnel are deployed to areas where rabies is epizootic. Personnel deployed to countries where rabies is enzootic should avoid unnecessary contact with wild or domesticated animals. It may be useful to establish an official "no-mascot" policy, strictly forbidding such contact.

A healthy dog or cat that bites a person should be confined and observed for at least 10 days. This recommendation is based on studies that observed that the virus was not isolated from the saliva of a rabid cat earlier than 1 day before the onset of clinical rabies and was not isolated from a rabid dog earlier than 3 days before onset.[215,216] There are a few reports of long periods of symptom-free virus excretion in dogs infected with rabies viruses from Ethiopia and India; this prompted some rabies-free areas to require a 6-month quarantine before entrance is allowed.[217] Following a long campaign for change by the US Army, Hawaii, a rabies-free state, recently reduced the quarantine time for dogs and cats from 4 months to 30 days if certain conditions are met.[218] Dogs and cats under quarantine should be evaluated by a veterinarian at the first sign of illness. If signs of rabies are present, the animal should be euthanized and the head carefully removed to prevent damage to the brain that would preclude proper pathologic examination. The head should then be packed in ice but not frozen and sent for histologic examination and culture for the rabies virus. Any stray or unwanted dog or cat that bites someone should be euthanized immediately and examined for rabies.

Data regarding viral shedding are limited in bats, raccoons, and skunks, and the risk of their harboring rabies is high; therefore, unlike pet dogs and cats, quarantine for these animals is not recommended. Prophylaxis should be started promptly after a documented exposure, whether the animal is caught or not. If the biting animal or its carcass is available, its tissue should be tested for the presence of rabies. If the results are negative, prophylaxis may be discontinued.

Preexposure Immunization Preexposure immunization with rabies vaccine usually results in protective antibody levels. Preexposure immunization is particularly important for individuals who are at high risk and for persons whose postexposure therapy might be delayed. It promotes a rapid anamnestic antibody response when booster doses of the vaccine are given to bite victims and eliminates the need for passive immunization with HRIG. It also reduces the number of vaccine doses needed if postexposure prophylaxis is necessary.

Preexposure immunization is recommended for anyone involved in wildlife management, veterinarians, animal handlers, laboratory workers handling rabies-infected specimens, military personnel whose assignments expose them to an unusual risk of rabies, Special Operations personnel because they may be delayed in getting postexposure prophylaxis, and others with frequent exposure to dogs, cats, foxes, raccoons, skunks, or bats.[219]

Of the number of rabies vaccines available, the most widely used preparation in the United States is the human diploid cell rabies vaccine (HDCV), which contains inactivated whole virus and is manufactured in France as a preexposure immunization. It is administered intramuscularly (only in the deltoid region) as a 1.0 mL dose on days 0, 7, and 28. Immunity is assumed after proper vaccination in a healthy individual. Additional primary doses are generally not necessary unless the vaccinee received an incomplete series, is immunocompromised, or has documented inadequate antibody titers. The World Health Organization considers a level of 0.5 IU or greater to be an adequate titer,[220] which is roughly equivalent to complete neutralization at the 1:25 level by the rapid fluorescent focus inhibition test that is recommended by the Centers for Disease Control and Prevention 2 to 4 weeks after primary vaccination. For those individuals at consistently high risk of exposure (eg, rabies researchers), titers should be checked every 6 months; for all others, titers should be checked every 2 years if a rabies risk persists.

The rabies vaccine adsorbed, produced in rhesus monkey diploid cell cultures by Bioport Corporation of Lansing, Mich, is also licensed and available in the United States. It is administered intramuscularly as a 1.0 mL dose and has been shown to be as effective as the HDCV for both preexposure and postexposure prophylaxis.[221] The purified chick embryo cell culture vaccine (RabAvert, Chiron Behring GmbH and Company) has been licensed by the Food and Drug Administration for both

preexposure and postexposure prophylactic use in humans. It provides another option for vaccine candidates who develop a sensitivity to one of the other vaccines. Preexposure vaccination for persons not previously vaccinated consists of three 1.0 mL doses injected intramuscularly in the deltoid region for

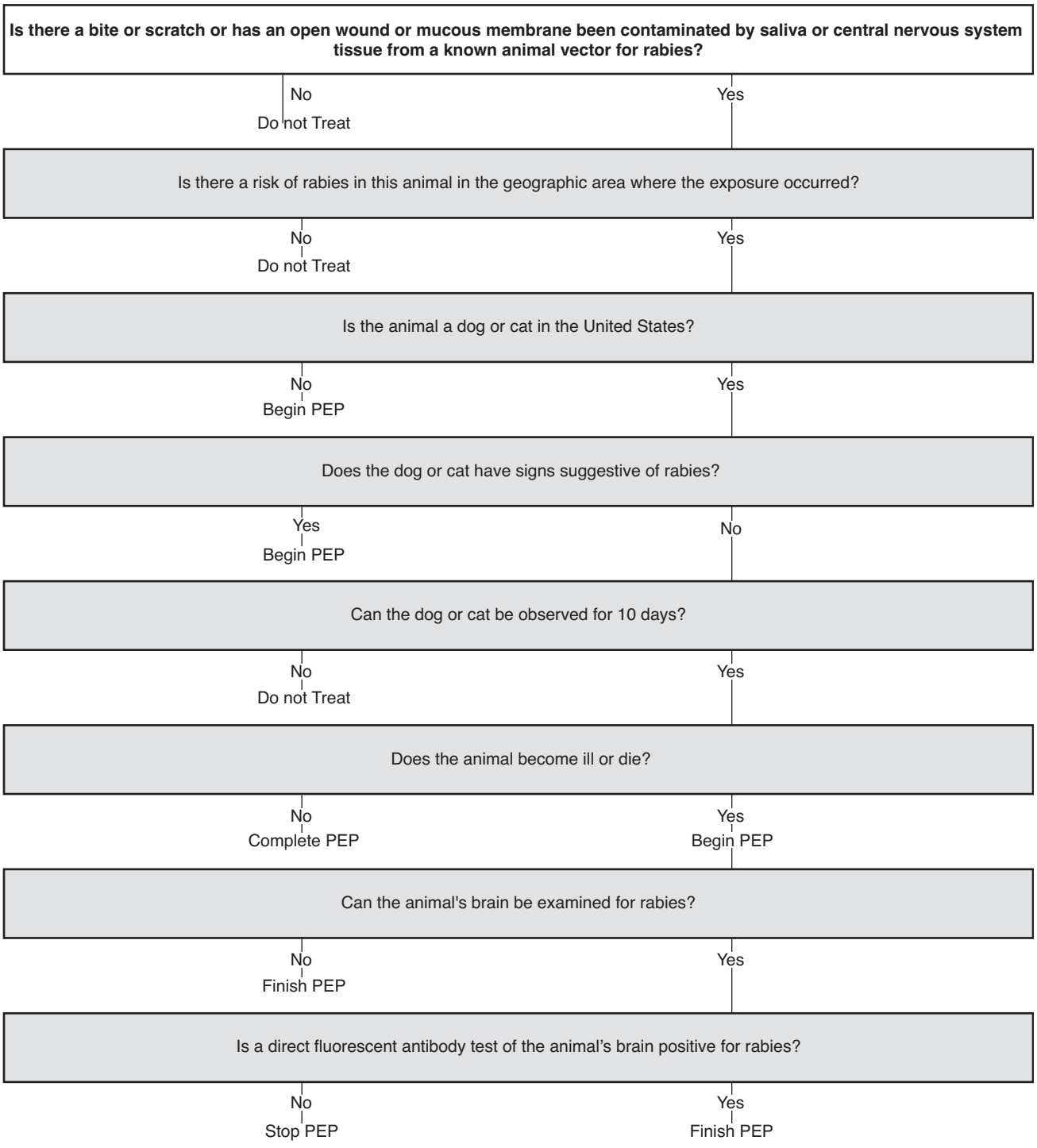

Fig. 36-7. An algorithm for human rabies postexposure prophylaxis

adults and in the anterolateral zone of the thigh for young children on days 0, 7, and either 21 or 28.[222]

Chloroquine phosphate, often given for malarial prophylaxis, may interfere with the antibody response to rabies vaccine.[186] Results of a randomized controlled study[223] indicated that chloroquine taken in the dose recommended for malaria prophylaxis can reduce the antibody response to primary immunization with HDCV; however, study participants were vaccinated by the reduced dose intradermal route, which is no longer recommended. It is believed that injecting rabies vaccine intramuscularly provides a reasonable margin of safety in this situation.

Postexposure Prophylaxis Very often the most difficult question facing the medical officer managing a possible rabies case is whether or not to initiate postexposure prophylaxis. A simplified approach to this management problem is illustrated in Figure 36-7. This process requires "yes" or "no" responses to several key questions designed to develop a rabies risk profile that addresses exposure and probability of the animal's being infected. For exposures to highly suspect animals, treatment should be started on the patient immediately and discontinued if fluorescent antibody testing of the animal brain is negative. In cases where the risk is low, treatment may be delayed for up to 48 hours pending the results of fluorescent antibody testing. Other factors often considered in deciding when to administer postexposure prophylaxis include suspicious animal behavior and whether the attack was provoked. Although such observations might suggest a higher likelihood of rabies, they are very subjective and are difficult to assess in wild animals.[224]

Expert guidance in making these decisions is provided at many military installations by a Rabies Advisory Board, which is composed of representatives from Preventive Medicine, Emergency Medicine, Pediatrics, Infectious Diseases (if available), and Veterinary Services. The chairman is usually the Preventive Medicine Officer, who is empowered to convene the board whenever there is suspicion of rabies exposure. Board members will have access to up-to-date information from local and state health departments on the occurrence of animal rabies in the area, which is critical in making decisions regarding postexposure prophylaxis. Local veterinary, animal control, and public health personnel may provide needed assistance in locating and quarantining animals suspected of having rabies.

The key elements of effective patient management following exposure to the rabies virus include local wound treatment, passive antibody administration, and vaccination (Table 36-6).

TABLE 36-6

RABIES POSTEXPOSURE PROPHYLAXIS RECOMMENDATIONS FOR ADULTS

Previously Vaccinated?	Treatment	Regimen
NO	Local wound care	Clean wound immediately with soap and water
	HRIG	20 IU/kg body weight: as much as possible infiltrated around wound site(s) and the remainder IM in buttocks
	Vaccine*	HDCV, RVA, or PCEC 1.0 mL IM (deltoid area) on days 0, 3, 7, 14, and 28
YES†	Local wound care	Clean wound immediately with soap and water
	Vaccine	HDCV, RVA, or PCEC 1.0 mL IM (deltoid), on days 0 and 3

*Do not inject using the same syringe as HRIG or into the same anatomic site

†Either (*a*) preexposure or postexposure vaccination with HDCV or RVA or (*b*) documentation of past antibody response to any other rabies vaccine

HRIG: human rabies immune globulin, HDCV: human diploid cell rabies vaccine, RVA: rabies vaccine adsorbed, PCEC: purified chick embryo cell culture

Passive immunization is achieved with the use of HRIG. This preparation has been well tolerated, with no reports of anaphylaxis or serum sickness and no reports of transmission of hepatitis or other viruses. A single dose of 20 IU/kg body weight is administered with as much of the total dose as is anatomically feasible infiltrated at the site of the wound. The remaining volume should be given intramuscularly in the upper outer quadrant of the buttocks or the anterolateral aspect of the thigh, but this should be an anatomical site distant from the site of vaccine administration. Local infiltration may, in some instances, be limited in wounds involving small anatomic areas such as fingertips. Any person with a previous history of preexposure or postexposure vaccination with HDCV or rabies vaccine adsorbed should not be given HRIG. The administration of HRIG is unnecessary and contraindicated because of the potential interference

with an anamnestic response to booster vaccination.

During some overseas deployments, HRIG may be unavailable. In the past, the only available alternative had been heterologous antiserum of equine origin (ARS, anti-rabies serum), but this preparation was associated with serum sickness in 16% of recipients in one study.[225] Although not licensed in the United States, a purified antirabies sera of equine origin (ERIG, equine rabies immune globulin) is available. In developing countries, ERIG may be the only viable alternative. The incidence of adverse reactions with ERIG has been low (0.8% to 6.0%), and the reactions have largely been minor.[226]

If in an area where only ERIG (or even ARS) is available, the decision to prophylax should take into account individual sensitivity to horse serum and the severity and likelihood of rabies exposure. Predeployment planning should include consideration for preexposure prophylaxis when the area of operation is located in countries where HRIG is not likely to be available.

Active immunization in persons not previously vaccinated consists of five 1.0 mL intramuscular doses of HDCV, RVA, or RabAvert vaccine into the deltoid region in adults or the anterolateral zone of the thigh for children; the first dose is given as soon as possible after exposure (day 0) and repeated on days 3, 7, 14, and 28. If the patient has been previously immunized, only two 1.0 mL injections on days 0 and 3 are needed. Vaccine should not be injected by the same syringe used to inject HRIG or at the same.

Postexposure treatment failures are uncommon, but they do occur and are more likely when one or more of these measures is omitted. One of the most common errors is the omission of passive immunization.[227] In other cases, local wound cleansing was omitted or post-exposure treatment was delayed.[228]

[Clifton A. Hawkes]

TULAREMIA

Introduction and Military Relevance

Tularemia (rabbit fever, deer-fly fever, Ohara disease) is a zoonotic bacterial disease caused by *Francisella tularensis*. Clinical recognition of tularemia and the epidemiology, diagnosis, treatment, and prevention of this disease are important military medicine capabilities. Transmission of tularemia can occur by direct inoculation of contaminated animal tissue, by ingestion of contaminated meat or water, and by bites from infected ticks, deer flies, and mosquitoes. The variety of clinical presentations of tularemia and the confusion of it with other infectious diseases underscore the importance of rapid diagnosis and a detailed history of travel and animal and arthropod exposure. In addition, the virulence of the bacterium, its transmissibility, and its capacity to induce significant morbidity make *F tularensis* a potential biological warfare agent.

Description of the Pathogen

The causative agent of tularemia is *Francisella tularensis. F tularensis* is a small, gram-negative, oxidase-negative, aerobic, nonmotile coccobacillus that can cause disease with as few as 10 organisms.[229] There are two distinct subspecies: *F tularensis*, biovar *tularensis* (type A), which produces virulent infections, and *F tularensis,* biovar *palaearctica* (type B), which produces infections that are often milder and indolent. *Francisella* organisms can survive for extended periods of time in water, mud, and animal carcasses.

Epidemiology

Transmission

Reservoirs for this organism in nature include many species of birds and wild animals (eg, rabbits, muskrats, squirrels, skunks, coyotes, beavers, water rats) and domesticated cats.[230] Direct and indirect transmission to humans typically occurs through the bite of infected animals[231] or the handling of blood or tissue or both while skinning and dressing rabbits or other infected animals.[232,233] Tularemia can be acquired from the consumption or handling of insufficiently cooked meat contaminated with the organism, through drinking or skin contact with contaminated water, and through contact with contaminated animal skins (Figure 36-8). *F tularensis* is resistant to freezing; rabbit meat remains infectious after being frozen for more than 3 years. Inhalation of bacteria from the dust of contaminated soil and occupational exposure from farming have been reported.[234] Along with the respiratory route, other mucous membranes (eg, ocular, oropharyngeal) are potential points of entry for the organism. There is no person-to-person transmission. The potential for laboratory acquisition from aerosolized organisms is high, and cultures should not be obtained because of the danger to laboratory personnel.

Vectors that can transmit the disease to humans include mosquitoes, deer flies (*Chrysops discalis*), and ticks (eg, *Dermacentor andersoni, D variabilis, Amblyoma americanum*). Ticks may be infected

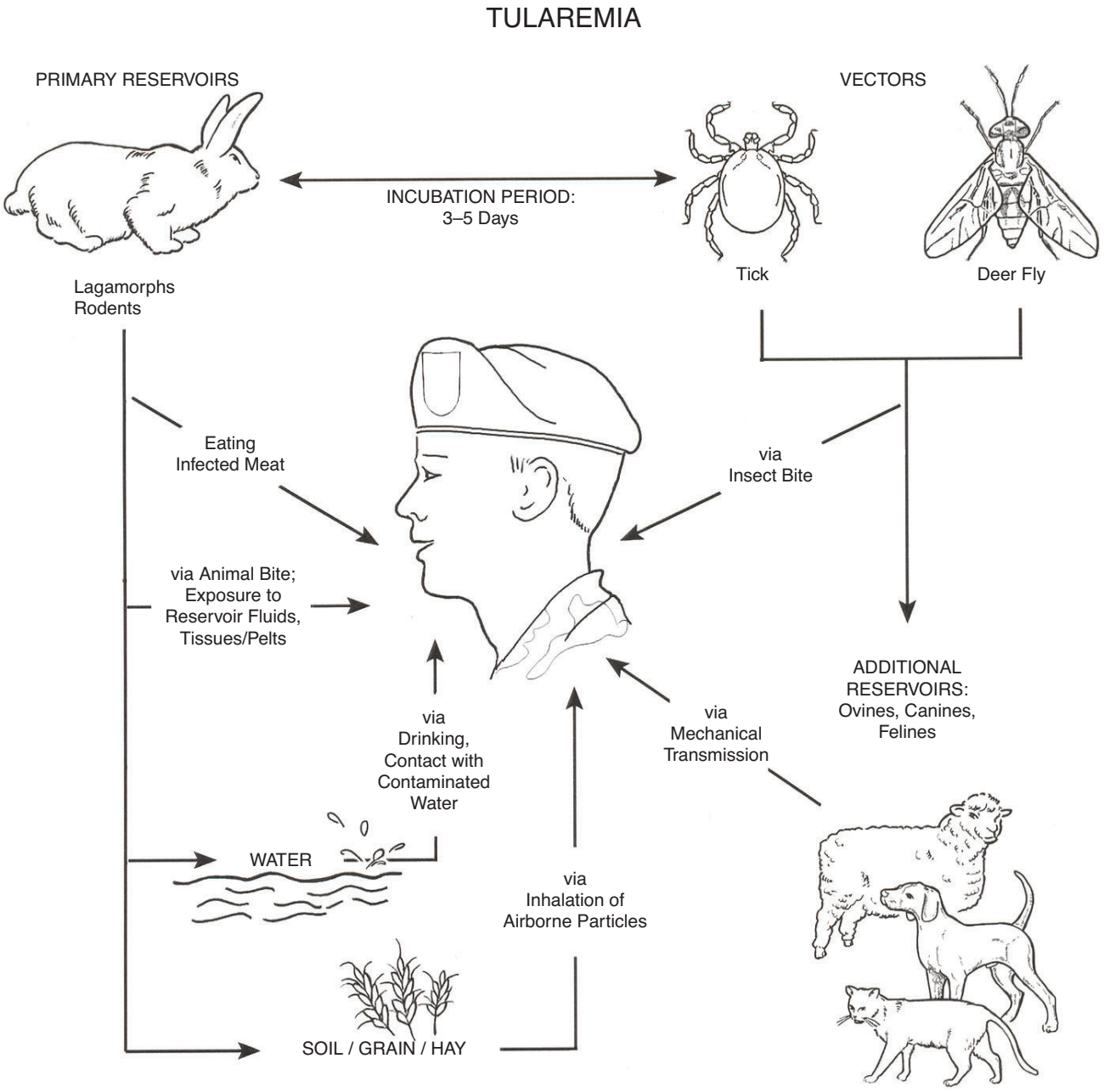

Fig. 36-8. The transmission cycle of *Francisella tularensis*. Art by Annabelle Wright, Walter Reed Army Institute of Research; research by Amelia Poisson.

throughout their lifetime and can transovarially pass the organisms to their progeny.

Geographic Distribution

Type A strains are limited to North America, particularly to the states of Arkansas, Oklahoma, South Dakota, and Missouri.[235] Type B strains are found in the northern hemisphere, including North America, Europe, Russia, China, and Japan. *F tularensis* has not been found in Africa or South America.

Incidence

Approximately 200 cases of tularemia are reported in the United States per year.[236] The highest incidence of tularemia occurs in the summer

months, when tick-borne transmission occurs. A second peak occurs in the winter, presumably with the increase in hunting wild animals such as rabbits. Transmission of the organism is not stable and can vary from year to year.

Pathogenesis and Clinical Findings

F tularensis is highly infectious; as few as 10 to 50 organisms can establish an infection through intracutaneous inoculation or via inhalation of aerosolized bacteria. Factors that contribute to the pathogenesis of the disease include the virulence of the organism, route of entry, age and immune status of the patient, and systemic involvement. Type A organisms are typically more virulent than Type B organisms, and less virulent strains may cause infections that resolve without treatment. The molecular basis for virulence of the organisms is unknown. *F tularensis* is an intracellular organism primarily of cells of the reticuloendothelial system, including macrophages, edothelial cells, and hepatocytes. The organism multiplies within macrophages, resisting intracellular destruction by preventing lysosome-phagosome fusion. A cellular-mediated immune response by the host attempts to control the infection by activation of T lymphocytes with consequent production of interferon-gamma and tumor necrosis factor. These cytokines activate macrophages through the production of reactive oxygen and nitrogen products that kill the intracellular organism.

The route of inoculation of the bacteria often predicts the clinical presentation of the disease, and the severity of illness depends on the virulence of the organism.[237,238] The clinical presentation of this disease can be confused with other infections, such as plague, staphylococcal and streptococcal infections, cat-scratch fever, and sporotrichosis, so an accurate clinical description that leads to a rapid diagnosis and the expeditious institution of therapy is required. There are six clinical forms of tularemia: glandular, ulceroglandular, typhoidal, pulmonary, oculoglandular, and oropharyngeal.

The most common forms of infection are classified as glandular or ulceroglandular tularemia. A localized, nonhealing, punched-out circular ulcer with raised borders forms at the site of inoculation in the skin and leads to lymphadenopathy and lymphadenitis in 90% of ulceroglandular tulaeria. In many cases, an ulcer or erythematous papule may not be clinically evident in glandular tularemia, and the only manifestation of infection is a relatively nonspecific regional lymphadenitis and systemic illness. The incubation period for the disease is approximately 3 days (range of 2 to 10 days). Fever is the primary sign of infection, and flu-like symptoms appear before or concurrent with swelling and pain in regional lymph nodes. Fever may last for up to 3 weeks in approximately 20% of cases treated with appropriate antibiotics.

The other major classification of tularemia is a systemic illness called typhoidal tularemia. It is the most frequently fatal form of the disease and results from rapid dissemination of the organism into multiple organs by hematogenous spread. This form of the disease may occur in patients with underlying illness, poor nutritional state, alcohol abuse, or chronic renal failure. Splenomegaly may be observed in 15% to 20% of the patients, but hepatomegaly is less common. Typhoidal tularemia in a healthy service member suggests an aerosol exposure, as may occur in a laboratory or during a bioterrorist event.

A major complication of typhoidal tularemia is the appearance of tularemic pneumonia, which may occur regardless of the initial route of entry but is three times more common in typhoidal disease than other forms of tularemia. Pulmonary symptoms are characterized by a nonproductive cough, pleuritic chest pain, dyspnea on exertion, malaise, fever, and myalgias. Radiographic findings may be normal or may show pulmonary infiltrates that mimic fungal and other bacterial pneumonias, tuberculosis, or malignancy.[236] Hilar adenopathy can be seen in 36% of tularemia pneumonia.[234] Pleural effusions complicating tularemic pneumonia are rare, but pleural-based granulomatous inflammation secondary to *F tularensis* infection has been reported and can be confused with the more common *Mycobacterium tuberculosis* infection.[238] The mortality rate from tularemic pneumonia is high.[236]

Other less common forms of tularemia include oropharyngeal tularemia; this results from the ingestion of infectious organisms and may induce pharyngitis, diarrhea, abdominal pain, and emesis. Oculoglandular tularemia is extremely rare and is acquired by the direct inoculation of bacteria into the conjunctival sac. Dermatological manifestations of the disease may include erythema nodosum and erythema multiforme.[237]

Rarely, *Francisella* organisms can cause meningitis, with cerebral spinal fluid findings of an elevated protein level and a predominance of mononuclear cells.[239] A precise history of rabbit and cat exposure should be elicited and therapy immediately initiated if other more common causes of meningitis are excluded.

Diagnostic Approaches

The current methods for the diagnosis of infections caused by *F tularensis* are unsatisfactory. The diagnosis of tularemia by serology, either by serum agglutination or enzyme-linked immunosorbent assay, remains the method of choice.[240] Serum agglutination titers documenting a 4-fold increase in titer between acute and convalescent sera or an absolute titer of 1:160 or greater aid in the diagnosis of exposure to *F tularensis*, but cross-reactions with *Brucella* species, *Proteus* species, and heterophile antibodies are known to occur. Antibodies to the *Francisella* organism typically appear after the second week of infection, and it is not unusual to have negative serologic agglutination tests despite acute infection. Therefore in the absence of definitive diagnosis, a presumptive diagnosis based on clinical presentation and epidemiologic exposure mandates aggressive treatment. The organism may be identified directly by fluorescent antibody assays on specimens obtained from an ulcer exudate or a lymph node aspirate. If a biopsy is done, appropriate antibiotics may be given to avoid the potentially resultant bacteremia.

In some rare cases, *F tularensis* can be directly cultured from aspirates of ulcers, from pleural fluid, or from blood.[241] However, culture of this highly infectious bacterium should only be attempted in a biosafety level 3 laboratory. Special media containing cysteine or cystine for growth are required to isolate the organism. Modified Muellar-Hinton broth, chocolate blood agar, and modified charcoal yeast extract agar have been used to isolate the bacterium.[240] The bacteria grow as smooth, blue-gray colonies, and identification as *Francisella* can be made by slide agglutination using commercially available antisera. The polymerase chain reaction has been used to rapidly and accurately identify *Francisella* from clinical specimens but is currently a research tool not generally available in the clinical microbiology laboratory.[242,243]

Recommendations for Therapy and Control

Therapy

Factors to be considered in the treatment of tularemia include the natural resistance of *F tularensis* to all penicillins and other β-lactams, the intracellular environment of the organism, the organism's tendency to cause suppurative adenitis, and the need for both parenteral antibiotics in severe infection and oral antibiotics for localized ulceroglandular disease. Another consideration when choosing an antibiotic regimen is whether the infection has been diagnosed with certainty or the diagnosis is based solely on the history or clinical examination. Since treatment with antibiotics that cannot penetrate cells fails to eliminate the bacteria and frequently results in clinical relapse, treatment should be directed toward the elimination of both extracellular and intracellular forms of the bacteria. Factors that increase the risk of clinical relapse include underlying immunosuppression, insufficient duration of therapy, and the use of bacteriostatic rather than bacteriocidal antibiotics.[236]

The antibiotic of choice in the treatment of tularemia is streptomycin (1 g intramuscularly four times a day for 10 to 14 days).[244,245] However, alternative antibiotics with comparable efficacy to streptomycin but with less potential for the adverse side effects of ototoxicity and nephrotoxicity have been sought. Gentamicin, another bactericidal aminoglycoside, is an adequate alternative to streptomycin and has been successfully used to treat the disease in both the adult[246] and pediatric population,[247] albeit with a greater relapse rate (6%).[248] Caution should be exercised when using gentamicin alone because of its variability in cell penetration, the higher frequency of relapses, and the need to maintain high blood levels of the antibiotic. Treatment with gentamicin (5 mg/kg) to maintain blood levels at approximately 5 μg/mL should continue for 10 to 14 days. Tetracycline is efficacious, but again, higher relapse rates occur, which may be a function of its bacteriostatic activity. Oral quinolones, such as ciprofloxacin (500-750 mg twice a day for 10 to 30 days), have been shown to be particularly efficacious, with resolution of fever within 2 days of beginning therapy and fewer documented relapses than tetracycline or the aminoglycosides.[248,249] Because of its excellent bactericidal activity, stability in an acidic environment, and levels of antibiotic achieved in soft tissues, ciprofloxacin is an excellent alternative in culture-proven *Francisella* infections and may become the antibiotic of choice for suspected but not proven infection.

In vitro antimicrobial susceptibility testing does not correlate with clinical response and should not be used to direct the choice of antibiotics. Cephalosporins, such as cefotaxime, ceftazidime, and ceftriaxone, have in vitro activity against *Francisella*[250] but no in†vivo efficacy.[251] For cases of suspected tularemic meningitis, chloramphenicol should be added to the streptomycin treatment regimen.[252] A single case report showed that imipenem (500 mg intravenously every 8 hours for 14 days) can achieve clini-

cal cure with no relapse.[253] The best indicator of response to therapy is the resolution of fever, which usually falls within 3 days of instituting therapy.[247]

Control

Protective measures that minimize contact with and infection by *F tularensis* include avoiding potentially contaminated drinking water, refraining from bathing and swimming in untreated water, and wearing protective clothing and insect repellent to protect against transmission by mosquitoes, ticks, and flies. Wild rabbit and rodent meat must be carefully handled and thoroughly cooked. For laboratory workers who may come in contact with the bacteria, the importance of the use of gloves and masks is obvious. Although quarantine is not indicated, universal secretion precautions should be taken when working with patients who have open lesions.

There is no licensed vaccine against tularemia. However, a live attenuated vaccine for laboratory personnel exposed to *F tularensis* has been shown to be safe and partially efficacious.[254] This vaccine is held under an investigational new drug protocol by the US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Md. Postexposure prophylaxis with doxycycline, as may occur after a biological warfare incident, may be beneficial.[255]

[Christian F. Ockenhouse]

ANTHRAX

Introduction

Anthrax, a zoonotic disease caused by *Bacillus anthracis*, occurs in domesticated and wild animals—primarily herbivores such as goats, sheep, cattle, horses, and swine. Humans usually become infected by contact with infected animals or contaminated animal products. Natural infection occurs most commonly via the cutaneous route and only very rarely via the respiratory or gastrointestinal routes. There are typical manifestations associated with each of these three routes.

The US military's primary concern with anthrax is with its potential use as a biological weapon, for which anthrax is suited because of the infectiousness of its spores by the respiratory route and the high mortality of inhalational anthrax. This concern was heightened after the revelation that the largest epidemic of inhalational anthrax in the 20th century occurred when anthrax spores were accidentally released from a military research facility in Sverdlovsk, USSR, in 1979. Cases were also reported in animals located more than 50 km from the site.[256,257] Another concern is that as the US military's mission expands to include more worldwide humanitarian efforts, service members are increasingly likely to encounter unvaccinated livestock that may have anthrax.

Description of the Pathogen

Bacillus anthracis is a large, gram-positive, spore-forming, nonmotile bacillus (1-1.5 μm by 3-10 μm) that forms a prominent capsule in tissue. The encapsulated bacterium occurs singly or in chains of two or three bacilli. The organism does not form spores in living tissue; sporulation occurs only after the infected body has been opened and exposed to oxygen. The spores are very stable and may survive in soil for decades under favorable conditions.

Epidemiology

Transmission

Anthrax in humans is nearly always associated with direct exposure to infected animals or to contaminated animal products. Animals, either domestic or wild, become infected when they ingest spores from the soil. Humans rarely, if ever, contract anthrax directly from the soil unless they are working with contaminated bone meal fertilizer. Exposure usually occurs through handling spore-laden carcasses, hides, wool, hair, or bones, often in an industrial setting. Cutaneous lesions, by far the most common form of anthrax, occur on exposed parts of the body (eg, arms, neck, face). Inhalation of a sufficient quantity of spores results in inhalational anthrax. This uncommon but lethal disease occurs most often in those processing contaminated wool or animal hair in an enclosed space. This is the form of anthrax that has potential as a biologic weapon. Finally, oropharyngeal or gastrointestinal anthrax can arise from eating poorly cooked, contaminated meat. This form of the disease is also quite rare.

There is no proven vector for transmission, although it has been suggested that anthrax in animals has been spread by biting flies carrying spores from one grazing area to another.[258] No evidence exists for a similar mechanism in humans. Human-to-human transmission does not occur in inhala-

tional anthrax and has been reported in only two cases of cutaneious disease.[259–261]

Geographic Distribution

Anthrax occurs worldwide, existing as a spore. Human cases of anthrax continue to be reported from Africa, Asia, Europe, and the Americas.[262] The threat of anthrax is increased when there is a concurrent epizootic in the region, particularly in cattle. Environmental conditions such as drought, which may promote trauma to the oral cavity of grazing animals, are thought to increase the chances of an animal acquiring anthrax.[263]

Incidence

In the United States, the annual incidence of human anthrax has steadily declined from approximately 127 cases in the early years of the 20th century to about 1 per year in the 1990s. The vast majority of cases have been cutaneous. Under natural conditions, inhalational anthrax is exceedingly rare, with only 18 cases having been reported in the United States in the 20th century.[264] During times of economic hardship and disruption of veterinary and human public health practices, such as occurs during war, there have been large epidemics of anthrax. The largest reported epidemic of human anthrax occurred in Zimbabwe from 1978 through 1980, with an estimated 10,000 cases. Nearly all these were cutaneous.[258,265,266] Incidence increases during the spring and summer months.

Units located in rural areas, particularly those in which there has been recent socioeconomic disruption leading to poor animal husbandry, should be aware of the increased risk of human anthrax in the presence of animal anthrax. If the stay is prolonged, it may be worthwhile to assist the community in immunizing their herds against anthrax.

Pathogenesis and Clinical Findings

B anthracis possesses three known virulence factors, which are an antiphagocytic capsule and two protein exotoxins (called the lethal and the edema toxins). The anthrax toxins possess two components: (1) a shared cell-binding, or B, domain and (2) a distinct active, or A, domain that has the toxic activity. Both components are required for biologic activity.[267–269]

Infection begins when the spores are inoculated through the skin or mucosa. It is thought that spores are ingested at the local site by macrophages and germinate into the vegetative bacilli that produce a capsule and toxins. At these sites, the bacilli are released from the macrophage, proliferate, and produce the edema and lethal toxins that impair host leukocyte function and lead to the distinctive pathological findings of edema, hemorrhage, tissue necrosis, and a relative lack of leukocytes.

In inhalational anthrax, the spores are ingested by alveolar macrophages, which transport them to the regional tracheobronchial lymph nodes, where germination occurs.[270] Once in the tracheobronchial lymph nodes, the local production of toxins by extracellular bacilli gives rise to the characteristic pathological picture of massive hemorrhagic, edematous, and necrotizing lymphadenitis, and the mediastinitis that is almost pathognomonic of this disease.[271] The bacilli can then spread to the blood, leading to septicemia with seeding of other organs and frequently causing hemorrhagic meningitis. Death is the result of respiratory failure that is associated with pulmonary edema, overwhelming bacteremia, and, often, meningitis.

Cutaneous Anthrax

More than 95% of cases of anthrax are cutaneous (Figure 36-9). After inoculation, the incubation period is 1 to 5 days. The disease first appears as a small papule that progresses over one to two days to a vesicle containing serosanguinous fluid with

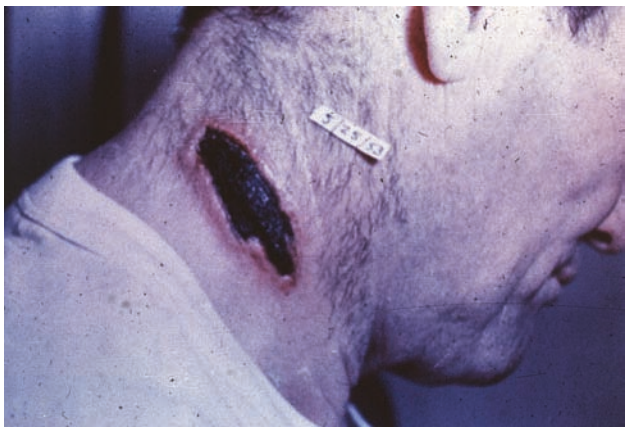

Fig. 36-9. A cutaneous lesion of anthrax with eschar on a patient's neck, occurring on approximately day 15 of disease. The patient had worked with air-dried goat skins from Africa.
Photograph: Courtesy of the Armed Forces Institute of Pathology, Washington, DC. AFIP Negative 75-4203-7.

many organisms and few leukocytes. The vesicle, which may be 1 to 2 cm in diameter, ruptures and leaves a necrotic ulcer. Satellite vesicles may also be present. The lesion is usually painless, and varying degrees of edema may surround it. The edema may occasionally be massive, encompassing the entire face or limb, and is called "malignant edema." Patients usually have fever, malaise, and headache, which may be severe in those with extensive edema. There may also be local lymphadenitis. The ulcer base develops a characteristic black eschar; after 2 to 3 weeks the eschar separates, usually leaving no scar. Septicemia is very rare, and with treatment mortality should be less than 1%. The case-fatality rate for untreated cutaneous anthrax is 5% to 20%.

Inhalational Anthrax

Inhalational anthrax begins after an incubation period of 1 to 6 days with nonspecific symptoms of malaise, fatigue, myalgia, and fever. There may be an associated nonproductive cough and mild chest discomfort. These symptoms usually persist for 2 or 3 days, and in some cases there may be a short period of improvement. This is followed by the sudden onset of increasing respiratory distress with dyspnea, stridor, cyanosis, increased chest pain, and diaphoresis. There may be associated edema of the chest and neck. Chest X-ray examination may show the characteristic widening of the mediastinum and pleural effusions (Figure 36-10). Bacterial pneumonia is an uncommon finding but can occur in some patients.[256] While cases of inhalational anthrax have been rare in the 20th century, several have occurred in patients with underlying pulmonary disease, suggesting increased susceptibility in these patients.[264] Meningitis is present in up to 50% of cases, and some patients may present with seizures. The onset of respiratory distress is followed by the rapid onset of shock and death within 24 to 36 hours. Mortality has been essentially 100% in reported cases, and there are no reliable human data on the effectiveness of current treatment regimens and supportive care.

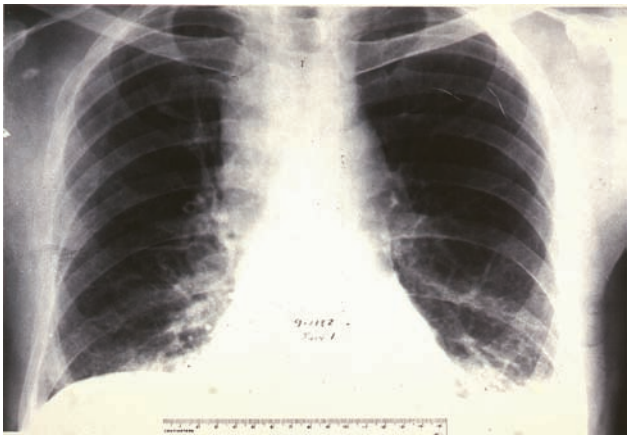

Fig. 36-10. This roentgenogram, taken on day 2 of illness, shows the lungs of a 51-year-old laborer with occupational exposure to airborne anthrax spores. Marked mediastinal widening is evident with small parenchymal infiltrate. Photograph: Courtesy of the Armed Forces Institute of Pathology, Washington, DC. AFIP Negative 71-12790-2.

Oropharyngeal and Gastrointestinal Anthrax

Oropharyngeal and gastrointestinal anthrax result from the ingestion of undercooked, infected meat. After an incubation period of 2 to 5 days, patients with oropharyngeal disease present with a severe sore throat or a local oral or tonsillar ulcer, usually associated with fever, toxicity, and swelling of the neck due to cervical or submandibular lymphadenitis and edema. Dysphagia and respiratory distress may also be present. Gastrointestinal anthrax begins with nonspecific symptoms of nausea, vomiting, and fever; these are followed in most cases by severe abdominal pain. The presenting sign may be an acute abdomen, which may be associated with hematemesis, massive ascites, and diarrhea. Mortality in each of these forms may be as high as 50% even with treatment.

Meningitis

Meningitis usually follows bacteremia as a complication of the disease. Meningitis may also occur, but very rarely, without a clinically apparent primary focus. It is very often hemorrhagic, which is important diagnostically, and is almost invariably fatal.

Diagnostic Approaches

The most critical aspect in making a diagnosis of anthrax is a high index of suspicion associated with a compatible history of exposure. Cutaneous anthrax should be considered following the development of a painless pruritic papule, vesicle, or ulcer that often is surrounded by edema and develops into a black eschar. With extensive or massive edema, such a lesion is almost pathognomonic. Gram's stain or culture of the lesion will usually

confirm the diagnosis. The differential diagnosis should include tularemia, staphylococcal or streptococcal disease, and orf (a zoonotic viral disease of sheep and goats).

The diagnosis of inhalational anthrax is extraordinarily difficult, but the disease should be suspected in those with a history of exposure to a *B anthracis*–containing aerosol. The early symptoms are entirely nonspecific, but two situations suggest the diagnosis: (1) the development of respiratory distress in association with radiographic evidence of a widened mediastinum due to hemorrhagic mediastinitis and (2) the presence of hemorrhagic pleural effusion or hemorrhagic meningitis. Sputum examination is not helpful in making the diagnosis, since pneumonia is not usually a feature of inhalational anthrax.

Gastrointestinal and oropharyngeal anthrax are exceedingly difficult to diagnose because of the rarity of the diseases and their nonspecific symptoms. Only with an appropriate epidemiologic history in the setting of an outbreak are these diagnoses usually considered. Microbiological cultures are not helpful in confirming the diagnosis. Gram's stain or Giemsa stain of the peripheral blood may allow visualization of the organism, and standard blood culture may yield the organism but not until late in the course of the disease.

Meningitis due to anthrax can be clinically indistinguishable from meningitis due to other causes. An important distinguishing feature is that the cerebral spinal fluid is grossly hemorrhagic in as many as 50% of the cases. The diagnosis can be confirmed by identifying the organism in cerebral spinal fluid by microscopy or culture.

Serology is generally only of use in making a retrospective diagnosis. Antibody to protective antigen or the capsule develops in 68% to 93% of reported cases of cutaneous anthrax.[272–275] Bacterial identification is confirmed by demonstration of the protective antigen toxin component, lysis by a specific bacteriophage, detection of capsule by fluorescent antibody, or virulence for mice and guinea pigs. Additional confirmatory tests to identify the organism by the presence of toxin and capsule genes using the polymerase chain reaction have also been developed as research tools. An enzyme-linked immunosorbent assay for circulating toxins will be positive but not until late in the course when bacteremia is present. A hand-held form of this assay has been shown to be of value in the field for rapid diagnosis in animals and is available on a research basis for use in human disease.

Recommendations for Therapy and Control

Therapy

Penicillin is the drug of choice for anthrax. Cutaneous anthrax without toxicity or systemic symptoms may be treated with oral penicillin. If spreading infection or systemic symptoms are present, then intravenous therapy with high-dose penicillin (2 million units administered every 6 hours) may be initiated until a clinical response is obtained. Effective therapy will reduce edema and systemic symptoms but will not change the evolution of the skin lesion itself. Treatment should be continued for 7 to 10 days. Tetracycline, erythromycin, and chloramphenicol have also been used successfully. These drugs may be used for treatment of the rare case caused by naturally occurring penicillin-resistant organisms or in penicillin-allergic patients. Other drugs, including the fluoroquinolones, first-generation cephalosporins, and doxycycline, are effective in vitro but have not been used in human cases.

Inhalational, oropharyngeal, and gastrointestinal anthrax should be aggressively treated with large doses of intravenous penicillin (2 million units administered every 2 hours), and appropriate vasopressors, oxygen, and other supportive therapy should also be used.

Illness after a suspected or known attack with *B anthracis* spores should be treated based on antibiotic sensitivities because of the possibility of inducing antibiotic resistance in weaponized spores. In the absence of information regarding antibiotic sensitivities, treatment should be instituted at the earliest signs of disease with intravenous ciprofloxacin (400 mg every 8 to 12 hours) or intravenous doxycycline (200 mg initially, followed by 100 mg every 12 hours).

Control

Service members on deployment should avoid contact with animals, hides, or products made from hide. To avoid potential cases of gastrointestinal anthrax, they should also take care to avoid the consumption of meat from infected animals. This issue applies primarily when there is local acquisition of food supplies. Additionally, all meat should be thoroughly cooked.

When service members encounter sick or dead animals, samples should be taken for microbiological diagnosis. If anthrax is confirmed, the dead animals should be incinerated and the remaining bones buried deeply. Caution must be used when handling

carcasses to avoid direct contact with skin, especially broken skin. If incineration is impossible, the animal should be buried to a depth of at least 6 feet and covered with lime.[259]

Prophylactic Treatment After Exposure

Experimental evidence from animals has demonstrated that postexposure antibiotic treatment, beginning 1 day after exposure to a lethal aerosol challenge with anthrax spores, can provide significant protection against death. All three drugs used in this study—ciprofloxacin, doxycycline, and penicillin—were effective.[276] In vitro sensitivity tests demonstrate very good activity against *B anthracis* with the quinolone antimicrobial agents.[277,278] Optimal protection is afforded by combining antibiotics with active immunization.[276] Oral antibiotics should be continued for at least 4 weeks, during which the unimmunized patient should be given 3 doses of vaccine 2 weeks apart. For those already immunized with at least 3 doses of the vaccine, the status of vaccination should be checked and any necessary boosters given. Regardless of immunization status, if the exposure has been confirmed and antibiotic supplies are sufficient, everyone exposed should be given 30 days of antibiotic treatment. Treatment for unimmunized personnel should be extended to 60 days if no vaccine is available, and the patient should be observed carefully for any signs and symptoms once antibiotic therapy has been discontinued.

Active Immunization

As of 1999, the only licensed human vaccine against anthrax is produced by BioPort Inc. (formerly the Michigan Biologic Products Institute). This vaccine was licensed in the early 1970s and has been given to thousands of people. This vaccine is made from sterile filtrates of microaerophilic cultures of an attenuated, unencapsulated, nonproteolytic, strain (V770-NP 1-R) of *B anthracis*. The recommended schedule for vaccination is 0.5 mL given subcutaneously at 0, 2, and 4 weeks, followed by doses at 6, 12, and 18 months. Annual boosters are recommended if the potential for exposure continues. When tested by an enzyme-linked immunosorbent assay, more than 95% of vaccinees seroconvert after the initial three doses.[279,280] Local side effects, consisting of erythema, tenderness, induration, and edema, may occur in up to 30% of recipients, but systemic side effects are rare.[281] Nontender subcutaneous nodules at the site of vaccination can occur and resolve without treatment. No long-term sequelae from the vaccine have been reported.[282]

The vaccine should be given to industrial workers exposed to potentially contaminated animal products imported from countries in which animal anthrax remains uncontrolled. People in direct contact with potentially infected animals and laboratory workers should also be immunized. Contraindications for use of the vaccine include a hypersensitivity reaction to the vaccine or one of its components. Reasons for temporary deferment of the vaccine include active febrile illness, corticosteroid or other immunosuppressive regimen, and pregnancy. Servicemembers who are scheduled to receive the vaccine and believe they may be pregnant should be referred for pregnancy testing, and vaccination should be suspended until the patient is no longer pregnant.

In 1998, the Department of Defense initiated the Anthrax Vaccination Immunization Program, a military-wide program to immunize all active duty and reserve forces against anthrax over several years. All immunizations given under the program are tracked centrally and documented in patient medical records. As of February 2001, more than 2 million doses have been given.

Anthrax Vaccination Immunization Program guidelines stipulate that if there is deviation from the vaccination schedule, the next dose should be given as soon as possible and then all remaining doses given based on the time of the last dose. The series should only be restarted if only one dose has been received and more than 2 years have elapsed since receiving that dose.

[Arthur M. Friedlander, Lisa A. Pearse, Julie Pavin]

BRUCELLOSIS

Introduction and Military Relevance

Brucellosis is a zoonotic infection of domesticated and wild animals caused by bacteria of the genus *Brucella*. Humans become infected by ingestion of animal food products, direct contact with infected animals, or inhalation of infectious aerosols. Onset is acute or insidious and is characterized by fever and a variety of systemic symptoms or by a local inflammatory process.

In 1751, Cleghorn, a British army surgeon stationed on the Mediterranean island of Minorca, described cases of a chronic, relapsing febrile illness and cited Hippocrates' description of a similar disease more

than 2,000 years earlier.[283] David Bruce isolated the causative organism from the spleens of five fatal cases in 1897 but termed it a micrococcus.[284] In 1897, Hughes coined the name "undulant fever" and described 844 cases.[285] In that same year, Bang identified a "Bacillus of abortion" in placentas and fetuses of cattle suffering from contagious abortion.[286] Twenty years later, Evans recognized that Bang's organism was identical to the causative agent of human brucellosis described by Hughes.

The organism infects a borad range of mammals, causing abortion, fetal death, and genital infection.[287,288] Humans, who are usually infected incidentally by contact with infected animals or ingestion of dairy foods, may develop numerous symptoms in addition to the usual ones of fever, malaise, and muscle pain that might incapacitate military personnel in a theater of operations. Disease frequently becomes chronic and may relapse, even with treatment. The ease of transmission by aerosol suggests that *Brucella* organisms might be a candidate for use as a biological warfare agent.

Description of the Pathogen

Brucellae are small, nonmotile, nonsporulating, nontoxigenic, nonfermenting, aerobic, gram-negative coccobacilli that may, based on DNA homology, represent a single species.[289] Conventionally, however, they are classified into six species, each comprising several biovars. Each species has a characteristic but not absolute predilection to infect certain animals (Table 36-7). A novel species (proposed nomen *B maris*) has recently been isolated from marine mammals.[290] Only *Brucella melitensis, B suis, B abortus, B canis* and *B manis* cause disease in humans.

TABLE 36-7

TYPICAL HOST SPECIFICITY OF *BRUCELLA* SPECIES

Brucella Species	Animal Host	Human Pathogenicity
B suis	Swine	High
B melitensis	Sheep, goats	High
B abortus	Cattle, bison	Intermediate
B canis	Dogs	Intermediate
B ovis	Sheep	None
B neotomae	Rodents	None
B maris	Marine mammals	Not known

Epidemiology

Transmission

Natural transmission is through contact with infectious tissues or secretions, through ingestion of raw milk and dairy products, or by inhalation of aerosolized bacteria in animal enclosures, laboratories, or slaughterhouses. Brucellosis is rarely, if ever, transmitted from person to person. The incidence of human disease is thus closely tied to the prevalence of infection in sheep, goats, swine, camels, and cattle and to practices that allow exposure of humans to potentially infected animals or their products.[291–293] In the United States, where most states are free of infected animals and where dairy products are routinely pasteurized, illness has historically occurred primarily in individuals who have occupational exposure to infected animals (eg, veterinarians, shepherds, cattlemen, slaughterhouse workers). In many other countries, humans more commonly acquire infection by ingestion of unpasteurized dairy products, especially cheese. In the last 2 decades, food-borne acquisition has also become relatively more frequent in the United States because of importation of contaminated food from Mexico.[294]

Less obvious exposures can also lead to infection. In Kuwait, for example, disease with a relatively high proportion of respiratory complaints has occurred in individuals who camped in the desert during the spring lambing season.[295] In Australia, an outbreak of *B suis* infection was noted in hunters of feral pigs.[296] Brucellae are also highly infectious in laboratory settings.

Geographic Distribution and Incidence

Brucella organisms are distributed worldwide. Fewer than 200 total cases per year (0.04 cases per 100,000 population) are reported in the United States. The incidence is much higher in the Middle East, countries bordering the Mediterranean Sea, China, India, Mexico, and Peru. For example, the incidence rate in Jordan was 33 cases per 100,000 population (1987)[297] and in Kuwait was 88 cases per 100,000 (1985).[298]

Pathogenesis and Clinical Findings

Brucellae can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, and the gastrointestinal tract.[299] In the gastrointestinal tract, the organisms are phagocytosed by lymphoepithelial cells of gut-associated lymphoid tissue, from which they gain access to the

submucosa.[300] Organisms are rapidly ingested by polymorphonuclear leukocytes, which generally fail to kill them,[301,302] and are also phagocytosed by macrophages. Bacteria transported in macrophages may eventually localize in lymph nodes, liver, spleen, mammary glands, joints, kidneys, and bone marrow. This wide distribution of infected macrophages throughout the mononuclear phagocyte system and other organs explains the prominence of nonspecific constitutional symptoms and diverse localization of abnormalities in this disease.

If unchecked by macrophages, the bacteria destroy their host cells and infect additional cells. Brucellae can also replicate extracellularly. The host cellular response may range from abscess formation to lymphocytic infiltration to granuloma formation with caseous necrosis.

In ruminants, *Brucella* organisms target embryonic and trophoblastic tissue. When septic abortion occurs, the intense concentration of bacteria and aerosolization of infected tissue during parturition often result in infection of other animals and people.

Clinical manifestations of brucellosis are diverse.[303] Patients may present with an acute, systemic febrile illness, an insidious chronic infection, or a localized inflammatory process. Disease may be abrupt or insidious in onset, with an incubation period of 3 days to several weeks. Patients usually complain of nonspecific symptoms such as fever, sweats, fatigue, anorexia, and muscle or joint aches (Table 36-8). Neuropsychiatric symptoms, notably depression, headache, and irritability, occur frequently. In addition, focal infection of bone, joints, or genitourinary tract may cause local pain. Cough, pleuritic chest pain, and dyspepsia may also be noted. Chronically infected patients frequently lose weight. Symptoms often last for 3 to 6 months and occasionally for a year or more. Physical examination is usually normal, although hepatomegaly, splenomegaly, or lymphadenopathy may occur. Brucellosis does not usually cause leukocytosis, and some patients may be moderately neutropenic. Although disease manifestations cannot be strictly related to the infecting species, *B melitensis* tends to cause more severe, systemic illness than the other brucellae, and *B suis* is more likely to cause localized, suppurative disease.

TABLE 36-8

SYMPTOMS AND SIGNS OF BRUCELLOSIS

Symptom or Sign	Patients Affected (%)
Fever	90-95
Malaise	80-95
Sweats	40-90
Body Aches	40-70
Hepatomegaly	10-70
Arthralgia	20-40
Splenomegaly	10-30

Data sources: Mousa AR, Elhag KM, Khogaii M, Marafie AA. The nature of human brucellosis in Kuwait: Study of 379 cases. *Rev Infect Dis.* 1988;10:211–217; Buchanan TM, Faber LC, Feldman RA. Brucellosis in the United States, 1960-1972: An abattoir-associated disease, I: Clinical features and therapy. *Medicine (Baltimore).* 1974;53:403–413; and Gotuzzo E, Alarcon GS, Bocanegra TS, et al. Articular involvement in human brucellosis: A retrospective analysis of 304 cases. *Semin Arthritis Rheum.* 1982;12:245–255.

Infection with *B melitensis* leads to bone or joint disease in about 30% of patients; sacroiliitis3 develops in 6% to 15%, particularly in young adults.[304,305] Arthritis of all large joints combined occurs with about the same frequency as sacroiliitis. Joint inflammation seen in patients with *B melitensis* is mild, and erythema of overlying skin is uncommon. Synovial fluid is exudative, but cell counts are in the low thousands with predominantly mononuclear cells. Spondylitis, another important osteoarticular manifestation of brucellosis, tends to affect middle-aged or elderly patients, causing back (usually lumbar) pain, local tenderness, and occasionally radicular symptoms.

Magnetic resonance imaging, computer assisted tomography, and bone scintigraphy are more sensitive than plain films for demonstration of bone and joint abnormalities.[306] In sacroiliitis and peripheral joint infections, destruction of bone is unusual. In spondylitis, erosion and sclerosis of the anterosuperior portion of the vertebrae, often accompanied by osteophyte formation, occur early. Magnetic resonance imaging shows an increased T2-weighted signal. Later, diffuse involvement of vertebrae may lead to further destruction of vertebral bodies and nuclear herniation into the softened vertebral bodies. Paravertebral abscess occurs rarely. In contrast with frequent infection of the axial skeleton, osteomyelitis of long bones is rare.[307]

Infection of the genitourinary tract may lead to signs and symptoms of disease in humans.[308] Pyelonephritis and cystitis and, in males, epididymoorchitis, may occur. Both diseases may mimic their tuberculous counterparts, with "sterile" pyuria on routine bacteriologic culture. With bladder and kidney infection, *Brucella* organisms can be cultured from the urine. Brucellosis in pregnancy can lead to placental and fetal infection.[309] Whether abortion is more common in brucellosis than in other severe bacterial infections, however, is unknown.

Lung infections have also been described, particularly before the advent of effective antibiotics. Although up to one quarter of patients with brucellosis may complain of respiratory symptoms (eg, cough, dyspnea, or pleuritic pain), chest X-ray examinations are usually normal.[310] Diffuse or focal infiltrates, pleural effusion, abscess, and granulomas may be noted.

Hepatitis and, rarely, liver abscess also occur. Mild elevations of serum lactate dehydrogenase and alkaline phosphatase are common. Biopsy may show well-formed granulomas or nonspecific hepatitis with collections of mononuclear cells.[303] Other sites of infection include the heart, central nervous system, and skin. Brucella endocarditis, a rare but most feared complication, accounts for 80% of deaths from brucellosis.[311] Central nervous system infection usually manifests itself as chronic meningoencephalitis, but subarachnoid hemorrhage and myelitis also occur.

Diagnostic Approaches

A thorough history that elicits details of possible exposure (eg, laboratories, animals, animal products, or environmental exposure to locations inhabited by potentially infected animals) is the most important diagnostic tool. Personnel with certain military occupations and symptoms compatible with brucellosis should be questioned particularly closely. Veterinary medicine officers assume responsibility for meat inspection and may visit slaughterhouses, especially during deployments. Special Forces personnel may subsist on the local economy during some actions and ingest unpasteurized dairy products. Brucellosis should also be strongly considered in the differential diagnosis of febrile illness if service members have been exposed to a presumed biological attack. Polymerase chain reaction and antibody-based antigen detection systems may demonstrate the presence of the organism in environmental samples collected from the attack area.

When the disease is considered, diagnosis is usually made by serology. The tube agglutination test remains the standard method.[312] Use of the tube agglutination test after treatment of serum with 2-mercaptoethanol or dithiothreitol to dissociate IgM into monomers detects IgG antibody. A titer of 1:160 or higher is considered diagnostic. Most patients already have high titers at the time of clinical presentation, so a 4-fold rise in titer may not occur. IgM rises early in disease and may persist at low levels (eg, 1:20) for months or years after successful treatment. Persistence or increase of 2-mercaptoethanol–resistant titers has been associated with persistent disease or relapse. Serum testing should always include dilution to at least 1:320, since inhibition of agglutination at lower dilutions may occur. The tube agglutination test does not detect antibodies to *B canis.* Enzyme-linked immunosorbent assays have been developed but are not well standardized.

Diagnosis should be pursued by microbiologic culture of blood or body fluid samples. Cultures should be held for at least 2 months and subcultured weekly onto solid medium. Because it is extremely infectious for laboratory workers, the organism should be subcultured only in a biohazard hood. The reported frequency of isolation from blood varies widely, from less than 10% to 90%; *B melitensis* is said to be more readily cultured than *B abortus*. Culture of bone marrow may increase the yield.[313]

Recommendations for Therapy and Control

Brucellae are sensitive in vitro to a number of oral antibiotics and to aminoglycosides. Therapy with a single drug has resulted in a high relapse rate, so combined regimens should be used whenever possible.[314] A 6-week regimen of doxycycline 200 mg per day administered orally with streptomycin 1 g per day administered intramuscularly for the first 2 to 3 weeks is effective therapy for adults with most forms of brucellosis.[315] Gentamicin, 2 to 5 mg per kg per day, is now often substituted for streptomycin.[291] A 6-week oral regimen of both rifampin (900 mg/day) and doxycycline (200 mg/day) is also effective and should result in nearly 100% response and a relapse rate of lower than 10%. Treatment with a combination of streptomycin and doxycycline may result in less frequent relapse than treatment with the combination of rifampin and doxycycline.[315]

Spondylitis, endocarditis, and central nervous system infections generally require prolonged therapy. Spondylitis should be treated with a regimen that includes an aminoglycoside, as notable failures have occurred in patients treated with the combination of rifampin and doxycycline. Endocarditis may best be treated with rifampin, streptomycin, and doxycycline; infected valves should be replaced early in therapy.[316] Central nervous system disease responds to a combination of rifampin and trimethoprim/sulfamethoxazole. This antibiotic combination is also effective for children under 8 years of age.[315] The Joint Food and Agriculture Organization–World Health Organization Expert Committee recommends treatment of

pregnant women with rifampin.[317] Trimethoprim/sulfamethoxazole is a reasonable alternative drug in pregnancy.[291]

Organisms used in a biological attack may be resistant to these first-line antimicrobial agents. Medical officers should make very effort to obtain tissue and environmental samples for bacteriological culture, so that the antibiotic susceptibility profile of the infecting brucellae may be determined and the therapy adjusted accordingly.

Effective livestock immunization programs will markedly reduce the incidence of human disease. Animal handlers should wear appropriate protective clothing when working with infected animals. Meat should be well-cooked, and milk should be pasteurized. Service members in endemic areas should be counseled to avoid eating uncooked foods, particularly dairy products, obtained from the local economy. Sheep, goats, cattle, swine, and camels in endemic areas should be considered infected. Locating camps close to these animals should be avoided, particularly during birthing seasons; animals should be segregated from military personnel. Laboratory workers should culture the organism only with appropriate biosafety level 2 (clinical samples) or 3 (research) containment.

In the event of a biological attack, the standard gas mask should adequately protect personnel from airborne brucellae, because the organisms are probably unable to penetrate intact skin. After personnel have been evacuated from the attack area, clothing, skin, and other surfaces can be decontaminated with standard disinfectants to minimize risk of infection by accidental ingestion or by conjunctival inoculation of viable organisms.

There is no commercially available vaccine for humans. In the event of known percutaneous or mucosal exposure to virulent or vaccinal strains of *Brucella*, prophylactic administration of rifampin and tetracycline for 3 to 6 weeks seems reasonable.[57p75–78,291]

[David Hoover]

REFERENCES

1. Berman SJ, Tsai C, Holmes K, Fresh JW, Watten RH. Sporadic anicteric leptospirosis in South Vietnam: A study in 150 patients. *Ann Intern Med*. 1973:79:167–173.

2. Takafuji ET, Kirkpatrick JW, Miller RN, et al. An efficacy trial of doxycycline chemoprophylaxis against leptospirosis. *N Engl J Med*. 1984;310:497–500.

3. Torten M. Leptospirosis. In: Steele JH, ed. *Handbook Series in Zoonoses*. Vol 1. Boca Raton, Fla: CRC Press; 1979: pp 363–421.

4. Feigin RD, Lobes LA, Anderson D, Pickering L. Human leptospirosis from immunized dogs. *Ann Intern Med*. 1973;79:777–785.

5. Kaufmann AF. Epidemiologic trends of leptospirosis in the United States, 1965-1974. In: Johnson RC, ed. *The Biology of Parasitic Spirochetes*. New York: Academic Press; 1976: pp 177-190.

6. Wilkins E, Cope A, Waitkins S. Rapids, rafts, and rats. *Lancet*. 1988;2:283–284.

7. Sanford JP. Leptospirosis—time for a booster. *N Engl J Med*. 1984;310:524–525.

8. Faine S, ed. *Guidelines for the Control of Leptospirosis*. Geneva: World Health Organization; 1982.

9. Bolin CA, Koellner P. Human-to-human transmission of *Leptospira interrogans* by milk. *J Infect Dis*. 1988;158:246–247.

10. Feigin RD, Anderson DC. Human leptospirosis. *CRC Crit Rev Clin Lab Sci*. 1975;5:413–467.

11. Shpilberg O, Shaked Y, Maier MK, Samra D, Samra J. Long-term follow-up after leptospirosis. *South Med J*. 1990;83:405–407.

12. Thiermann AB: Leptospirosis: Current developments and trends. *J Am Vet Med Assoc*. 1984;184;722–725.

13. McClain JB, Ballou WR, Harrison SM, Steinweg DL. Doxycycline therapy for leptospirosis. *Ann Intern Med*. 1984;100:696–698.

14. Kocen RS. Leptospirosis: A comparison of symptomatic and penicillin therapy. *Br Med J*. 1962;1:1181.

15. Watt G, Padre LP, Tuazon ML, Everard CO, Callender J. Placebo-controlled trial of intravenous penicillin for severe and late leptospirosis. *Lancet*. 1988;1:433–435.

16. Edwards CN, Nicholson GD, Hassell TA, et al. Penicillin therapy in icteric leptospirosis. *Am J Trop Med Hyg*. 1988;39:388–390.

17. Alexander AD, Rule PL. Penicillins, cephalosporins, and tetracyclines in treatment of hamsters with fatal leptospirosis. *Antimicrob Agents Chemother*. 1986;30:835–839.

18. Gajdusek DC. Virus hemorrhagic fevers with special reference to hemorrhagic fever with renal syndrome (epidemic hemorrhagic fever). *J Peds*. 1962;60:841–857.

19. Lahdevirta J. Nephropathia epidemica in Finland: A clinical, histological and epidemiological study. *Ann Clin Res (Helsinki)*. 1971;3(suppl 8):1–154.

20. Trencseni T, Keleti B. *Clinical Aspects and Epidemiology of Haemorrhagic Fever with Renal Syndrome: Analysis of Clinical and Epidemiological Experiences in Hungary.* Budapest: Akademiai Kiado; 1971.

21. McKee KT Jr, LeDuc JW, Peters CJ. Hantaviruses. In: Belshe RB, ed. *Textbook of Human Virology*. 2nd ed. St. Louis: Mosby Year Book; 1991: 615–632.

22. Lee HW, Lee P-W, Johnson KM. Isolation of the etiological agent of Korean hemorrhagic fever. *J Infect Dis*. 1978;137:298–308.

23. Pon E, McKee KT Jr, Diniega BM, Merrel B, Corwin A, Ksiazek TG. Outbreak of hemorrhagic fever with renal syndrome among U.S. Marines in Korea. *Am J Trop Med Hyg*. 1990;42:612–619.

24. Underwood PK. Chief, Preventive Medicine Service, US Army Medical Department Activity, Neurnberg. Personal communication, 1992.

25. Schmaljohn CS, Hasty SE, Dalrymple JM, et al. Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. *Science*. 1985;227:1041–1044.

26. LeDuc JW. Epidemiology of Hantaan and related viruses. *Lab Anim Sci*. 1987;37:413–418.

27. Chapman LE, McKee KT Jr, Peters CJ. Hantaviruses. In: Feigin RD, Cherry JD, eds. *Textbook of Pediatric Infectious Diseases*. 4th ed. Philadelphia: W.B. Saunders; 1998: 2141–2150.

28. Schmaljohn C, Hjelle B. Hantaviruses: A global disease problem. *Emerg Infect Dis.* 1997;3:95–104.

29. Peters CJ. Hantavirus pulmonary syndrome—the Americas. Scheld WM, Craig WA, Hughes JM, eds. *Emerging Infections*. Washington, DC: ASM Press; 1998.

30. Lee HW, French GR, Lee PW, Baek LJ, Tsuchiya K, Foulke RS. Observations on natural and laboratory infection of rodents with the etiologic agent of Korean hemorrhagic fever. *Am J Trop Med Hyg*. 1981;30:477–482.

31. Yanagihara R. Hantavirus infection in the United States: Epizootology and epidemiology. *Rev Infect Dis.* 1990;12:449–457.

32. Lee HW, Lee PW, Baek LJ, Chu YK. Geographical distribution of hemorrhagic fever with renal syndrome and hantaviruses. *Arch Virol*. 1990;115 (Suppl 1):5–18.

33. Settergren B. Nephropathia epidemica (hemorrhagic fever with renal syndrome) in Scandinavia. *Rev Infect Dis*. 1991;13:736–744.

34. Avsic-Zupanc T, Likar M, Navakovic S, et al. Evidence of the presence of two hantaviruses in Slovenia. *Arch Virol*. 1990;115(Suppl 1):87–94.

35. Avsic-Zupanc T, Xiao SY, Stojanovic R, Gligic A, van der Groen G, LeDuc JW. Characterization of Dobrava virus: A hantavirus from Slovenia, Yugoslavia. *J Med Virol*. 1992;38:132–137.

36. Gligic A, Dimkovic N, Xiao SY, et al. Belgrade virus: A new hantavirus causing severe hemorrhagic fever with renal syndrome in Yugoslavia. *J Infect Dis*. 1992;166:113–120.

37. Nichol ST, Spiropoulou CF, Morzunov S, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science*. 1993;262:914–917.

38. Elliott LH, Ksiazek TG, Rollin PE, et al. Isolation of the causative agent of hantavirus pulmonary syndrome. *Am J Trop Med Hyg*. 1994;51:102–108.

39. Ksiazek TG, Peters CJ, Rollin PE, et al. Identification of a new North American hantavirus that causes acute pulmonary insufficiency. *Am J Trop Med Hyg*. 1995;52:117–123.

40. Rollin PE, Ksiazek TG, Elliott LH, et al. Isolation of Black Creek Canal virus, a new hantavirus from *Sigmodon hispidus* in Florida. *J Med Virol.* 1995;46:35–39.

41. Morzunov SP, Feldmann H, Spiropoulou CF, et al. A newly recognized virus associated with a fatal case of hantavirus pulmonary syndrome in Louisiana. *J Virol.* 1995;69:1980–1983.

42. Khan AS, Ksiazek TG, Peters CJ. Hantavirus pulmonary syndrome. *Lancet.* 1996;347:739–741.

43. Lee HW. Epidemiology. In: Lee HW, Dalrymple JM, eds. *Manual of Hemorrhagic Fever with Renal Syndrome*. Seoul: World Health Organization Collaborating Center for Virus Reference and Research Institute for Viral Diseases and Korea University; 1989: 39–48.

44. Desmyter J, LeDuc JW, Johnson KM, Brasseur, Deckers C, van Ypersele de Strihou C. Laboratory rat associated outbreak of haemorrhagic fever with renal syndrome due to Hantaan-like virus in Belgium. *Lancet.* 1983;2:1445–1448.

45. Glass GE, Watson AJ, LeDuc JW, Kelem GD, Quinn TC, Childs JE. Infection with ratborne hantavirus in US residents is consistently associated with hypertensive renal disease. *J Infect Dis.* 1993;167:614–620.

46. Butler JC, Peters CJ. Hantaviruses and hantavirus pulmonary syndrome. *Clin Infect Dis*. 1994;19:387–394.

47. Chapman LE. Hantaviruses. *Sem Ped Infect Dis*. 1996;7:97–100.

48. Koster FT. Professor of Medicine, University of New Mexico Health Sciences Center, June 1997. Personal communication

49. Lee PW, Meegan JM, LeDuc JW, et al. Serologic techniques for detection of Hantaan virus infection, related antigens and antibodies. In: Lee HW, Dalrymple JM, eds. *Manual of Hemorrhagic Fever with Renal Syndrome*. Seoul: World Health Organization Collaborating Center for Virus Reference and Research Institute for Viral Diseases, and Korea University; 1989: 75–106.

50. Feldmann H, Sanchez A, Morzunov S, et al. Utilization of autopsy RNA for the synthesis of the nucleocapsid antigen of a newly recognized virus associated with hantavirus pulmonary syndrome. *Virus Res*. 1993;30:351–367.

51. Enria D, Padula P, Segura EL, et al. Hantavirus pulmonary syndrome in Argentina: Possibility of person to person transmission. *Medicina (B Aires)* 1996; 56: 709–711.

52. Huggins JW, Hsiang CM, Cosgriff TM, et al. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. *J Infect Dis*. 1991;164:1119–1127.

53. Armed Forces Pest Management Board. *Protection from Rodent-Borne Diseases, With Special Emphasis on Occupational Exposure to Hantavirus*. Washington, DC: Defense Pest Management and Information and Analysis Center and the Deputy Under Secretary of Defense for Environmental Security; 1999. Technical Information Memorandum 41.

54. Benenson MW, Takafuji ET, Lemon SM, Greenup RL, Sulzer AJ. Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. *N Engl J Med*. 1982;307:666–669.

55. Nicolle C, Manceaux LH. Sur une infection á corps de Leishman (ou organismes voisins) du gondi. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*. 1908;147:763–766.

56. Jank J. Pathogenesa a pathologická anatomie tak nazuného vrozeného kolobome zluté skvrny v oku normálne velikém a mikrophthalmickém s nálezem parazitu v sítnici. *Cas Lék Ces*. 1923;62:1021–1027,1054–1059,1081–1085,1111–1115,1138–1144.

57. Chin J, ed. *Control of Communicable Diseases Manual*. 17th ed. Washington, DC: American Public Health Association; 2000.

58. Krick JA,Remington JS. Toxoplasmosis in the adult—an overview. *N Engl J Med*. 1978;298:550–553.

59. Nussenblatt RB, Belfort R. Ocular toxoplasmosis: An old disease revisited. *JAMA*. 1994;271:304–307.

60. Jackson MH, Hutchison WM. The prevalence and source of *Toxoplasma* infection in the environment. *Adv Parasitol*. 1989;28:55–105.

61. Scott RJ. Toxoplasmosis. *Trop Dis Bull*. 1978;75:809–827.

62. Garcia LS, Sulzer AJ, Healy GR, Grady KK, Brukner DA. Blood and tissue protozoa. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. *Manual of Clinical Microbiology*. 6th ed. Washington, DC: ASM Press; 1995: 1171–1195.

63. Sibley LD, Boothroyd JC. Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. *Nature*. 1992;359:82–85.

64. Frenkel JK, Ruiz A, Chinchilla M. Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. *Am J Trop Med Hyg*. 1975;24:439–443.

65. Yilmaz SM, Hopkins SH. Effects of different conditions on duration of infectivity of *Toxoplasma gondii* oocysts. *J Parasitol*. 1972;58:938–939.

66. Dubey JP, Beattie CP. Toxoplasmosis of animals and man. Boca Raton, Fla: CRC Press; 1988.

67. Glaser CA, Angulo FJ, Rooney JA. Animal-associated opportunistic infections among persons infected with the human immunodeficiency virus. *Clin Infect Dis*. 1994;18:14–24.

68. Dubey JP. Reshedding of *Toxoplasma* oocysts by chronically infected cats. *Nature*. 1976;262:213–214.

69. Beaman MH, McCabe RE, Wong SY, Remington JS. *Toxoplasma gondii*. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases*. 4th ed. New York: Churchill Livingstone; 1995: 2455–2475.

70. Feldman HA. Toxoplasmosis. *N Engl J Med*. 1968;279:1370–1375.

71. Jewell ML, Frenkel JK, Johnson KM, Reed V, Ruiz A. Development of *Toxoplasma* oocysts in neotropical *Felidae*. *Am J Trop Med Hyg*. 1972;21:512–517.

72. Frenkel JK. Toxoplasmosis. In: Binford CH, Connor DH, eds. *Pathology of Tropical and Extraordinary Diseases*. Vol 1. Washington, DC: Armed Forces Institute of Pathology; 1976: 284–300.

73. Feldman HA, Miller LT. Serological study of toxoplasmosis prevalence. *Am J Hyg*. 1956;64:320–335.

74. Smith KL, Wilson M, Hightower AW, et al. Prevalence of *Toxoplasma gondii* antibodies in US military recruits in 1989: Comparison with data published in 1965. *Clin Infect Dis*. 1996;23:1182–1183.

75. Feldman HA. A nationwide serum survey of United States military recruits, 1962, VI: *Toxoplasma* antibodies. A*m J Epidemiol*. 1965;81:385–391.

76. Feldman HA. Toxoplasmosis. *N Engl J Med*. 1968;279:1431–1437 (conclusion).

77. McCabe RE, Brooks RG, Dorfman RF, Remington JS. Clinical spectrum in 107 cases of toxoplasmic lymphadenopathy. *Rev Infect Dis*. 1987;9:754–774.

78. Wong SY, Remington JS. Toxoplasmosis in pregnancy. *Clin Infect Dis*. 1994;18:853–862.

79. Remington JS. Toxoplasmosis in the adult. *Bull N Y Acad Med*. 1974;50:211–227.

80. Elliot DL, Tollf SW, Goldberg L, Miller JB. Pet-associated illness. *N Engl J Med*. 1985;313:985–995.

81. Remington JS, McLeod R, Desmonts G. Toxoplasmosis. In: Remington JS, Klein JO, eds. *Infectious Diseases of the Fetus and Newborn Infant*. 4th ed. Phildelphia: W.B. Saunders; 1995: 140–267.

82. Sabin AB, Feldman HA. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (*Toxoplasma*). *Science*. 1948;108:660–663.

83. Sulzer AJ, Franco EL, Takafuji ET, Benenson MW, Walls KW, Greenup RL. An oocyst-transmitted outbreak of toxoplasmosis: Patterns of immunoglobulin G and M over one year. *Am J Trop Med Hyg*. 1986;35:290–296.

84. Brooks RG, McCabe RE, Remington JS. Role of serology in the diagnosis of toxoplasmic lymphadenopathy. *Rev Infect Dis*. 1987;9:1055–1062.

85. Tomasi JP, Schlit AF, Stadtsbaeder S. Rapid double-sandwich enzyme-linked immunosorbent assay for detection of human immunoglobulin M anti-*Toxoplasma gondii* antibodies. *J Clin Microbiol*. 1986;24:849–850.

86. Desmonts G, Couvreur J. Congenital toxoplasmosis: A prospective study of 378 pregnancies. *N Engl J Med*. 1974;290:1110–1116.

87. Ho-Yen DO, Joss AWL, Balfour AH, Smyth ET, Baird D, Chatterton JM. Use of the polymerase chain reaction to detect *Toxoplasma gondii* in human blood samples. *J Clin Pathol*. 1992;45:910–913.

88. Depouy-Camet J, de Souza SL, Maslo C, et al. Detection of *Toxoplasma gondii* in venous blood from AIDS patients by polymerase chain reaction. *J Clin Microbiol*. 1993;31:1866–1869.

89. Dannemann BR, Israelski DM, Remington JS. Treatment of toxoplasmic encephalitis with intravenous clindamycin. *Arch Intern Med*. 1988;148:2477–2482.

90. Dannemann B, McCutchan JA, Israelski D, et al. Treatment of toxoplasmic encephalitis in patients with AIDS: A randomized trial comparing pyrimethamine plus clindamycin to pyrimethamine plus sulfadizine. *Ann Intern Med*. 1992;116:33–43.

91. Spicer AJ. Military significance of Q fever: A review. *J R Soc Med*. 1978;71:762–767.

92. Robbins FC, Ragan CA. Q fever in the Mediterranean area: Report of its occurrence in allied troops, I: Clinical features of the disease. Am J Hyg. 1946;44:6–22.

93. Robbins FC, Gauld RL, Warner FB. Q fever in the Mediterranean area: Report of its occurrence in allied troops, II: Epidemiology. Am J Hyg. 1946;44:23–50.

94. The Commission of Acute Respiratory Diseases. Epidemics of Q fever among troops returning from Italy in the spring of 1945, III: Etiological studies. Am J Hyg. 1946;44:88–102.

95. Feinstein M, Yesner R, Marks JL. Epidemics of Q fever among troops returning from Italy in the spring of 1945, I: Clinical aspects of the epidemic at Camp Patrick Henry, Virginia. *Am J Hyg.* 1946;44:72–87.

96. Spicer AJ, Crawther RW, Vella EE, Bengtsson E, Miles R, Ritzolis G. Q fever and animal abortion in Cyprus. Trans R Soc Trop Med Hyg. 1977;71:16–20.

97. Rombo L, Bengtsson E, Grandien M. Serum Q fever antibodies in Swedish UN soldiers in Cyprus: Reflecting a domestic or foreign disease? *Scand J Infect Dis.* 1978;10:157–158.

98. Ferrante MA, Dolan MJ. Q fever meningoencephalitis in a soldier returning from the Persian Gulf War. *Clin Infect Dis.* 1993;16:489–496.

99. World Health Organization. *Health Aspects of Chemical and Biological Weapons: Report of a WHO Group of Consultants*. Geneva: WHO; 1970: 72, 99.

100. Department of the Army. *US Army Activity on the US Biological Warfare Programs: 1942-1977: Annexes*. Vol 2. DA; 1977.

101. Weisburg WG, Dobson ME, Samuel JE, et al. Phylogenetic diversity of the rickettsias. *J Bacteriol*. 1989;171:4202–4206.

102. Williams JC. Infectivity, virulence, and pathogenicity of *Coxiella burnetii* for various hosts. In: Williams JC, Thompson HA, eds. *Q Fever: The Biology of* Coxiella burnetii. Boca Raton, Fla: CRC Press; 1991.

103. McCaul TF, Dare AJ, Gannon JP, Galbraith AJ. In vivo endogenous spore formation by *Coxiella burnetii* in Q fever endocarditis. *J Clin Pathol.* 1994;47:978–981.

104. Stoker MGP, Fiset P. Phase variation of the Nine Mile and other strains of *Rickettsia burnetii. Can J Microbiol*. 1956;2:310–321.

105. Tigertt WD, Benenson AS. Studies on Q fever in man. *Trans Assoc Am Phys.* 1956;69:98–104.

106. Oliphant JW, Gordon DA, Meis A, Parker RR. Q fever in laundry workers presumably transmitted from contaminated clothing. *Am J Hyg.* 1949;49:76–82.

107. Marrie TJ, Langille D, Papukna V, Yates L. Truckin' pneumonia—an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens. *Epidemiol Infect*. 1989;102:119–127.

108. Salmon MM, Howells B, Glencross EJ, Evans AD, Palmer SR. Q fever in an urban area. *Lancet*. 1982;1:1002–1004.

109. Mann JS, Douglas JG, Inglis JM, Leitch AG. Q fever: Person to person transmission within a family. *Thorax.* 1986;41:974–975.

110. Kaplan MM, Bertagna P. The geographical distribution of Q fever. *Bull World Health Organ.* 1955;13:829–860.

111. Wisniewski HJ, Piraino FF. Review of virus respiratory infections in the Milwaukee area, 1955–1965. *Public Health Reports*. 1969;84:175–181.

112. Epidemiology of a Q fever outbreak in Los Angeles County, 1966. *HSMHA Health Reports.* 1972;87:71–74.

113. Pinsky RL, Fishbein DB, Greene CR, Gensheimer KF. An outbreak of cat-associated Q fever in the United States. J Infect Dis. 1991;164:202–204.

114. Langley JM, Marrie TJ, Covert A, Waag DM, Williams JC. Poker players' pneumonia: An urban outbreak of Q fever following exposure to a parturient cat. *N Engl J Med.* 1988;319:354–356.

115. Centers for Disease Control. Q fever among slaughterhouse workers—California. *MMWR*. 1986;35:223–226.

116. Meiklejohn G, Reimer LG, Graves PS, Helmick C. Cryptic epidemic of Q fever in a medical school. *J Infect Dis.* 1981;144:107–113.

117. Rauch AM, Tanner M, Pacer RE, Barrett MJ, Brokopp CD, Schonberger LB. Sheep-associated outbreak of Q fever, Idaho. *Arch Intern Med.* 1987;147:341–344.

118. Graham CJ, Yamauchi T, Rountree P. Q fever in animal laboratory workers: An outbreak and its investigation. *Am J Infect Control.* 1989;17:345–348.

119. Hamadeh GN, Turner BW, Trible W Jr, Hoffmann BJ, Anderson RM. Laboratory outbreak of Q fever. *J Fam Pract.* 1992;35:683–685.

120. D'Angelo LJ, Baker EF, Schlosser W. Q fever in the United States, 1948–1977. *J Infect Dis.* 1979;139:613–615.

121. Sawyer LA, Fishbein DB, McDade JE. Q fever in patients with hepatitis and pneumonia: Results of a laboratory-based surveillance in the United States. *J Infect Dis.* 1988;158:497–498.

122. Sienko DG, Bartlett PC, McGee HB, Wentworth BB, Herndon JL, Hall WN. Q fever: A call to heighten our index of suspicion. *Arch Intern Med.* 1988;148:609–612.

123. Dupuis G, Petite J, Peter O, Vouilloz M. An important outbreak of human Q fever in a Swiss Alpine valley. *Int J Epidemiol.* 1987;16:282–287.

124. Raoult D, Marrie TJ. State-of-the-art clinical lecture: Q fever. *Clin Infect Dis.* 1995;20:489–496.

125. Tissot Dupont H, Raoult D, Brouqui P. Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. *Am J Med.* 1992;93:427–434.

126. Smith DL, Ayres JG, Blair I, et al. A large Q fever outbreak in the West Midlands: Clinical aspects. *Respir Med.* 1993;87:509–516.

127. Derrick EH. The course of infection with *Coxiella burnetii. Med J Aust.* 1973;1:1051–1057.

128. Smith DL, Wellings R, Walker C, Ayres JG, Burge PS. The chest x-ray in Q fever: A report on 69 cases from the 1989 West Midlands outbreak. *Br J Radiol.* 1991;64:1101–1108.

129. Marmion BP, Shannon M, Maddocks I, Storm P, Penttila I. Protracted fatigue and debility after Q fever. *Lancet*. 1996;347:977–978.

130. Ayres JG, Smith EG, Flint N. Protracted fatigue and debility after acute Q fever. *Lancet*. 2996;347:978–979.

131. Ayres JG, Flint N, Smith EG, et al. Post-infection fatigue syndrome following Q fever. *QJM*. 1998;91:105–123.

132. Peter O, Dupuis G, Peacock MG, Burgdorfer W. Comparison of enzyme-linked immunosorbent assay and complement fixation and indirect fluorescent-antibody tests for detection of *Coxiella burnetti* antibody. *J Clin Microbiol*. 1987;25:1063–1067.

133. Uhaa IJ, Fishbein DB, Olson JG, Rives CC, Waag DM, Williams JC. Evaluation of specificity of indirect enzyme-linked immunosorbent assay for diagnosis of human Q fever. *J Clin Microbiol.* 1994;32:1560–1565.

134. Waag D, Chulay J, Marrie T, England M, Williams J. Validation of an enzyme immunoassay for serodiagnosis of acute Q fever. *Eur J Clin Microbiol Infect Dis.* 1995;14:421–427.

135. Hoover TA, Vodkin MH, Williams JC. A *Coxiella burnetii* repeated DNA element resembling a bacterial insertion sequence. *J Bacteriol*. 1992;174:5540–5548.

136. Stein A, Raoult D. Detection of *Coxiella burnetii* by DNA amplification using polymerase chain reaction. *J Clin Microbiol.* 1992;30:2462–2466.

137. Willems H, Thiele D, Krauss H. Plasmid based differentiation and detection of *Coxiella burnetii* in clinical samples. *Eur J Epidemiol.* 1993;9:411–418.

138. Raoult D. Treatment of Q fever. *Antimicrob Agents Chemother*. 1993;37:1733–1736.

139. Tselentis Y, Gikas A, Kofteridis D. Q fever in the Greek island of Crete: Epidemiologic, clinical, and therapeutic data from 98 cases. *Clin Infect Dis.* 1995;20:1311–1316.

140. Sobradillo V, Zalacain R, Capelastegui A, Uresandi F, Corral J. Antibiotic treatment in pneumonia due to Q fever. *Thorax*. 1992;47:276–278.

141. Benenson AS. Q fever vaccine: Efficacy and present status. In: Smadel JE, ed. *Symposium on Q Fever by the Committee on Rickettsial Diseases*. Washington, DC: Armed Forces Epidemiology Board; 1959: 47–60.

142. Bell JF, Lackman DB, Meis A, Hadlow WJ. Recurrent reaction at site of Q fever vaccination in a sensitized person. *Mil Med.* 1964;124:591–595.

143. Lackman DB, Bell EJ, Bell JF, Pickens EG. Intradermal sensitivity testing in man with a purified vaccine for Q fever. *Am J Public Health.* 1962;52:87–93.

144. Scott GH, Williams JC. Susceptibility of *Coxiella burnetii* to chemical disinfectants.

145. Monath TP, Newhouse VF, Kemp GE, Setzer HW, Cacciapouti A. Lassa virus isolation from *Mastomys natalensis* rodents during an epidemic in Sierra Leone. *Science*. 1974;185:263–265.

146. Ter Meulen J, Lukashevich I, Sidibe K, et al. Hunting of peridomestic rodents and consumption of their meat as possible risk factors for rodent-to-human transmission of Lassa virus in the Republic of Guinea. *Am J Trop Med Hyg*. 1996;55:661–666.

147. Carey DC, Kemp GE, White HA, et al. Lassa fever—epidemiological aspects of the 1970 epidemic, Jos, Nigeria. *Trans R Soc Trop Med Hyg*. 1972;66:402–408.

148. Monath TP, Mertens PE, Patton R, et al. A hospital epidemic of Lassa fever in Zorzor, Liberia, March-April 1972. *Am J Trop Med Hyg*. 1973;22:773–779.

149. McCormick JB. Epidemiology and control of Lassa fever. *Curr Top Microbiol Immunol*. 1987;134:69–78.

150. McCormick JB, King IJ, Webb PA, et al. A case-control study of the clinical diagnosis and course of Lassa fever. *J Infect Dis*. 1987;155:445–455.

151. Webb PA, McCormick JB, King IJ, et al. Lassa fever in children in Sierra Leone, West Africa. *Trans R Soc Trop Med Hyg*. 1986;80:577–582.

152. Price ME, Fisher-Hoch SP, Craven RB, McCormick JB. A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy. *BMJ*. 1988;297:584–587.

153. Solbrig MV. Lassa virus and central nervous system diseases. In: Salvato MS, ed. *The Arenaviruses*. New York: Plenum Press; 1993: 325–330.

154. Cummins D, McCormick JB, Bennet D, et al. Acute sensorineural deafness in Lassa fever. *JAMA*. 1990;264: 2093–2096.

155. Fisher-Hoch S, McCormick JB, Sasso D, Craven RB. Hematologic dysfunction in Lassa fever. *J Med Virol*. 1988;26:127–135.

156. Johnson KM, McCormick JB, Webb PA, Smith EC, Elliott LH, King IJ. Clinical virology of Lassa fever in hospitalized patients. *J Infect Dis*. 1987;155:456–464.

157. McCormick JB, King IJ, Webb PA, et al. Lassa fever: Effective therapy with ribavirin. *N Eng J Med*. 1986;314:20–26.

158. Maiztegui JI, Feuillade M, Briggiler A. Progressive extension of the endemic area and changing incidence of Argentine hemorrhagic fever. *Med Microbiol Immunol*. 1986;175:149–152.

159. Peters CJ, Kuehne RW, Mercado RR, Le Bow RH, Spertzel RO, Webb PA. Hemorrhagic fever in Cochabamba, Bolivia, 1971. *Am J Epidimiol*. 1974;99:425–433.

160. Centers for Disease Control and Prevention. Bolivian hemorrhagic fever—El Beni Department, Bolivia, 1994. *MMWR*. 1994;43:943–946.

161. MacKenzie RB. Epidemiology of Machupo virus infection, I: Pattern of human infection, San Joachin, Bolivia, 1962–1964. *Am J Trop Med Hyg*. 1965;14:808–813.

162. Salas R, de Manzione N, Tesh RB, et al. Venezuelan haemorrhagic fever. *Lancet*. 1991;338:1033–1036.

163. Fulhorst CE, Bowen MD, Salas RA, et al. Isolation and characterization of Pirital virus, a newly discovered South American arenavirus. *Am J Trop Med Hyg*. 1997;56:548–553.

164. Lisieux T, Coimbra M, Nassar ES, et al. New arenavirus isolated in Brazil. *Lancet*. 1994;343:391–392.

165. Maiztegui JI, Fernandez NJ, de Damilano AJ. Efficacy of immune plasma in treatment of Argentine haemorrhagic fever and association between treatment and a late neurological syndrome. *Lancet*. 1979;2:1216–1217.

166. Enria DA, Briggiler AM, Fernandez NJ, Levis SC, Maiztegui JI. Importance of dose of neutralizing antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *Lancet*. 1984;2:255–256.

167. Maiztegui JI, McKee, KT Jr, Barrera Oro JG, et al. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. *J Infect Dis*. 1998;177:277–283.

168. World Health Organization. Ebola haemorrhagic fever. *Wkly Epidemiol Rec*. 1995;70:149–151.

169. Martini GA, Siegert R, eds. *Marburg Virus Disease*. New York: Springer-Verlag; 1971.

170. Peters CJ, Sanchez A, Rollin PE, Ksiazek TG, Murphy FA. *Filoviridae*: Marburg and Ebola viruses. In: Fields BN, Knipe DM, Channock RM, Melnick JL, Roizman B, Shope RE, eds. *Virology*. 3rd ed. New York: Raven Press; 1996: 1161–1176.

171. Jahrling PB, Geisbert TW, Dalgard DW, et al. Preliminary report: Isolation of Ebola virus from monkeys imported to USA. *Lancet*. 1990;335:502–505.

172. Casals J. Antigenic similarity between the virus causing Crimean hemorrhagic fever and Congo virus. *Proc Soc Exp Biol Med*. 1969;131:233–236.

173. Burney MI, Ghafoor A, Saleen M, Webb PA, Casals J. Nosocomial outbreak of viral hemorrhagic fever caused by Crimean hemorrhagic fever-Congo virus in Pakistan, January 1976. *Am J Trop Med Hyg*. 1980;29:941–947.

174. Watts DM, Ksiazek TG, Linthicum KJ, Hoogstraal H. Crimean-Congo hemorrhagic fever. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 2. Boca Raton, Fla: CRC Press; 1986: 177–222.

175. Swanepoel R, Gill DE, Shepherd AJ, Leman PA, Mynhardt JH, Harvey S. The clinical pathology of Crimean-Congo hemorrhagic fever. *Rev Infect Dis*. 1989;11(Suppl 4):S794–S800.

176. Fisher-Hoch SP, Khan JA, Rehman S, Mirza S, Khurshid M, McCormick JB. Crimean Congo-haemorrhagic fever treated with oral ribavirin. *Lancet*. 1995;346:472–475.

177. Steele JH, Fernandez PJ. History of rabies and global aspects. In: Baer GM, ed. *The Natural History of Rabies*. 2nd ed. Boca Raton, Fla: CRC Press; 1991: 1–24.

178. Steele JH. Rabies in the Americas and remarks on global aspects. *Rev Infect Dis*. 1988;10(Suppl 4):S585–S597.

179. Miller EB, Caldwell GL, Coates JB, eds. *United States Army Veterinary Service in World War II*. Washington, DC: Office of the Surgeon General, Department of the Army; 1961.

180. Kelser RA. Chloroform-treated rabies vaccine: Preliminary report. *Veterinary Bull*. 1928;22:95–98.

181. Kaplan C, Turner GS, Warrell DA. *Rabies: The Facts*. 2nd ed. Oxford: Oxford University Press; 1986.

182. Centers for Disease Control. Human rabies death—Alabama. *MMWR*. 1963;12:300.

183. Constantine DG. Rabies transmission by the non-bite route. *Public Health Rep*. 1962;77:287–289.

184. Winkler WG, Fashinell TR, Leffingwell L, Howard P, Conomy P. Airborne rabies transmission in a laboratory worker. *JAMA*. 1973;226:1219–1221.

185. Centers for Disease Control and Prevention. Human rabies—Washington, 1995. *MMWR*. 1995;44:625–627.

186. Houff SA, Burton RC, Wilson RW, et al. Human-to-human transmission of rabies virus by corneal transplant. *N Engl J Med*. 1979;300:603–604.

187. Hanlon CA, Koprowski H. Rabies: Issues in transmission and infectivity. *Mediguide to Infectious Diseases*. 1997;17(4):1–7.

188. McColl KA, Gould AR, Selleck PW, Hooper PT, Westbury HA, Smith JS. Polymerase chain reaction and other laboratory techniques in the diagnosis of long incubation rabies in Australia. *Aust Vet J*. 1993;70:84–89.

189. World Health Organization. Global health situation, IV: Selected infectious and parasitic diseases due to identified organisms. *Wkly Epidemiol Rec*. 1993;68:43–44.

190. Centers for Disease Control. Human rabies—Michigan. *MMWR*. 1983;32:159–160.

191. Centers for Disease Control. Human rabies—Pennsylvania. *MMWR*. 1984;33:633–635.

192. Centers for Disease Control. Human rabies—Texas, 1991. *MMWR*. 1991;40:132–133.

193. Centers for Disease Control. Human rabies—Texas, Arkansas, and Georgia, 1991. *MMWR*. 1991;40:765–769.

194. Centers for Disease Control and Prevention. Human rabies—New York, 1993. *MMWR*. 1993;42:805–806.

195. Tsiang H. Pathophysiology of rabies virus infection of the nervous system. *Adv Virus Res*. 1993;42:375–412.

196. Fishbein DB, Bernard KW. Rabies virus. In: Mandel G, Dolin RG, Bennett J, eds. *Principles and Practice of Infectious Diseases*. 4th ed. New York: Churchill Livingstone; 1995: 1527–1543.

197. Warrell DA. The clinical picture of rabies in man. *Trans R Soc Trop Med Hyg*. 1976;70:188–195.

198. Blenden DC, Creech W, Torres-Anjel MJ. Use of immunofluorescence examination to detect rabies virus antigen in the skin of humans with clinical encephalitis. *J Infect Dis*. 1986;154:698–701.

199. Schneider LG. The cornea test; a new method for the intra-vitam diagnosis of rabies. *Zentralbl Veterinarmed (B).* 1969;16:24–31.

200. Zaidman GW, Billingsley A. Corneal impression test for the diagnosis of acute rabies encephalitis. *Ophthalmology*. 1998;105:249–251.

201. Larghi OP, Gonzalez E, Held JR. Evaluation of the corneal test as a laboratory method for rabies diagnosis. *Appl Microbiol*. 1973;25:187–189.

202. Mathuranayagam D, Rao PV. Antemortem diagnosis of human rabies by corneal impression smears using immunofluorescent technique. *Indian J Med Res*. 1984;79:463–467.

203. Swoveland PT, Johnson KP. Identification of rabies antigen in human and animal tissues. *Ann N Y Acad Sci*. 1983;420:185–191.

204. Smith JS, Yager PA, Baer GM. A rapid reproducible test for determining rabies neutralizing antibody. *Bull World Health Organ*. 1973;48:535–541.

205. Kamolvarin N, Tirawatnpong T, Rattanasiwamoke R, Tirawatnpong S, Panpanich T, Hemachudha T. Diagnosis of rabies by polymerase chain reaction with nested primers. *J Infect Dis*. 1993;167:207–210.

206. Dean DJ, Baer GM, Thompson WR. Studies on the local treatment of rabies infected wounds. *Bull World Health Organ*. 1963;28:477–486.

207. Lin FT, Chen SB, Wang YZ, Sun CZ, Zeng FZ, Wang GF. Use of serum and vaccine in combination for prophylaxis following exposure to rabies. *Rev Infect Dis*. 1988;10(suppl 4):S766–S770.

208. Merigan TC, Baer GM, Winkler WG, et al. Human leukocyte interferon administration to patients with symptomatic and suspected rabies. *Ann Neurol*. 1984;16:82–87.

209. Bergen GA, Fitzmorris K. Viral encephalitis. In: *Infection Control and Applied Epidemiology: Principles and Practice*. St. Louis: Mosby; 1996.

210. Helmick CG, Tauxe RV, Vernon AA. Is there a risk to contacts of patients with rabies? *Rev Infect Dis*. 1987;9:511–518.

211. Centers for Disease Control and Prevention. Compendium of animal rabies control, 1997: National Association of State Public Health Veterinarians, Inc. *MMWR*. 1997;46(RR-4):1–9.

212. Brochier B, Kieny MP, Costy F, et al. Large-scale eradication of rabies using recombinant vaccinia-rabies vaccine. *Nature*. 1991;354:520–522.

213. Rosatte RC, Power MJ, MacInnes CD, Campbell JB. Trap-vaccinate-release and oral vaccination for rabies control in urban skunks, raccoons and foxes. *J Wildl Dis*. 1992;28:562–571.

214. National Association of State Public Health Veterinarians. Compendium of animal rabies control, 1994. *J Am Vet Assoc*. 1994;204:173–176.

215. Vaughn JB, Gerhardt P, Newell KW. Excretion of street rabies virus in the saliva of dogs. *JAMA*. 1965:193:363–368.

216. Vaughn JB Gerhardt P, Paterson J. Excretion of rabies virus in saliva of cats. *JAMA*. 1963;184:705.

217. Fekadu M, Shaddock JH, Baer GM. Intermittent excretion of rabies virus in the saliva of a dog two and six months after it had recovered from experimental rabies. *Am J Trop Med Hyg*. 1981;30:1113–1115.

218. Lopez T. Quarantine changes take effect in Hawaii. *J Am Vet Med Assoc*. 1997;211:817,819.

219. Roumiantzeff M, Ajjan N, Vincent-Falquet JC. Experience with preexposure rabies vaccination. *Rev Infect Dis*. 1988;10(suppl 4):S751–S757.

220. Centers for Disease Control. Rabies prevention—United States, 1991: Recommendations of the Immunization Practices Advisory Committee. *MMWR*. 1991;40:1–19.

221. Trimarchi CV, Safford M Jr. Poor response to rabies vaccination by the intradermal route. *JAMA*. 1992;268:874.

222. Centers for Disease Control and Prevention. Availability of new rabies vaccine for human use. *MMWR*. 1998;47:12,19.

223. Pappaioanou M, Fishbein DB, Dreesen DW, et al. Antibody response to preexposure human diploid-cell rabies vaccines given concurrently with chloroquine. *N Engl J Med*. 1986;314:280–284.

224. Siwasontiwat D, Lumlertdacha B, Polsuwan C, Hemachudha T, Chutvongse S, Wilde H. Rabies: Is provocation of the biting dog relevant for risk assessment? *Trans R Soc Trop Med Hyg*. 1992;86:443.

225. Karliner JS, Belaval GS. Incidence of adverse reactions following administration of antirabies serum: A study of 562 cases. *JAMA*. 1965:193:359.

226. Wilde H, Chomchey P, Punyaratabandhu P, Phanupak P, Chutivongse S. Purified equine rabies immune globulin: A safe and affordable alternative to human rabies immune globulin. *Bull World Health Organ*. 1989;67:731–736.

227. Centers for Disease Control. Human rabies despite treatment with rabies immune globulin and human diploid cell rabies vaccine—Thailand. *MMWR*. 1987;36:759–760,765.

228. Hatz CF, Bidaux JM, Eichenberger K, Mikulics U, Junghanss T. Circumstances and management of 72 animal bites among long-term residents in the tropics. *Vaccine*. 1995;13:811–815.

229. Schriker RL, Eigelsbach HT, Mitten JQ, Hall WC. Pathogenesis of tularemia in monkeys aerogenically exposed to *Francisella tularensis* 425. *Infect Immun*. 1972;5:734–744.

230. Sanford JP. Landmark perspective: Tularemia. *JAMA.* 1983;250:3225–3226.

231. Capellan J, Fong IW. Tularemia from a cat bite: Case report and review of feline-associated tularemia. *Clin Infect Dis*. 1993;16:472–475.

232. Francis E. Tularemia. *JAMA.* 1925;84:1243–1250.

233. Boyce JM. Recent trends in the epidemiology of tularemia in the United States. *J Infect Dis*. 1975;131:197–199.

234. Syrjala H, Kujala P, Myllyla V, Salminen A. Airborne transmission of tularemia in farmers. *Scand J Infect Dis.* 1985;17:371–375.

235. Centers for Disease Control and Prevention. Cases of selected notifiable diseases: United States, weeks ending December 11, 1993, and December 5, 1992. *MMWR*. 1993;42:955–958.

236. Gill V, Cunha BA. Tularemia pneumonia. *Semin Respir Infect*. 1997;12:61–67.

237. Evans ME, Gregory DW, Schaffner W, McGee ZA. Tularemia: A 30-year experience with 88 cases. *Medicine (Baltimore).* 1985;64:251–269.

238. Schmid GP, Catino D, Suffin SC, Martone WJ, Kaufmann AF. Granulomatous pleuritis caused by *Francisella tularensis*: Possible confusion with tuberculous pleuritis. *Am Rev Respir Dis*. 1983;128:314–316.

239. Lovell VM, Cho CT, Lindsey NJ, Nelson PL. *Francisella tularensis* meningitis: A rare clinical entity. *J Infect Dis.* 1986;154:916–918.

240. Stewart SJ. *Francisella.* In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. *Manual of Clinical Microbiology*. Washington, DC: ASM Press; 1995: 545–548.

241. Provenza JM, Klotz SA, Penn RL. Isolation of *Francisella tularensis* from blood. *J Clin Microbiol*. 1986;24:453–455.

242. Fulop M, Leslie D, Titball R. A rapid, highly sensitive method for the detection of *Francisella tularensis* in clinical samples using the polymerase chain reaction. *Am J Trop Med Hyg.* 1996;54:364–366.

243. Sjostedt A, Eriksson U, Berglund L, Tarnvik A. Detecton of *Francisella tularensis* in ulcers of patients with tularemia by PCR. *J Clin Microbiol*. 1997;35:1045–1048.

244. Enderlin G, Morales L, Jacobs RF, Cross JT. Streptomycin and alternative agents for the treatment of tularemia: Review of the literature. *Clin Infect Dis*. 1994;19:42–47.

245. Penn RL. *Francisella tularensis* (tularemia). In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases*. 4th ed. New York: Churchill Livingstone; 1995: 2060–2068.

246. Mason WL, Eigelsbach HT, Little SF, Bates JH. Treatment of tularemia, including pulmonary tularemia, with gentamicin. *Am Rev Respir Dis.* 1980;121:39–45.

247. Cross JT, Schutze GE, Jacobs RF. Treatment of tularemia with gentamicin in pediatric patients. *Pediatr Infect Dis J*. 1995;14:151–152.

248. Risi GF, Pombo DJ. Relapse of tularemia after aminoglycoside therapy: Case report and discussion of therapeutic options. *Clin Infect Dis*. 1995;20:174–175.

249. Syrjala H, Schildt R, Raisainen S. In vitro susceptibility of *Francisella tularensis* to fluoroquinolones and treatment of tularemia with norfloxacin and ciprofloxacin. *Eur J Clin Microbiol Infect Dis*. 1991;10:68–70.

250. Baker CN, Hollis DG, Thornsberry C. Antimicrobial susceptibility testing of *Francisella tularensis* with a modified Mueller-Hinton broth. *J Clin Microbiol*. 1985;22:212–215.

251. Cross JT, Jacobs RF. Tularemia: Treatment failures with outpatient use of ceftriaxone. *Clin Infect Dis*. 1993; 17:976–980.

252. Hill B, Sandstrom G, Schroder S, Franzen C, Tarnvik A. A case of tularemia meningitis in Sweden. *Scand J Infect Dis*. 1990;22:95–99.

253. Lee HC, Horowitz E, Linder W. Treatment of tularemia with imipenem/cilastatin sodium. *South Med J.* 1991;84:1277–1278.

254. Saslow S, Eigelsbach HT, Prior JA, Wilson HE, Carhrt S. Tularemia vaccine study. *Arch Intern Med*. 1961;107:134–146.

255. Franz DR, Jahrling PB, Friedlander AM, et al. Clinical recognition and management of patients exposed to biological warfare agents. *JAMA*. 1997;278:399–411.

256. Abramova FA, Grinberg LM, Yampolskaya OV, Walker DH. Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979. *Proc Natl Acad Sci USA.* 1993;90:2291–2294.

257. Walker DH, Yampolska O, Grinberg LM. Death at Sverdlovsk: What have we learned? *Am J Pathol.* 1994;144: 1135-1141.

258. Davies JC. A major epidemic of anthrax in Zimbabwe, part II: Distribution of cutaneous lesions. *Cent Afr J Med.* 1983;29:8–12.

259. Brachman PS. Anthrax. In: Evans AS, Brachman PS, eds. *Bacterial Infections of Humans: Epidemiology and Control.* 2nd ed. New York: Plenum Medical Book Co; 1991: 75–86.

260. Christie AB. *Infectious Diseases: Epidemiology and Clinical Practice*. 2 Vols. New York: Churchill Livingston; 1987: 992.

261. Nunanusont D, Limpakarnjanarat K, Foy HM. Outbreak of anthrax in Thailand. *Ann Trop Med Parasitol*. 1990;84: 507–512.

262. Fujikura T. Current occurrence of anthrax in man and animals. *Salisbury Med Bull Suppl.* 1990;68:1.

263. Wilson GS, Miles AA. *Topley and Wilson's Principles of Bacteriology and Immunity.* Vol 2. Baltimore: Williams & Wilkins; 1955: 1940.

264. Brachman PS. Inhalation anthrax. *Ann N Y Acad Sci.* 1980:353:83–93.

265. Davies JC. A major epidemic of anthrax in Zimbabwe. *Cent Afr J Med*. 1982;28:291–298.

266. Davies JC. A major epidemic of anthrax in Zimbabwe: The experience at the Beatrice Road Infectious Diseases Hospital, Harare. *Cent Afr J Med*. 1985;31:176-180.

267. Ivins BE, Ezzell JW Jr, Jemski J, Hedlund KW, Ristroph JD, Leppla SH. Immunization studies with attenuated strains of *Bacillus anthracis. Infect Immun.* 1986;52:454-458.

268. Mikesell P, Ivins BE, Ristroph JD, Dreier TM. Evidence for plasmid-mediated toxin production in *Bacillus anthracis. Infect Immun.* 1983;39:371–376.

269. Cataldi A, Labruyere E, Mock M. Construction and characterization of a protective antigen-deficient *Bacillus anthracis* strain. *Mol Microbiol.* 1990;4:1111-1117.

270. Ross JM. The pathogenesis of anthrax following the administration of spores by the respiratory route. *J Pathol Bacteriol.* 1957;73:485–494.

271. Dutz W, Kohout E. Anthrax. *Pathol Annu.* 1971;6:209–248.

272. Turnbull PC, Leppla SH, Broster MG, Quinn CP, Melling J. Antibodies to anthrax toxin in humans and guinea pigs and their relevance to protective immunity. *Med Microbiol Immunol (Berl).* 1988;177:293–303.

273. Buchanan TM, Feeley JC, Hayes PS, Brachman PS. Anthrax indirect microhemagglutination test. *J Immunol*. 1971;107:1631–1636.

274. Sirisanthana T, Nelson KE, Ezell J, Abshire TG. Serological studies of patients with cutaneous and oral-oropharyngeal anthrax from northern Thailand. *Am J Trop Med Hyg.* 1988;39:575–581.

275. Harrison LH, Ezzell JW Jr, Abshire TG, Kidd S, Kaufmann AF. Evaluation of serologic tests for diagnosis of anthrax after an outbreak of cutaneous anthrax in Paraguay. *J Infect Dis.* 1989;160:706–710.

276. Friedlander AM, Welkos SL, Pitt ML, et al. Postexposure prophylaxis against experimental inhalation anthrax. *J Infect Dis*. 1993;167:1239–1243.

277. Doganay M, Aydin N. Antimicrobial susceptibility of *Bacillus anthracis*. *Scand J Infect Dis*. 1991;23:333–335.

278. Lightfoot NF, Scott RJ, Turnbull PC. Antimicrobial susceptibility of *Bacillus anthracis*. *Salisbury Med Bull Suppl*. 1990;68:95.

279. Turnbull PC, Broster MG, Carman JA, Manchee RJ, Melling J. Development of antibodies to protective antigen and lethal factor components of anthrax toxin in humans and guinea pigs and their relevance to protective immunity. *Infect Immun.* 1986;52:356–363.

280. Pittman PR. Lieutenant Colonel, Medical Corps, US Army. Chief, Clinical Investigation, Medical Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, January 1994.

281. Anthrax vaccine adsorbed. Lansing, Mich: Michigan Dept of Public Health; 1987. Package insert.

282. Brachman PS, Gold H, Plotkin SA, Fekety FR, Werrin M, Ingraham NR. Field evaluation of a human anthrax vaccine. *Am J Public Health.* 1962;52:632–645.

283. Cleghorn G. *Observations of the Epidemical Diseases of Minorca (From the Years 1744 to 1749).* London, England; 1751. As cited in: Evans AC. Comments on the early history of human brucellosis. Larson CH, Soule MH, eds. *Brucellosis.* Baltimore, Md: Waverly Press; 1950: 1–8.

284. Bruce D. Note on the discovery of a micro-organism in Malta fever. *Practitioner* (*London*). 1887;39:161–170. As cited in: Evans AC. Comments on the early history of human brucellosis. Larson CH, Soule MH, eds. *Brucellosis.* Baltimore, Md: Waverly Press; 1950: 1–8.

285. Hughes ML. *Mediterranean, Malta or Undulant Fever.* London, England: Macmillan and Co; 1897. As cited in: Evans AC. Comments on the early history of human brucellosis. Larson CH, Soule MH, eds. *Brucellosis.* Baltimore, Md: Waverly Press; 1950: 1–8.

286. Bang B. Die Aetiologie des seuchenhaften ("infectiösen") Verwerfens. *Z Thiermed* (*Jena*). 1897;1:241–278. As cited in: Evans AC. Comments on the early history of human brucellosis. Larson CH, Soule MH, eds. *Brucellosis.* Baltimore, Md: Waverly Press; 1950: 1–8.

287. Meador VP, Hagemoser WA, Deyoe BL. Histopathologic findings in *Brucella abortus*–infected, pregnant goats. *Am J Vet Res.* 1988;49(2):274–280.

288. Nicoletti P. The epidemiology of bovine brucellosis. *Adv Vet Sci Comp Med.* 1980;24:69–98.

289. Grimont F, Verger JM, Cornelis P, et al. Molecular typing of *Brucella* with cloned DNA probes. *Res Microbiol.* 1992;143:55–65.

290. Jahans KL, Foster G, Broughton ES. The characterisation of Brucella strains isolated from marine mammals. *Vet Microbiol*. 1997;57:373–382.

291. Young EJ. Brucellosis: Current epidemiology, diagnosis, and management. *Curr Clin Top Infect Dis*. 1995;15:115–128.

292. Radwan AI, Bekairi SI, Prasad PV. Serological and bacteriological study of brucellosis in camels in central Saudi Arabia. *Rev Sci Tech*. 1992;11:837–844.

293. Yagoub IA, Mohamed AA, Salim MO. Serological survey of *Brucella abortus* antibody prevalence in the one-humped camel (*Camelus dromedarius*) from eastern Sudan. *Rev Elev Med Vet Pays Trop*. 1990;43:167–171.

294. Chomel BB, DeBess EE, Mangiamele DM, et al. Changing trends in the epidemiology of human brucellosis in California from 1973 to 1992: A shift toward foodborne transmission. *J Infect Dis*. 1994;170:1216–1223.

295. Mousa AR, Elhag KM, Khogali M, Marafie AA. The nature of human brucellosis in Kuwait: Study of 379 cases. *Rev Infect Dis.* 1988;10:211–217.

296. Robson JM, Harrison MW, Wood RN, Tilse MH, McKay AB, Brodribb TR. Brucellosis: Re-emergence and changing epidemiology in Queensland. *Med J Aust*. 1993;159:153–158.

297. Dajani YF, Masoud AA, Barakat HF. Epidemiology and diagnosis of human brucellosis in Jordan. *J Trop Med Hyg.* 1989;92:209–214.

298. Mousa AM, Elhag KM, Khogali M, Sugathan TN. Brucellosis in Kuwait: A clinico-epidemiological study. *Trans R Soc Trop Med Hyg.* 1987;81:1020–1021.

299. Buchanan TM, Hendricks SL, Patton CM, Feldman RA. Brucellosis in the United States, 1960–1972: An abattoir-associated disease, III: Epidemiology and evidence for acquired immunity. *Medicine (Baltimore).* 1974;53:427–439.

300. Ackermann MR, Cheville NF, Deyoe BL. Bovine ileal dome lymphoepithelial cells: Endocytosis and transport of *Brucella abortus* strain 19. *Vet Pathol.* 1988;25:28–35.

301. Elsbach P. Degradation of microorganisms by phagocytic cells. *Rev Infect Dis.* 1980;2:106–128.

302. Braude AI. Studies in the pathology and pathogenesis of experimental brucellosis, II: The formation of the hepatic granulomas and its evolution. *J Infect Dis.* 1951;89:87–94.

303. Young EJ. An overview of human brucellosis. *Clin lnfect Dis.* 1995;21:283–289.

304. Gotuzzo E, Alarcon GS, Bocanegra TS, et al. Articular involvement in human brucellosis: A retrospective analysis of 304 cases. *Semin Arthritis Rheum.* 1982;12:245–255.

305. Mousa AR, Muhtaseb SA, Almudallal DS, Khodeir SM, Marafie AA. Osteoarticular complications of brucellosis: A study of 169 cases. *Rev Infect Dis.* 1987;9:531–543.

306. al Shahed MS, Sharif HS, Haddad MC, Aabed MY, Sammak BM, Mutairi MA. Imaging features of musculoskeletal brucellosis. *Radiographics*. 1994;14:333–348.

307. Rotes-Querol J. Osteo-articular sites of brucellosis. *Ann Rheum Dis.* 1957;16:63–68.

308. Ibrahim AI, Awad R, Shetty SD, Saad M, Bilal NE. Genito-urinary complications of brucellosis. Br J Urol. 1988;61:294–298.

309. Lubani MM, Dudin KI, Sharda DC, et al. Neonatal brucellosis. *Eur J Pediatr.* 1988;147:520–522.

310. Buchanan TM, Faber LC, Feldman RA. Brucellosis in the United States, 1960–1972: An abattoir-associated disease, I: Clinical features and therapy. *Medicine (Baltimore).* 1974;53:403–413.

311. Peery TM, Belter LF. Brucellosis and heart disease, II: Fatal brucellosis. *Am J Pathol.* 1960;36:673–697.

312. Young EJ. Serologic diagnosis of human brucellosis: Analysis of 214 cases by agglutination tests and review of the literature. *Rev Infect Dis*. 1991;13:359–372.

313. Gotuzzo E, Carrillo C, Guerra J, Llosa L. An evaluation of diagnostic methods for brucellosis—the value of bone marrow culture. *J Infect Dis*. 1986:153:122–125.

314. Hall WH. Modern chemotherapy for brucellosis in humans. *Rev Infect Dis.* 1990;12:1060–1099.

315. Luzzi GA, Brindle R, Sockett PN, Solera J, Klenerman P, Warrell DA. Brucellosis: Imported and laboratory-acquired cases, and an overview of treatment trials. *Trans R Soc Trop Med Hyg.* 1993;87:138–141.

316. Chan R, Hardiman RP. Endocarditis caused by *Brucella melitensis. Med J Aust.* 1993;158:631–632.

317. *Joint FAO/WHO Expert Committee on Brucellosis, Sixth Report*. Geneva: FAO/WHO; 1986. WHO Technical Report Series 740.