Chapter 7

BRUCELLOSIS

BRET K. PURCELL, PhD, MD*; R. MARTIN ROOP II, PhD†; ARTHUR M. FRIEDLANDER, MD‡; and DAVID L. HOOVER, MD§

*Colonel, Medical Corps, US Army; Deputy Chief, Bacteriology Division, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702

†Professor, Microbiology and Immunology, Brody School of Medicine, East Carolina University, 600 Moye Boulevard, Room 118, Biotechnology Building, Greenville, North Carolina 27834

‡Colonel (Retired), Medical Corps, US Army; Senior Scientist, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702

§Colonel (Retired), Medical Corps, US Army; Senior Scientific Advisor, Clinical Research Management, 1265 Ridge Road, Hinckley, Ohio 44233; formerly Medical Director, Dynport Vaccine Company LLC, a CSC Company, 64 Thomas Johnson Drive, Frederick, Maryland, and Scientific Coordinator, Brucella Program, Department of Bacterial Diseases, Walter Reed Army Institute of Research, Silver Spring, Maryland

INTRODUCTION

Brucellosis is a zoonotic infection of domesticated and wild animals caused by bacteria of the genus *Brucella*. Humans become infected by ingesting animal food products directly contacting infected animals or inhaling infectious aerosols either inadvertently or by intentional means through bioterrorism. Brucellosis is currently considered to be one of the world's leading zoonoses.[1]

Military medicine has played a large role in discovering and defining brucellosis in humans.[2] In 1751 G Cleghorn, a British army surgeon stationed on the Mediterranean island of Minorca, described cases of chronic, relapsing febrile illness and cited Hippocrates' description of a similar disease more than 2,000 years earlier.[3] Three additional British army surgeons working on the island of Malta during the 1800s were responsible for important descriptions of the disease. JA Marston described clinical characteristics of his own infection in 1861.[4] In 1887 David Bruce, for whom the genus *Brucella* is named, isolated the causative organism from the spleens of five fatal cases and placed this bacterium within the genus *Micrococcus*.[5] Ten years later, ML Hughes, who had coined the name "undulant fever," published a monograph that detailed clinical and pathological findings in 844 patients.[6]

In that same year, Bernhard Bang, a Danish investigator, identified a bacterium, which he called the "bacillus of abortion," in placentas and fetuses of cattle suffering from contagious abortion.[7] In 1917 Alice C Evans recognized that Bang's organism was identical to that described by Bruce as the causative agent of human brucellosis. The bacterium infects mainly cattle, sheep, goats, and other ruminants, in which it causes abortion, fetal death, and genital infections.[8,9] 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. With the worldwide distribution of brucellosis, international travel and military deployments increase the risk of exposure to this disease.[10] In particular, the deployment of US military and coalition forces into Iraq, Afghanistan, Libya, and other Middle Eastern countries has posed particular risk from environmental and food source animals.[11–13] The disease frequently becomes chronic and may relapse, even with treatment. Laboratory-acquired infections have been documented as awareness of this disease has increased,[14–17] and as biodefense research expands in the academic and biotechnology industries, laboratory accidents may unfortunately become more frequent and significant.[18] Strict adherence to proper engineering controls, good laboratory and microbiology techniques, and the use of personal protective equipment significantly reduces the incidence of laboratory-acquired infections.[19,20] No vaccine is available that can safely be used to prevent laboratory-acquired brucellosis.

The ease of transmission by aerosol underscores the concern that *Brucella* might be used as a biological warfare agent. The United States began developing *Brucella 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 with animal targets. By 1969 the United States terminated its offensive program for development and deployment of *Brucella* as a weapon and destroyed all of its biological weapon munitions. Although the munitions developed were never used in combat, studies conducted under the offensive program reinforced the concern that *Brucella* might be used against US troops as a biological warfare agent.[21] Even before the post–September 11, 2001 attacks, civilian populations were recognized as potential high yield targets. In 1997 a model of aerosol attack with *Brucella* on an urban population estimated an economic impact of $477.7 million per 100,000 persons exposed.[22] *Brucella* represents one of many biological agents of zoonotic disease that could pose threats as terrorist weapons against human or agricultural targets.[23] Several reviews that focus on the potential use of the brucellae as agents of bioterrorism or biowarfare have been published.[24–26]

THE INFECTIOUS AGENT

Brucellae are small, nonmotile, nonsporulating, nontoxigenic, nonfermenting, facultatively intracellular, gram-negative bacteria that represent a single "genospecies" from a phylogenetic perspective.[27] However, for epidemiologic purposes and ease and accuracy of communication, *Brucella* strains are classified as separate "nomenspecies" based on readily distinguished phenotypic characteristics that include host specificity.[28] There are presently 10 of these recognized "nomenspecies" (Table 7-1). *Brucella melitensis*, *B suis*, *Brucella abortus*, and *Brucella canis* are the classic causative agents of disease in humans. Human infections with the marine mammal strain *Brucella ceti*[29,30] and a strain (*Brucella inopinata*) of unknown origin[31–33] have also recently been described, but prevalence of such infections is unclear.

Human infections with *Brucella ovis*, *Brucella neotomae*, *Brucella pinnipedialis*, and *Brucella microti* have not been described. Brucellae grow best on trypticase soy-based media or other enriched media with a typical doubling time of 2 hours in liquid culture. Although *B melitensis* bacteremia can be detected within 1 week by using automated culture systems,[34] cultures should be maintained for at least 4 weeks, with weekly subculture, for diagnostic purposes. Most biovars of *B abortus* require incubation in an atmosphere of 5% to 10% carbon dioxide for growth. Brucellae may produce urease and may oxidize nitrite to nitrate; they 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 (LPS) epitopes; and by susceptibility to lysis by bacteriophage. *Brucella* can grow on blood agar plates and does not require X or V factors for growth.

Serological agglutinating antibodies have been used worldwide as the definitive diagnostic test for brucellosis infection. The standard tube agglutination test is the modified *Brucella* microagglutination test.[35] This test uses direct agglutination of bacterial antigens by specific antibodies of the immunoglobulin (Ig), IgG, and IgA classes. Acute infection is indicated by the presence of antigen-specific IgM antibodies, but these antibodies decline rapidly within weeks of the onset of infection. Chronic or relapsing disease is characterized by elevated or increasing levels of IgG and IgA classes.[36] A four-fold or greater rise in *Brucella* agglutination titers demonstrated between acute and convalescent serum specimens collected at least 2 weeks apart in conjunction with clinically compatible illness is considered a confirmatory test for brucellosis infection. Additional confirmatory tests for infection include the isolation of *Brucella* from clinical specimens or the identification of *Brucella* bacteria in tissue cultures by specific immunohistochemical staining.[37] Although highly sensitive and specific, occasionally false positive tests and cross reactions do occur using *Brucella* antibody tests. The cell wall lipopolysaccharide of the *Brucella* organism is antigenically similar to other gram-negative bacteria. Antibodies to *Moraxella phenylpyruvica*, *Yersinia enterocolitica*, *Escherichia coli* O157, and specific *Salmonella* strains are known to provide false positive reactions.[38,39]

Analysis of fragment lengths of DNA cut by various restriction enzymes has also been used to differentiate brucellae groupings.[33] Single nucleotide polymorphism analyses using real time polymerase chain reaction (PCR) have been used to rapidly identify *Brucella* isolates to the species level.[40] Both the multiple loci variable number of tandem repeat analysis and the Bruce-ladder multiplex PCR assays have been recently used to type a variety of marine *Brucella* isolates and differentiate by biovar typing of *B suis* and *B canis*.[41,42] Recent studies using proteomics, complete genomic sequencing, and multi-locus analysis of variable number tandem repeats have rapidly expanded the information on virulence determinants, identification of pathogenicity islands, and evolutionary relatedness among the *Brucella* species.[43–47] Microarrays have now been developed to phylogenetically classify and forensically identify unknown pathogens as well as genotype *Brucella* species.[48,49] The LPS component of the outer cell membranes of the brucellae is different—both structurally and functionally—from that of other gram-negative organisms.[31,32] For instance, in addition to its capacity to provide resistance to complement and potentially serve as a ligand for binding to host cells, experimental evidence indicates that the O-chain of LPS of "smooth" (fully expressed O-chain versus "rough" strains with substantially reduced or absent O-chain) *Brucella* strains directly interferes with the capacity of host macrophages to process antigens via the major histocompatibility complex class II pathway[50] and influences in the intracellular trafficking of the *Brucella* containing vacuoles in host macrophages preventing their fusion with lysosomes.[51] The chemical compositions of the

TABLE 7-1

TYPICAL HOST SPECIFICITY OF *BRUCELLA* SPECIES

Brucella Species	Animal Host	Human Pathogenicity
B melitensis	Sheep, goats	High
B suis	Swine	High
B abortus	Cattle, bison	Intermediate
B canis	Dogs	Low
B ceti	Dolphins, porpoises	Unknown*
B inopinata	Humans	Unknown*
B pinnipedialis	Seals	Not reported
B ovis	Sheep	Not reported
B neotomae	Rodents	Not reported
B microti	Rodents	Not reported

**B ceti* and *B inopinata* strains have been isolated from human disease, but the importance of these strains as human pathogens is presently unknown.

lipid A and core moieties of the *Brucella* LPS are also distinct from those found in the enteric and many other gram-negative bacteria, and these differences greatly reduce the "recognition" of the brucellae by the Toll-like receptors on host macrophages, which allows these bacteria to induce a dampened inflammatory response and use a "stealthy" approach for establishing infections.[52]

One of the unique features of *Brucella* strains is that unlike most pathogenic bacteria, these bacteria produce relatively few "classical" virulence factors.[53] Probably the most widely studied virulence determinants in the *Brucella* strains are the LPS and the Type IV secretion system.[54] The brucellae use this transport system to secrete effector proteins into the cytoplasm of infected mammalian cells. These effector proteins interfere with the activity of the host cell proteins that control the intracellular membrane trafficking. The net result is that the phagosomes within which the brucellae reside in host macrophages avoid extensive interactions with lysosomes and eventually fuse with the host cell endoplasmic reticulum. The formation of these so-called replicative *Brucella* containing vacuoles (or rBCVs) is essential for the virulence of the naturally occurring smooth *Brucella* strains such as *B melitensis*, *B suis*, and *B abortus*. The capacity of *Brucella* strains to survive and replicate in host macrophages is critical for their virulence. Accordingly, in addition to gene products such as these that overtly interfere with biology of the host cell, the brucellae also produce numerous proteins that allow them to successfully resist the environmental stresses they encounter during their intracellular residence in host macrophages. These stresses include exposure to acidic pH, reactive oxygen species and antimicrobial peptides, and nutrient deprivation.[55]

EPIDEMIOLOGY

Animals may transmit *Brucella* organisms during septic abortion, at the time of slaughter, and in their milk. For infected patients, no conclusive evidence indicates that brucellosis can be transmitted from person to person. The incidence of human disease is thus closely tied to the prevalence of infection in sheep, goats, pigs, and cattle, and to practices that allow exposure of humans to potentially infected animals or their products. In the United States, where all 50 states are considered to be "free" of bovine brucellosis and 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 ingesting unpasteurized dairy products, especially cheese.

Less obvious exposures can also lead to infection. In the United States and Australia, for example, hunters have acquired *B suis* infection from feral swine.[56,57] It was also not uncommon for veterinarians to develop brucellosis after accidental exposure to *B abortus* Strain 19 in the United States when this strain was being used as a live vaccine in cattle.[58] Another bovine vaccine strain, Brucella Abortus Vaccine, Strain RB-51, has been used to eradicate brucellosis from the US livestock herds.[59] Accidental human infections with this vaccine cannot be identified using the standard LPS-based diagnostics assay. Brucellae are also highly infectious in laboratory settings; numerous laboratory workers who culture the organism become infected. Disease with a relatively high proportion of respiratory complaints has also been reported in individuals who have camped in the desert during the spring lambing season.[46] *B canis*, a naturally rough strain that typically causes genital infection in dogs, can also infect humans.[60] Although *B canis* infections were once considered rare, it has become apparent that in some areas of the world these infections were probably unrecognized.[60] In the United States the total number of cases of brucellosis remains very low (0.02 to 0.09 cases per 100,000 person-years).[61,62] A major contributing factor to this low incidence of brucellosis can be attributed to a national eradication campaign to eliminate brucellosis in domestic cattle herds. When implemented the human incidence of disease dropped from a high of 6,321 cases in 1947 to 136 cases in 2001 (0.48 cases per million). These few cases are primarily caused by infections with *B melitensis* and now most human cases are distributed in Hispanic populations residing on either side of the Mexico border.[61] The endemic regions located in Latin America, Europe, Africa, and Asia account for most of the human cases of brucellosis with the highest incidences occurring in the former Yugoslav Republic of Macedonia, Algeria, Peru, Iraq, Iran, Syria, Turkey, Kyrgyzstan, and Mongolia.[61,62] With the improvement of diagnostic methods, ever increasing international tourism, and establishment of new eradication programs, the epidemiology of brucellosis will continue to shift and evolve requiring constant vigilance for new foci of disease. Unfortunately, with the rapidly changing political, international, and financial environments, worldwide eradication of this zoonotic disease will be extremely difficult.

PATHOGENESIS

Brucellae can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, and the respiratory tract, and, unlike enteric pathogens such as *Salmonella* or *Shigella* species that infect the lower gastrointestinal tract, the most likely site of bacterial entry is the mucosae of the upper gastrointestinal tract.[63,64] Organisms are rapidly ingested by polymorphonuclear leukocytes, which generally fail to kill them,[65,66] and are also phagocytosed by macrophages (Figure 7-1). Bacteria transported in macrophages, which traffic to lymphoid tissue draining the upper gastrointestinal mucosa, may eventually disseminate to lymph nodes, liver, spleen, mammary glands, joints, kidneys, and bone marrow. As noted previously, the brucellae are resistant to the microbicidal activity of macrophages, and it is their capacity to survive and replicate for prolonged periods in these phagocytes that underlies their ability to produce chronic infections.[55] Histopathologically, the host cellular response may range from abscess formation to lymphocytic infiltration to granuloma formation with caseous necrosis.[58]

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.[67,68] Administration of antibody to mice before challenge with rough or smooth strains of brucellae reduces the number of organisms that appear in liver and spleen. This effect is caused mainly by antibodies directed against LPS, with little or no contribution of antibodies directed against other cellular components.[69]

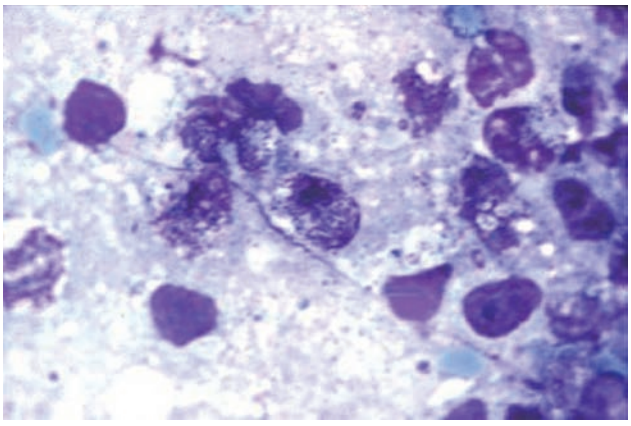

Figure 7-1. Impression tissue smear from a bovine aborted fetus infected with *Brucella abortus*. The bacteria appear as lightly stained, gram-negative cells.
Photograph: Courtesy of John Ezzell, PhD, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

The intensity of an infection in mice can be reduced by transferring from immune to nonimmune animals differentiated $CD4^+$and $CD8^+$ T cells[70] or by the Ig fractions of serum. In particular, the T-cell response to *Brucella* appears to play a key role in the development of immunity and protection against chronic disease.[71,72] Neutralization of *B abortus*-induced host interferon gamma (IFN-γ) during infection in pregnant mice prevents abortion.[73] Moreover, macrophages treated with IFN-γ in vitro inhibit intracellular bacterial replication.[74] Studies in humans support a role for IFN-γ in protection; homozygosity for the IFN-γ +874A allele is associated with about a two-fold increase in incidence of brucellosis.[75] In ruminants, vaccination with live vaccines is required in order to provide protection.[76–78]

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 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.[79] 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 of erythritol in the placenta 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.[80] When septic abortion occurs, the intense concentration of bacteria and aerosolization of infected body fluids during parturition often results in infection of other animals and people.

CLINICAL MANIFESTATIONS

Clinical manifestations of brucellosis are diverse and the course of the disease is variable.[81] Patients with brucellosis may present with an acute, systemic febrile illness; an insidious chronic infection; or a localized inflammatory process. However, in the absence of suspicion for brucellosis, many cases seen in the United States are not diagnosed in the early stage of disease, but they are discovered once a focal complication has developed, such as a joint infection. 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 7-2). 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 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[82]; however, cases of pancytopenia have been noted.[83] In addition, bone marrow hypoplasia, immune thrombocytopenic purpura, and erythema nodosum may occur during brucellosis infections.[84–86] Disease manifestations cannot be strictly related to the infecting species.

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.[87–89] 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.[90] Radiographic findings, similar to those of tuberculous infection, typically include disk space narrowing and epiphysitis, particularly of the antero-superior 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.[91]

Infection of the genitourinary tract, an important target in ruminant animals, also may lead to signs and symptoms of disease in humans.[92–94] Pyelonephritis, cystitis, Bartholin's gland abscess and, in males, epididymo-orchitis, 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.[95] 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 radiograph examinations are usually normal.[96] 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. Serum transaminases are frequently elevated.[97] Biopsy may show well-formed

TABLE 7-2

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;10:211–217. (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;53:403–413. (3) Gotuzzo E, Alarcon GS, Bocanegra TS, et al. Articular involvement in human brucellosis: a retrospective analysis of 304 cases. *Semin Arthritis Rheum.* 1982;12:245–255.

granulomas or nonspecific hepatitis with collections of mononuclear cells.[81] Spontaneous bacterial peritonitis has also been reported.[98,99]

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.[100,101] Central nervous system infection usually manifests itself as chronic meningoencephalitis, but subarachnoid hemorrhage and myelitis also occur. Guillain-Barré syndrome has been associated with acute neurobrucellosis and involvement of spinal roots has been noted on magnetic resonance imaging.[102,103] A few cases of skin abscesses have been reported.

DIAGNOSIS

A thorough history that describes 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. The differential diagnosis for brucellosis is broad and includes noninfectious causes such as vasculitis, sacroiliitis, lumbar disk disorders, thrombotic thrombocytopenic purpura, ankylosing spondylitis, abortion complications, depression/suicide, collagen-vascular disease, erythema nodosum, pediatric chronic fatigue syndrome, and malignancy. The infectious disease differential includes fever of unknown origin, rickettsial diseases, bacterial and viral pneumonia, bronchitis, cat scratch fever, cryptococcosis, acute epididymitis, cystitis in females, gastroenteritis, hepatitis, histoplasmosis, infectious mononucleosis, infective endocarditis, influenza, leptospirosis, malaria, meningitis, osteomyelitis, Epstein-Barr virus infection, spontaneous bacterial peritonitis, tuberculosis, tularemia, typhoid fever, and urinary tract infections in men. Brucellosis should also be strongly considered in differential diagnosis of febrile illness if troops have been exposed to a presumed biological attack. PCR 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 based on clinical history, bacterial isolation from clinical samples, biochemical identification of the organism, and by serology. The Centers for Disease Control and Prevention's clinical description of brucellosis is "an illness characterized by acute or insidious onset of fever, night sweats, undue fatigue, anorexia, weight loss, headache and arthalgia."[104] Cultivation of *Brucella* poses a significant hazard to clinical laboratory personnel.[105–108] Rapid detection of the organism in clinical samples using PCR-enzyme-linked immunosorbent assays (ELISAs) or real-time PCR assays can be used to detect *Brucella* DNA in clinical specimens as well as cultivated bacteria and may eventually prove to be the optimal method for identification of these infections.[109–112] Although PCR may have many advantages, a positive PCR is not proof of viable *Brucella*. Many of the assays used are not standardized and have led to false "outbreak" investigations in the United States and, therefore, these assays require proper validation and standardization by the testing laboratory. Typically, the most reliable and simple PCR identification uses a single pair of primers directed against the 16S-23S rRNA operon containing the IS711 or BCSP31 genes.[111] To identify four of the major *Brucella* species, combination primers directed against the BCSP31, OMP3B, OMP2A, and OMP31 external membrane protein genes are used.[111] Multiplex PCR provides a method to identify all known species of *Brucella*. Despite these technical advances, PCR has sensitivity and specificity limitations that depend heavily on the quality of DNA isolated and potential inhibitors present within the clinical samples.[109–111]

According to the Centers for Disease Control and Prevention case definition for brucellosis, the infection may be diagnosed if any of the following laboratory criteria is met:

- isolation of the organism from a clinical specimen;
- fourfold or greater rise in *Brucella* agglutination titer between acute and convalescent-phase serum obtained greater than 2 weeks apart; and
- demonstration by immunofluorescence of *Brucella* in a clinical specimen.[104,112]

Although several serologic techniques such as the Coombs test have been developed and tested, the tube agglutination test remains the standard method.[113] 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 treating serum with 2-mercaptoethanol or dithiothreitol to dissociate IgM immunoglobulin into monomers makes these antibodies inactive and permits agglutination by immunoglobulin G antibodies that are resistant to dissolution by chemical agents. A titer of 1:160 or higher is considered diagnostic. Most patients already have high titers at the time of clinical presentation, so a fourfold rise in titer may not occur. Immunoglobulin M 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 (essentially immunoglobulin G) antibody titers has been associated with persistent disease or relapse.[114] Serum testing should always include dilution to at least 1:320, as 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. Unfortunately, given the need for trained personnel and standardization of the test reagents and control sera, only some references laboratories, such as the Centers for Disease Control and Prevention in Atlanta, Georgia, and the ARUP National Reference Laboratory in Utah, perform the tube agglutination test. ELISAs have been developed for use with *B canis*, but are not well standardized. Although ELISAs developed for other brucellae similarly suffer from lack of standardization, recent improvements have resulted in greater sensitivity and specificity. ELISAs will probably replace the serum agglutination and Coombs tests, thus allowing for screening and confirmation of brucellosis in one test.[115,116]

In addition to serologic testing, diagnosis should be pursued by microbiologic culture of blood or body fluid samples. If unautomated systems are used, blood cultures should be incubated for 21 days, with blind subculturing every 7 days and terminal subculturing of negative blood cultures. For automated systems, incubation of cultures for 10 days with blind culture at 7 days is recommended.[117] Because it is extremely infectious for laboratory workers, the organism should be subcultured only in a biohazard hood. Appropriate personal protective equipment such as a powered air purifying respirator with hood, gown, and gloves should be used when working with cultures or preparing and manipulating bacteria for studies. 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*. A recent study indicated that BACTEC™ Myco/F lytic medium (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD), pediatric Peds Plus/F or adult Plus Aerobic/F medium in conjunction with BACTEC™ 9240 blood culture system yielded detection rates of 80% and 100%, respectively.[34] Culture of bone marrow may increase the yield and is considered superior to blood.[118] In addition, direct fluorescent antibody tests under development may offer a method of rapidly identifying these organisms in clinical specimens (Figure 7-2). The case classification of "probable" is defined as a clinically compatible case that is epidemiologically linked to a confirmed case or that has supportive serology (ie, *Brucella* agglutination titer greater than or equal to 160 in one or more serum specimens obtained after the onset of symptoms), and a "confirmed" is a clinically compatible case that is laboratory confirmed.[104,119]

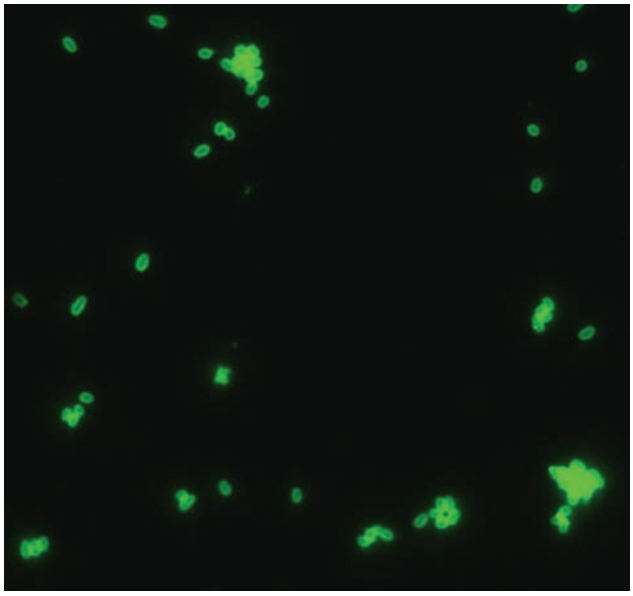

Figure 7-2. Direct fluorescent antibody staining of *Brucella abortus*.
Photograph: Courtesy of Dr John W Ezzell and Terry G Abshire, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

Future trends on rapid identification may use sophisticated protein microarrays to rapidly screen clinical samples or bacterial isolates.[111] However, many of these state-of-the-art identification methods will remain out of reach for resource and fiscally constrained, endemic countries, and thus for many of these areas the primary methods of identification of *Brucella* infections will remain the clinical presentation and traditional diagnostic methods.

TREATMENT

Brucellae are sensitive in vitro to a number of oral antibiotics and to aminoglycosides. In June 2005 at the Clinical Laboratory Standards Institute (CLSI formally known as National Committee for Clinical Laboratory Standards or NCCLS) meeting, the minimum inhibitory concentration breakpoints were established (Table 7-3) for *Brucella* along with the standard procedures for in vitro testing.[120] Therapy with a single drug has resulted in a high relapse rate, so combined regimens should be used whenever possible.[104,121–125] A 6-week regimen of doxycycline (200 mg/day administered orally) and streptomycin (1 g/day administered intramuscularly for

TABLE 7-3

BRUCELLOSIS MINIMUM INHIBITORY CONCENTRATION BREAKPOINT RANGES

Antimicrobial	Minimum Inhibitory Concentration range (μg/mL)
Azithromycin	0.25 – > 64
Chloramphenicol	0.5 – 4
Ciprofloxacin	0.25 – 8
Streptomycin*	≤ 8
Tetracycline	0.03 – 0.5
Doxycycline	≤ 1
Gentamicin	0.5 – 4
Rifampin	< 0.12 – 2
Levofloxacin	< 0.06 – 4
Trimethoprim – Sulfamethoxazole	≤ 2/38

*The streptomycin-susceptible breakpoint is > 16 μg/mL when the test is incubated in CO_2 and > 8 μg/mL when incubated in room air. Data sources: (1) Jorgensen JH. CLSI M45-A2: Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline-Second Edition, M45A2. 2010, Clinical and Laboratory Standards Institute, ISBN(s):1562387324. (2) Patel J, Heine H, oral personal communication between these principal investigators at the Clinical and Laboratory Standards Institute Guideline Meeting, June 2005.

the first 2 to 3 weeks) is effective therapy for adults with most forms of brucellosis.[125,126] However, a randomized, double-blind study using doxycycline plus rifampin or doxycycline plus streptomycin demonstrated that 100 mg twice daily oral doxycycline plus 15 mg/kg body weight of oral rifampin once a day for 45 days was as effective as the classical doxycycline plus streptomycin combination, provided these patients did not have evidence of spondylitis.[127] A 6-week oral regimen of both rifampin (900 mg/day) and doxycycline (200 mg/day) is an effective therapeutic treatment with a relapse rate lower than 10%.[128] Several studies, however, suggest that treatment with a combination of streptomycin and doxycycline is more successful and may result in less frequent relapse than treatment with the combination of rifampin and doxycycline.[126–130] Although it is a highly effective component of therapy for complicated infections, streptomycin has disadvantages of limited availability and requirement for intramuscular injection. Other aminoglycosides (netilmicin and gentamicin), which can be given intravenously and may be more readily available, have been substituted for streptomycin with success in a limited number of studies.[97] Fluoroquinolones in combination with rifampin have demonstrated efficacy similar to the doxycycline-rifampin regimen and may replace doxycycline plus rifampin due to potential doxycycline–rifampin interactions.[125,131–134]

Endocarditis may best be treated with rifampin, streptomycin, and doxycycline for 6 weeks; infected valves may need to be replaced early in therapy.[125,135] However, if patients do not demonstrate congestive heart failure, valvular destruction, abscess formation, or a prosthetic valve, conservative therapy with three antibiotics—(1) doxycycline, fluoroquinolone and trimethoprim/sulfamethoxazole, (2) tetracycline or doxycycline plus rifampin, and (3) aminoglycoside or trimethoprim/sulfamethoxazole—may be effective therapy.[136] Patients with spondylitis may require treatment for 3 months or longer. Central nervous system disease responds to a combination of rifampin and trimethoprim/sulfamethoxazole, but patients may need prolonged therapy. The latter antibiotic combination is also effective for children younger than 8 years old.[137] The Joint Food and Agriculture Organization–World Health Organization Expert Committee recommends treating pregnant women with rifampin.[128]

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, depending on the stage of bacterial identification (diagnostic sample verses isolated culture).[138] Chemoprophylaxis is not generally recommended for possible exposure to endemic disease.

In the event of a biological attack, the M40 mask (3M, St Paul, MN) should adequately protect personnel from airborne brucellae, as 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. A 3- to 6-week course of therapy with one of the treatments listed above should be considered after a confirmed biological attack or an accidental exposure in a research laboratory.[138,139] There is no safe and effective vaccine currently available to use in humans.

SUMMARY

Brucellosis is a naturally occurring disease in a wide variety of wild and domestic mammals. Although humans are not natural hosts for *Brucella* strains, they can be infected by ingesting contaminated foods (oral route) or slaughtering infected animals (percutaneous route). The brucellae are highly infectious by the airborne route, and this is the route of infection that is presumed to be of the biggest threat to military personnel. Laboratory workers can easily become infected when *Brucella* cultures are handled outside of a biosafety cabinet. Individuals presumably infected by aerosol have symptoms indistinguishable from patients infected by other routes: fever, chills, and myalgia are most common.

Because the brucellae disseminate throughout the reticuloendothelial system, they 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 LPS. This test, however, is not useful to diagnose infection caused by *B canis*, a naturally O-polysaccharide-deficient strain. Although ELISAs can more easily be standardized and performed in most clinical laboratories, these tests tend to have a higher degree of false-positive results,[139] and therefore the Rose Bengal (slide-type) agglutination test[140] or Brucella microagglutination test[141] continue to be considered the gold standards for diagnosis. 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. Alternatively, doxycycline plus fluoroquinolone may be as effective for treating this disease. Six weeks of doxycycline plus streptomycin for the first 3 weeks is also effective therapy; the limited availability of streptomycin may be overcome by substitution of netilmicin or gentamicin. No vaccine is available for humans.

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