Chapter 25

BRUCELLOSIS

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INTRODUCTION

Brucellosis is a zoonotic infection of domesticated and wild animals, caused by organisms of the genus *Brucella*. Humans become infected by ingestion of animal food products, direct contact with infected animals, or inhalation of infectious aerosols.

Brucellosis in humans has a strong association with military medicine.[1] In 1751, Cleghorn, a British army surgeon stationed on the Mediterranean island of Minorca, described cases of chronic, relapsing febrile illness and cited Hippocrates's description of a similar disease more than 2,000 years earlier.[2] Three additional British army surgeons working on the island of Malta during the 1800s were responsible for important observations of the disease. J. A. Marston described clinical characteristics of his own infection in 1861.[3] In 1887, David Bruce, for whom the genus *Brucella* is named, isolated the causative organism from the spleens of five fatal cases and placed it within the genus *Micrococcus*.[4] Ten years later, M. L. Hughes, who had coined the name "undulant fever," published a monograph that detailed clinical and pathological findings in 844 patients.[5]

In that same year, B. Bang, a Danish investigator, identified an organism, which he called the "Bacillus of abortion," in placentas and fetuses of cattle suffering from contagious abortion.[6] In 1917, A. C. Evans recognized that Bang's organism was identical to that described by Bruce as the causative agent of human brucellosis. The organism infects mainly cattle, sheep, goats, and other ruminants, in which it causes abortion, fetal death, and genital infection.[7,8] 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. 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. Indeed, the United States began development of *B suis* as a biological weapon in 1942. The agent was formulated to maintain long-term viability, placed into bombs, and tested in field trials during 1944–1945 using animal targets. By 1967, the United States terminated its offensive program for development and deployment of *Brucella* as a biological weapon. Although the munitions developed were never used in combat, the studies reinforced the concern that *Brucella* organisms might be used against U.S. troops as a biological warfare agent.[9]

THE INFECTIOUS AGENT

Brucellae are small, nonmotile, nonsporulating, nontoxigenic, nonfermenting, aerobic, Gram-negative coccobacilli that may, based on DNA homology, represent a single species.[10] Conventionally, however, they are classified into six species, each comprising several biovars. Each species has a characteristic, but not an absolute, predilection to infect certain animal species (Table 25-1). Only *Brucella melitensis*, *B suis*, *B abortus*, and *B canis* cause disease in man. Infection of humans with *B ovis* and *B neotomae* has not been described.

Brucellae grow best on trypticase, soy-based, or other enriched media with a typical doubling time of 2 hours. Most biovars of *B abortus* require incubation in an atmosphere of 5% to 10% carbon dioxide for growth. Brucellae may produce urease, oxidize nitrite to nitrate, and are oxidase and catalase positive. Species and biovars are differentiated by their carbon dioxide requirements; ability to use glutamic acid, ornithine, lysine, and ribose; hydrogen sulfide production; growth in the presence of thionine or basic fuchsin dyes; agglutination by antisera directed against certain lipopolysaccharide epitopes; and by susceptibility to lysis by bacte-

TABLE 25-1

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

riophage. Recently, analysis of fragment lengths of deoxyribonucleic acid (DNA) cut by various restriction enzymes has also been used to differentiate brucellae groupings.[10]

The lipopolysaccharide (LPS) component of the outer cell membranes of brucellae is quite different—both structurally and functionally—from that of other Gram-negative organisms.[11,12] The lipid A portion of a *Brucella* organism LPS contains fatty acids 16 carbons long, and lacks the 14-carbon myristic acid typical of lipid A of Enterobacteriaceae. This unique structural feature may underlie the remarkably reduced pyrogenicity (less than 1/100th) of *Brucella* LPS, compared with the pyrogenicity of *Escherichia coli* LPS.[13] In addition, the O-polysaccharide portion of LPS from smooth organisms contains an unusual sugar, 4,6-dideoxy-4-formamido-alpha-D-mannopyranoside, which is expressed either as a homopolymer of alpha-1,2-linked sugars (A type), or as 3 alpha-1,2 and 2 alpha-1,3-linked sugars (M type). These variations in O-polysaccharide linkages lead to specific, taxonomically useful differences in immunoreactivity between A and M sugar types.[14]

THE DISEASE

Epidemiology

Animals may transmit *Brucella* organisms during septic abortion, at the time of slaughter, and in their milk. 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, and cattle, and to practices that allow exposure of humans to potentially infected animals or their products. In the United States, where most states are free of infected animals and where dairy products are routinely pasteurized, illness occurs primarily in individuals such as veterinarians, shepherds, cattlemen, and slaughterhouse workers who have occupational exposure to infected animals. In many other countries, humans more commonly acquire infection by ingestion of unpasteurized dairy products, especially cheese.

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 have camped in the desert during the spring lambing season.[15] In Australia, an outbreak of *B suis* infection was noted in hunters of infected feral pigs.[16] *B canis,* a naturally rough strain that typically causes genital infection in dogs, can rarely infect man.

Brucellae are also highly infectious in laboratory settings; numerous laboratory workers who culture the organism become infected. 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 other regions such as the Middle East; countries bordering the Mediterranean Sea; and China, India, Mexico, and Peru; for example, 33 cases per 100,000 population in Jordan (1987) and 88 cases per 100,000 population in Kuwait (1985), respectively.[17,18]

Pathogenesis

Brucellae can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, and the gastrointestinal tract.[19] In the gastrointestinal tract, the organisms are phagocytosed by lymphoepithelial cells of gut-associated lymphoid tissue, from which they gain access to the submucosa.[20] Organisms are rapidly ingested by polymorphonuclear leukocytes, which generally fail to kill them,[21,22] and are also phagocytosed by macrophages (Figure 25-1). Bacteria transported in macrophages, which traffic to lymphoid tissue draining the infection site, may eventually localize

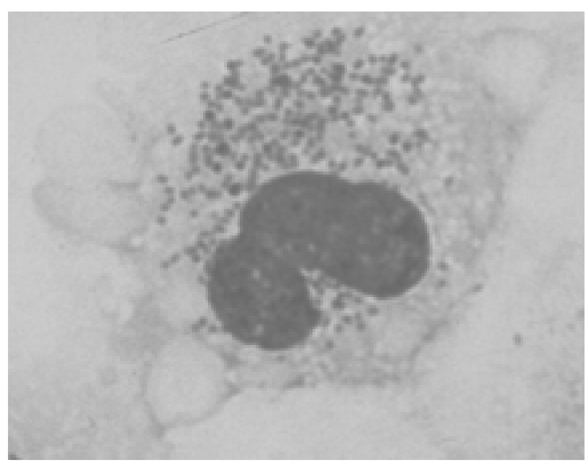

Fig. 25-1. Cultured human monocyte-derived macrophage infected with *Brucella melitensis*. The bacteria, which replicate in phagolysosomes, have a coccobacillary appearance (eosin Y–methylene blue–azure A, original magnification x 1,000). Photograph: Courtesy of Robert Crawford, Ph.D., Senior Scientist, American Registry of Pathology, Washington, DC.

in lymph nodes, liver, spleen, mammary gland, joints, kidneys, and bone marrow.

In macrophages, brucellae may inhibit fusion of phagosomes and lysosomes, and replicate in the phagosome.[23] If unchecked by macrophage microbicidal mechanisms, the bacteria destroy their host cells and infect additional cells. Brucellae can also replicate extracellularly in host tissues. Histopathologically, the host cellular response may range from abscess formation to lymphocytic infiltration to granuloma formation with caseous necrosis.

Studies in experimental models have provided important insights into host defenses that eventually control infection with *Brucella* organisms. Serum complement effectively lyses some rough strains (ie, those that lack O-polysaccharide side chains on their LPS), but has little effect on smooth strains (ie, bacteria with a long O-polysaccharide side chain); *B melitensis* may be less susceptible than *B abortus* to complement-mediated killing.[24,25] Administration of antibody to mice prior to challenge with rough or smooth strains of brucellae reduces the number of organisms that appear in liver and spleen. This effect is due mainly to antibodies directed against LPS, with little or no contribution of antibody directed against other cellular components.[26]

Reduction in intensity of infection in mice can be transferred from immune to nonimmune animals by both cluster of differentiation 4+ (CD4+) and CD8+ T cells[27] or by immunoglobulin (Ig) fractions of serum. Administration of antibody to interferon gamma (IFN-γ) worsens experimental infection.[28] Moreover, macrophages treated with IFN-γ in vitro inhibit intracellular bacterial replication.[29] In ruminants, vaccination with killed bacteria provides some protection against challenge, but live vaccines are much more effective.

These observations suggest that brucellae, like other facultative or obligate intramacrophage pathogens, are primarily controlled by macrophages activated to enhanced microbicidal activity by IFN-γ and other cytokines produced by immune T lymphocytes. It is likely that antibody, complement, and macrophage-activating cytokines produced by natural killer (NK) cells play supportive roles in early infection or in controlling growth of extracellular bacteria.

In ruminants, *Brucella* organisms bypass the most effective host defenses by targeting embryonic and trophoblastic tissue. In cells of these tissues, the bacteria grow not only in the phagosome but also in the cytoplasm and the rough endoplasmic reticulum.[30] In the absence of effective intracellular microbicidal mechanisms, these tissues permit exuberant bacterial growth, which leads to fetal death and abortion. In ruminants, the presence in the placenta of erythritol may further enhance growth of brucellae. Products of conception at the time of abortion may contain up to 10^{10} bacteria per gram of tissue.[31] When septic abortion occurs, the intense concentration of bacteria and aerosolization of infected body fluids during parturition often result in infection of other animals and people.

Clinical Manifestations

Clinical manifestations of brucellosis are diverse and the course of the disease is variable.[32] Patients with brucellosis 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 25-2). 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. Symptoms of patients infected by aerosol are indistinguishable from those of patients infected by other routes. Chronically infected patients fre-

TABLE 25-2

SYMPTOMS AND SIGNS OF BRUCELLOSIS

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

Data sources: (1) Mousa AR, Elhag KM, Khogali M, Marafie AA. The nature of human brucellosis in Kuwait: Study of 379 cases. *Rev Infect Dis*. 1988;10(1):211–217. (2) 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(6):403–413. (3) Gotuzzo E, Alarcon GS, Bocanegra TS, et al. Articular involvement in human brucellosis: A retrospective analysis of 304 cases. *Semin Arthritis Rheum*. 1982;12(2):245–255.

quently 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.[33] Although disease manifestations cannot be strictly related to the infecting species, *B melitensis* tends to cause more severe, systemic illness than the other brucellae; *B suis* is more likely to cause localized, suppurative disease.

Infection with *B melitensis* leads to bone or joint disease in about 30% of patients; sacroiliitis develops in 6% to 15%, particularly in young adults.[34–36] Arthritis of large joints occurs with about the same frequency as sacroiliitis. In contrast to septic arthritis caused by pyogenic organisms, 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. In both sacroiliitis and peripheral joint infections, destruction of bone is unusual. Organisms can be cultured from fluid in about 20% of cases; culture of the synovium may increase the yield. 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.[37]

Radiographic findings, similar to those of tuberculous infection, typically include disk space narrowing and epiphysitis, particularly of the anterosuperior quadrant of the vertebrae, and presence of bridging syndesmophytes as repair occurs. Bone scan of spondylitic areas is often negative or only weakly positive. Paravertebral abscess occurs rarely. In contrast with frequent infection of the axial skeleton, osteomyelitis of long bones is rare.[38]

Infection of the genitourinary tract, an important target in ruminant animals, also may lead to signs and symptoms of disease in man.[39,40] 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.[41] 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 may complain of respiratory symptoms, mostly cough, dyspnea, or pleuritic pain, chest X-ray examinations are usually normal.[42] 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.[32]

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.[43] Central nervous system infection usually manifests itself as chronic meningoencephalitis, but subarachnoid hemorrhage and myelitis also occur. A few cases of skin abscesses have been reported.

Diagnosis

A thorough history that elicits details of appropriate exposure (eg, laboratories, animals, animal products, or environmental exposure to locations inhabited by potentially infected animals) is the most important diagnostic tool. Brucellosis should also be strongly considered in differential diagnosis of febrile illness if troops 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. Although a number of serologic techniques have been developed and tested, the tube agglutination test remains the standard method.[44] This test, which measures the ability of serum to agglutinate killed organisms, reflects the presence of anti–O-polysaccharide antibody. 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.[45] 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* because this rough organism does not have O-polysaccharide on its surface. Immunoenzymatic assays (eg, enzyme-linked immunosorbent assays [ELISAs]) have been developed for use with *B canis*, but are not well standardized. ELISAs developed for other brucellae similarly suffer from lack of standardization.

In addition to serologic testing, diagnosis should be pursued by microbiologic culture of blood or body fluid samples. Cultures should be held for at least 2 months, with weekly subcultures 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.[46]

Treatment

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.[47] A 6-week regimen of doxycycline 200 mg/d administered orally, with the addition of streptomycin 1 g/d administered intramuscularly for the first 2 to 3 weeks is effective therapy for adults with most forms of brucellosis.[48] Patients with spondylitis may require longer treatment. A 6-week oral regimen of both rifampin 900 mg/d and doxycycline 200 mg/d is also effective, and should result in nearly 100% response and a relapse rate lower than 10%.[49] Several studies,[48,50,51] however, suggest that treatment with a combination of streptomycin and doxycycline may result in less frequent relapse than treatment with the combination of rifampin and doxycycline. Notable failures have occurred when spondylitis was treated with the latter combination.[50]

Endocarditis may best be treated with rifampin, streptomycin, and doxycycline for 6 weeks; infected valves should be replaced early in therapy.[52] Central nervous system disease responds to a combination of rifampin and trimethoprim/sulfamethoxazole, but may need prolonged therapy. The latter antibiotic combination is also effective for children under 8 years of age.[53] The Joint Food and Agriculture Organization–World Health Organization Expert Committee recommends treatment of pregnant women with rifampin.[49]

Organisms used in a biological attack may be resistant to these first-line antimicrobial agents. Medical officers should make every 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.

PROPHYLAXIS

To prevent brucellosis, animal handlers should wear appropriate protective clothing when working with infected animals. Meat should be well-cooked; milk should be pasteurized. Laboratory workers should culture the organism only with appropriate Biosafety Level 2 or 3 containment (see Chapter 19, The U.S. Biological Warfare and Biological Defense Programs, for a discussion of the biosafety levels that are used at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland).

In the event of a biological attack, the standard gas mask should adequately protect personnel from airborne brucellae, since 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.

SUMMARY

Brucellosis is a zoonosis of large animals, especially cattle, camels, sheep, and goats. Although humans usually acquire *Brucella* organisms by ingestion of contaminated foods (oral route) or slaughter of animals (percutaneous route), the organism is highly infectious by the airborne route; this is the presumed route of infection of the military threat. Laboratory workers commonly become infected when cultures are handled outside a biosafety cabinet. Individuals presumably infected by aerosol have symptoms indistinguishable from patients infected by other routes: fever, chills, and myalgia are most common, occurring in more than 90% of cases.

Since the bacterium disseminates throughout the reticuloendothelial system, it may cause disease in virtually any organ system. Large joints and the axial skeleton are favored targets; arthritis appears in approximately one third of patients. Fatalities occur rarely, usually in association with central nervous system or endocardial infection.

Serologic diagnosis uses an agglutination test that detects antibodies to lipopolysaccharide. This test, however, is not useful to diagnose infection caused by *B canis*, a naturally O-polysaccharide deficient strain. Infection can be most reliably confirmed by culture of blood, bone marrow, or other infected body fluids, but the sensitivity of culture varies widely.

Nearly all patients respond to a 6-week course of oral therapy with a combination of rifampin and doxycycline; fewer than 10% of patients relapse. Six weeks of doxycycline with addition of streptomycin for the first 3 weeks is also effective therapy. No vaccine is available for humans.

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