# Chapter 9

# BRUCELLOSIS

BRET K. PURCELL, PHD, MD[*]; DAVID L. HOOVER, MD[†]; AND ARTHUR M. FRIEDLANDER, MD[‡]



[*] *Lieutenant Colonel, Medical Corps, US Army; Chief, Bacterial Therapeutics, Division of Bacteriology, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702*

[†] *Colonel, Medical Corps, US Army (Ret); Medical Director, Dynport Vaccine Company LLC, A CSC Company, 64 Thomas Johnson Drive, Frederick, Maryland 21702; formerly, Scientific Coordinator, Brucella Program, Department of Bacterial Diseases, Walter Reed Army Institute of Research, Silver Spring, Maryland*

[‡] *Colonel, Medical Corps, US Army (Ret); Senior Scientist, Division of Bacteriology, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702; and Adjunct Professor of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814*

## INTRODUCTION

Brucellosis is a zoonotic infection of domesticated and wild animals caused by organisms of the genus *Brucella*. Humans become infected by ingesting animal food products, directly contacting infected animals, or inhaling infectious aerosols either by accident or as a result of bioterrorism.

Military medicine has played a major role in studying and describing brucellosis in humans.[1] 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.[2] Three additional British army surgeons working on the island of Malta during the 1800s were responsible for important observations of the disease. JA 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 patients who died from the disease and placed the microorganism within the genus *Micrococcus*.[4] Ten years later, ML Hughes, who coined the name "undulant fever," published a monograph that detailed clinical and pathological findings in 844 patients.[5]

That same year, Danish investigator B Bang identified an organism, which he called the "bacillus of abortion," in the placentas and fetuses of cattle suffering from contagious abortion.[6] In 1917 AC 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 infections.[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. Because of the worldwide distribution of brucellosis, international travel and military deployments increase the risk of exposure.[9] The disease frequently becomes chronic and may relapse, even with treatment. Laboratory-acquired infections have been documented as awareness of this disease has increased.[10-13] Laboratory accidents may become more frequent and significant as biodefense research expands in the academic and biotechnology industries. Strict adherence to proper engineering controls, good laboratory and microbiology techniques, and personal protective equipment, in addition to vaccination (when possible), significantly reduce the incidence of laboratory-acquired infections.[14,15] However, no human brucellosis vaccine is available for laboratory workers.

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 in 1944 and 1945 with animal targets. By 1969 the United States terminated its offensive *Brucella* program and destroyed all its biological weapon munitions. Although the munitions developed were never used in combat, studies conducted under the offensive program reinforced the concern that *Brucella* organisms might be used against US troops as a biological warfare agent.[16] Even before the 2001 anthrax attacks, civilian populations were recognized as potential high-yield targets. A 1997 model of aerosol attack with *Brucella* on an urban population included an estimated economic impact of $477.7 million per 100,000 persons exposed.[17] *Brucella* represents one of many biological agents of zoonotic disease that could pose a threat as a terrorist weapon against human or agricultural targets.[18] An excellent review of brucellosis was published in 2005.[19]

## INFECTIOUS AGENT

Brucellae are small, nonmotile, nonsporulating, nontoxigenic, nonfermenting, facultative, intracellular, gram-negative coccobacilli parasites that may, based on DNA homology, represent a single species.[20,21] Taxonomically, brucellae are classified as α-*Proteobacteria* and subdivided into six species, each comprising several biovars.[22] Each species has a characteristic, but not absolute, predilection to infect certain animal species (Table 9-1). *Brucella melitensis*, *B suis*, *B abortus*, and *B canis* are the classic causative agents of disease in humans. Human infection with recently discovered marine strains (see Table 9-1) has also been noted.[23]

Human infections with *Brucella ovis* and *Brucella neotomae* 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,[24] 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

**TABLE 9-1**

**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 |
| Marine species | Marine mammals | Rare |
| *B ovis* | Sheep | None |
| *B neotomae* | Rodents | None |

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; production of hydrogen sulfide; growth in the presence of thionine or basic fuchsin dyes; agglutination by antisera directed against certain lipopolysaccharide (LPS) epitopes; and susceptibility to lysis by bacteriophage. *Brucella* can grow on blood agar plates and does not require X or V factors for growth. Analysis of fragment lengths of DNA cut by various restriction enzymes has also been used to differentiate brucellae groupings.[21] Recent studies using proteomics, complete genomic sequencing, and multilocus analysis of variable number tandem repeats have rapidly expanded information on virulence determinants, identification of pathogenicity islands, and evolutionary relatedness among the *Brucella*.[25-30]

The LPS component of the outer cell membranes of brucellae is different—both structurally and functionally—from that of other gram-negative organisms.[31,32] The lipid A portion of a *Brucella* organism LPS contains fatty acids that are 16-carbons long, and it lacks the 14-carbon myristic acid typical of lipid A of *Enterobacteriaceae*. This unique structural feature may underlie the remarkably reduced pyrogenicity of *Brucella* LPS, compared with the pyrogenicity of *Escherichia coli* LPS (less than 1/100th).[33] 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 a repetitive series of 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.[34] A unique feature of this organism, unlike most pathogenic bacteria, is the lack of many classical virulence factors, such as exotoxins; capsule; flagella; fimbriae; plasmids; lysogenic phage; antigenic variation; cytolysins; pathogenic islands; or type I, II, or III secretion systems; making characterization of pathogenic mechanisms in this organism highly challenging. Recently, however, a type IV secretion system[35] has been identified as an important contributor to virulence.

## DISEASE

### Epidemiology

Animals may transmit *Brucella* organisms during septic abortion, during slaughter, and through 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 who have occupational exposure to infected animals, such as veterinarians, shepherds, cattlemen, and slaughterhouse workers. 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 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.[36] In Australia an outbreak of *B suis* infection was noted in hunters of infected feral pigs.[37] *B canis*, a naturally rough strain that typically causes genital infection in dogs, can rarely infect humans.[38]

Brucellae are highly infectious in laboratory settings; numerous laboratory workers who culture the organism have become infected. However, 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. Jordan, for example, had 33 cases per 100,000 persons in 1987; Kuwait had 88 cases per 100,000 persons in 1985; and Iran had 469 cases from 1997 to 2002.[39-41]

**Pathogenesis**

Brucellae can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, and the gastrointestinal tract.[42] In the gastrointestinal tract, the organisms are phagocytosed by lymphoepithelial cells of gut-associated lymphoid tissue, from which they gain access to the submucosa.[43] Organisms are rapidly ingested by polymorphonuclear leukocytes, which generally fail to kill them,[44,45] and are also phagocytosed by macrophages (Figure 9-1). Bacteria transported in macrophages, which travel to lymphoid tissue draining the infection site, may eventually localize in lymph nodes, liver, spleen, mammary glands, joints, kidneys, and bone marrow.

In macrophages, brucellae inhibit fusion of phagosomes and lysosomes,[46] and replicate within compartments that contain components of endoplasmic reticulum[47] via a process facilitated by the type IV secretion system.[35] 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.[48,49] Administration of antibody to mice before challenge with rough or smooth strains of brucellae reduces the number of organisms that appear in the liver and spleen. This effect is attributable mainly to antibodies directed against LPS, with little or no contribution of antibody directed against other cellular components.[50]

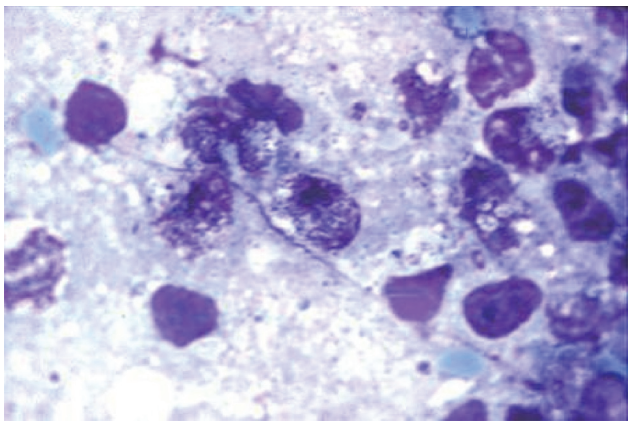

**Fig. 9-1.** Impression tissue smear from a bovine aborted fetus infected with *Brucella abortus*. The bacteria appear as lightly stained, gram-negative cells.
Photograph: Courtesy of John Ezzell, PhD, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

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[51] or by the immunoglobulin (IgG) 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.[52,53] Neutralization of *B abortus*-induced host interferon gamma (IFN–γ) during infection in pregnant mice prevents abortion.[54] Moreover, macrophages treated with IFN-γ in vitro inhibit intracellular bacterial replication.[55] Studies in humans support a role for IFN-γ in protection; homozygosity for the IFN-γ + 874A allele is associated with about a 2-fold increase in the incidence of brucellosis.[56] In ruminants, vaccination with killed bacteria provides some protection against challenge, but live vaccines are more effective.[57-59] The most efficacious live vaccines express surface O-polysaccharide; at a minimum, a complete LPS core is required for rough mutant vaccine efficacy against *B abortus* and *B ovis* infections in the mouse model.[60]

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.[61] 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.[62] 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 humans.

## Clinical Manifestations

Clinical manifestations of brucellosis are diverse, and the course of the disease is variable.[63] 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 9-2). 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 occur. 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 be found. Brucellosis does not usually cause leukocytosis. Some patients may be moderately neutropenic[64]; however, cases of pancytopenia have been noted.[65] In addition, bone marrow hypoplasia, immune thrombocytopenic purpura, and erythema nodosum may occur during brucellosis infections.[66-68] 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% of patients, particularly in young adults.[69-71] 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.[72] 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 rarely occurs. In contrast with frequent infection of the axial skeleton, osteomyelitis of long bones is rare.[73]

**TABLE 9-2**

**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.

Infection of the genitourinary tract (an important target in ruminant animals) may lead to pyelonephritis, cystitis, Bartholin's gland abscess and, in males, epididymoorchitis. Both pyelonephritis and cystitis may mimic their tuberculous counterparts, with "sterile" pyuria on routine bacteriologic culture.[74-76] With bladder and kidney infection, *Brucella* organisms can be cultured from the urine. Brucellosis in pregnancy can lead to placental and fetal infection.[77] 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, including mostly cough, dyspnea, or pleuritic pain, chest radiograph examinations are usually normal.[78] Diffuse or focal infiltrates, pleural effusion, abscess, and granulomas may be seen.

Hepatitis and, rarely, liver abscess also occur. Mild elevations of serum lactate dehydrogenase and alkaline phosphatase are common. Serum transaminases are frequently elevated.[79] Biopsy may show well-formed granulomas or nonspecific hepatitis with collections of mononuclear cells.[63] Spontaneous bacterial peritonitis has been reported.[80,81]

Other sites of infection include the heart, central nervous system, and skin. Although rare, *Brucella* endocarditis is the most feared complication and accounts for 80% of deaths from brucellosis.[82,83] Central nervous system infection usually manifests itself as chronic meningoencephalitis, but subarachnoid hemorrhage and myelitis also occur. Guillain-Barre syndrome has

been associated with acute neurobrucellosis, and involvement of spinal roots has been noted on magnetic resonance imaging.[84,85] A few cases of skin abscesses have been reported.

**Diagnosis**

A thorough history with details of likely 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 the differential diagnosis of febrile illness in troops who are presumed to have been exposed to a biological attack. Polymerase chain reaction and antibody-based antigen-detection systems may demonstrate the presence of the organism in environmental samples collected from an attack area.

When the disease is considered, diagnosis is based on clinical history, bacterial isolation from clinical samples, biochemical identification of the organism, and 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 arthralgia."[86] Handling specimens for cultivation of *Brucella* poses a significant hazard to clinical laboratory personnel.[87-90] Rapid detection of the organism in clinical samples using polymerase chain reaction–enzyme-linked immunosorbent assays (ELISA) or real-time polymerase chain reaction assays may eventually prove to be the optimal method for identification of these infections.[91] According to the Centers for Disease Control and Prevention's 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;
- 4-fold 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.[86]

Although several serologic techniques have been developed and tested, the tube agglutination test remains the standard method.[92] 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 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 (essentially IgG) antibody titers has been associated with persistent disease or relapse.[93] Serum testing should always include dilution to at least 1:320 because 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. 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, which will allow for screening and confirmation of brucellosis in one test.[94,95]

In addition to serologic testing, diagnosis should be pursued by microbiologic culture of blood or body fluid samples. If nonautomated 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, cultures should be incubated for at least 10 days with blind culture at 7 days.[96] The samples should be subcultured in a biohazard hood because it is extremely infectious. The reported frequency of isolation from blood varies from less than 10% to 90%; *B melitensis* is said to be more readily cultured than *B abortus*. A recent study indicated that BACTEC (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md) Myco/F lytic medium, 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.[24] Culture of bone marrow may increase the yield and is considered superior to blood cultures.[97] In addition, direct fluorescent antibody tests under development may offer a method of rapidly identifying these organisms in clinical specimens (Figure 9-2). The case classification of "probable" is defined as a clinically compatible case that is epidemiologically linked to a confirmed case or 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.[98]

**Treatment**

Brucellae are sensitive in vitro to a number of oral antibiotics and to intravenous/intramuscular 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 for Brucella (Table 9-3) and the standard procedures for in-vitro testing were established. These breakpoints and procedures were published in the new CLSI (NCCLS) guidelines in September–October 2005.[99] Therapy with a single drug has resulted in a high relapse rate; therefore, combined regimens should be used whenever possible.[98] A 6-week regimen of doxycycline at 200 mg per day administered orally, with the addition of streptomycin at 1 gram per day administered intramuscularly for the first 2 to 3 weeks, is effective therapy in adults with most forms of brucellosis.[100] However, a randomized, double-blind study using doxycycline plus rifampin or doxycycline plus streptomycin demonstrated that 100 mg of oral doxycycline twice daily plus 15 mg/kg body weight of oral rifampin once daily for 45 days was as effective as the classical doxycycline plus streptomycin combination, provided these patients did not have evidence of spondylitis.[101] A 6-week oral regimen of both rifampin at 900 mg per day and doxycycline at 200 mg per day should result in nearly 100% response and a relapse rate lower than 10%.[102] Several studies,[100,103-105] 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. Although it is a highly effective component of therapy for complicated infections, streptomycin has the 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.[79] Fluoroquinolones in combination with rifampin have demonstrated efficacy similar to the doxycycline-rifampin regimen and may replace it because of potential doxycycline-rifampin interactions.[106-109]

Endocarditis may best be treated with rifampin, streptomycin, and doxycycline for 6 weeks. Infected valves may need to be replaced early in therapy.[110] However, if patients do not demonstrate congestive heart failure, valvular destruction, abscess formation, or have a prosthetic valve, therapy with three antibiotics—(1) tetracycline or doxycycline, plus (2) rifampin, plus (3) aminoglycoside or trimethoprim/sulfamethoxazole for a mean duration of 3 months—may be effective.[111] 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 under 8 years old.[112]

**TABLE 9-3**

**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 | 1 – 16 |
| Tetracycline | 0.03 – 0.5 |
| Doxycycline | ≤ 0.015 – 1 |
| Gentamicin | 0.5 – 4 |
| Rifampin | < 0.12 – 2 |
| Levofloxacin | < 0.06 – 4 |
| Trimethoprim – Sulfamethoxazole | 0.25 – 2 |

Data sources: (1) Patel J, Heine H. Personal communication from Clinical Laboratory Standards Institute (CLSI, formally known as National Committee for Clinical Laboratory Standards or NCCLS) June 2005 Guideline Meeting. (2) Patel J, et al. *J Clin Microbiol*. Publication pending.

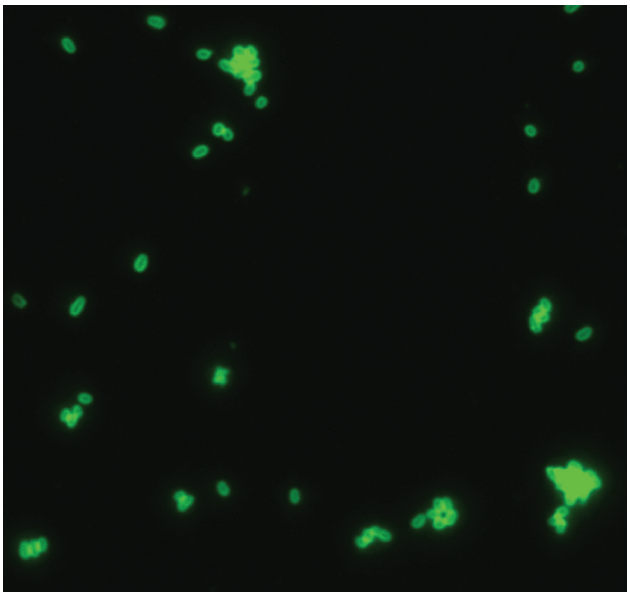

**Fig. 9-2.** Direct fluorescent antibody staining of *Brucella abortus*.
Photograph: Courtesy of Dr John W Ezzell and Terry G Abshire, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

The Joint Food and Agriculture Organization–World Health Organization Expert Committee recommends treating pregnant women with rifampin.[102] In the case of a biological attack, the organisms used may be resistant to these first-line antimicrobial agents. Medical officers should 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 22) for a discussion of the biosafety levels that are used at the US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Md. Chemoprophylaxis is not generally recommended for possible exposure to endemic disease.

In the event of a biological attack, the M40 mask (ILC Dover, Frederica, Del) 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. 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.[113] There is no commercially available vaccine for humans.

## SUMMARY

Brucellosis is a zoonotic infection of large animals, especially cattle, camels, sheep, and goats. Although humans can acquire *Brucella* organisms by ingesting contaminated foods (oral route) or slaughtering 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.

Because the bacterium disseminates throughout the reticuloendothelial system, brucellosis 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. ELISAs are more sensitive and specific for brucellosis but have not been validated for standard laboratory use. 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 a 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.

## REFERENCES

1. Evans AC. Comments on the early history of human brucellosis. In: Larson CH, Soule MH, eds. *Brucellosis*. Baltimore, Md: Waverly Press; 1950: 1–8.

2. Cleghorn G. Observations of the epidemical diseases of Minorca (From the Years 1744–1749). London, England; 1751. Cited in: Evans AC. Comments on the early history of human brucellosis. In: Larson CH, Soule MH, eds. *Brucellosis*. Baltimore, Md: Waverly Press; 1950: 1–8.

3. Marston JA. Report on fever (Malta). Army Medical Report 1861;3:486–521. Cited in: Evans AC. Comments on the early history of human brucellosis. In: Larson CH, Soule MH, eds. *Brucellosis*. Baltimore, Md: Waverly Press; 1950: 1–8.

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

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

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

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

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

9. Memish ZA, Balkhy HH. Brucellosis and international travel. *J Travel Med*. 2004;11:49–55.

10. Olle-Goig JE, Canela-Soler J. An outbreak of *Brucella melitensis* infection by airborne transmission among laboratory workers. *Am J Public Health*. 1987;77:335–338.

11. Memish ZA, Mah MW. Brucellosis in laboratory workers at a Saudi Arabian hospital. *Am J Infect Control*. 2001;29:48–52.

12. Staszkiewicz J, Lewis CM, Colville J, Zervos M, Band J. Outbreak of *Brucella melitensis* among microbiology laboratory workers in a community hospital. *J Clin Microbiol*. 1991;29:287–290.

13. Fiori PL, Mastrandrea S, Rappelli P, Cappuccinelli P. *Brucella abortus* infection acquired in microbiology laboratories. *J Clin Microbiol*. 2000;38:2005–2006.

14. Hawley RJ, Eitzen EM Jr. Biological weapons—A primer for microbiologists. *Annu Rev Microbiol*. 2001;55:235–253.

15. Rusnak JM, Kortepeter MG, Hawley RJ, Anderson AO, Boudreau E, Eitzen E. Risk of occupationally acquired illness from biological threat agents in unvaccinated laboratory workers. *Biosecur Bioterror*. 2004;2:281–293.

16. Department of the Army. *US Army Activity in the US Biological Warfare Programs*. Vols 1 and 2. Washington, DC: HQ, DA; February 24, 1977.

17. Kaufmann AF, Meltzer MI, Schmid GP. The economic impact of a bioterrorist attack: are prevention and postattack intervention programs justifiable? *Emerg Infect Dis*. 1997;3:83–94.

18. Kortepeter MG, Parker GW. Potential biological weapons threats. *Emerg Infect Dis*. 1999;5:523–527.

19. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. *N Engl J Med*. 2005;352:2325–2336.

20. Moreno E, Moriyon I. *Brucella melitensis*: a nasty bug with hidden credentials for virulence. *Proc Natl Acad Sci U S A*. 2002;99:1-3.

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

22. Moreno E, Moriyon I. *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt I, eds. New York, NY: Springer; 2001.

23. Sohn AH, Probert WS, Glaser CA, et al. Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. *Emerg Infect Dis*. 2003;9:485–488.

24. Yagupsky P. Use of BACTEC MYCO/F LYTIC medium for detection of *Brucella melitensis* bacteremia. *J Clin Microbiol*. 2004;42:2207–2208.

25. Mujer CV, Wagner MA, Eschenbrenner M, et al. Global analysis of *Brucella melitensis* proteomes. *Ann NY Acad Sci*. 2002;969:97–101.

26. DelVecchio VG, Kapatral V, Redkar RJ, et al. The genomic sequence of the facultative intracellular pathogen *Brucella melitensis*. *Proc Natl Acad Sci U S A*. 2002;99:443–448.

27. Paulsen IT, Seshadri R, Nelson KE, et al. The *Brucella suis* genome reveals fundamental similarities between animal and plant pathogens and symbionts. *Proc Natl Acad Sci U S A*. 2002;99:13148–13153.

28. Halling SM, Peterson-Burch BD, Bricker BJ, et al. Completion of the genome sequence of *Brucella abortus* and comparison to the highly similar genomes of *Brucella melitensis* and *Brucella suis*. *J Bacteriol*. 2005;187:2715–2726.

29. Bricker BJ, Ewalt DR, Halling SM. *Brucella* HOOF-Prints: strain typing by multi-locus analysis of variable number tandem repeats (VNTRs). *BMC Microbiol*. 2003;3:15.

30. Bricker BJ, Ewalt DR. Evaluation of the HOOF-Print assay for typing *Brucella abortus* strains isolated from cattle in the United States: results with four performance criteria. *BMC Microbiol*. 2005;5:37.

31. Bundle DR, Cherwonogrodzky JW, Caroff M, Perry MB. The lipopolysaccharides of *Brucella abortus* and *B melitensis*. *Ann Inst Pasteur Microbiol*. 1987;138:92–98.

32. Moreno E, Borowiak D, Mayer H. *Brucella* lipopolysaccharides and polysaccharides. *Ann Inst Pasteur Microbiol*. 1987;138:102–105.

33. Goldstein J, Hoffman T, Frasch C, et al. Lipopolysaccharide (LPS) from *Brucella abortus* is less toxic than that from *Escherichia coli*, suggesting the possible use of *B abortus* or LPS from *B abortus* as a carrier in vaccines. *Infect Immun*. 1992;60:1385–1389.

34. Cherwonogrodzky JW, Perry MB, Bundle DR. Identification of the A and M antigens of *Brucella* as the O-polysaccharides of smooth lipopolysaccharides. *Can J Microbiol*. 1987;33:979–981.

35. Boschiroli ML, Ouahrani-Bettache S, Foulongne V, et al. Type IV secretion and *Brucella* virulence. *Vet Microbiol*. 2002;90:341–348.

36. 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.

37. 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.

38. Wallach JC, Giambartolomei GH, Baldi PC, Fossati CA. Human infection with M-strain of *Brucella canis*. *Emerg Infect Dis*. 2004;10:146–148.

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

40. 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.

41. Roushan MRH, Mohrez M, Gangi SMS, Amiri MJS, Hajiahmadi M. Epidemiological features and clinical manifestations in 469 adult patients with brucellosis in Babol, Northern Iran. *Epidemiol Infect*. 2004;132:1109–1114.

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

43. 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.

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

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

46. Harmon BG, Adams LG, Frey M. Survival of rough and smooth strains of *Brucella abortus* in bovine mammary gland macrophages. *Am J Vet Res*. 1988;49:1092–1097.

47. Pizarro-Cerda J, Meresse S, Parton RG, et al. *Brucella abortus* transits through the autophagic pathway and replicates in the endoplasmic reticulum of nonprofessional phagocytes. *Infect Immun*. 1998;66:5711–5724.

48. Young EJ, Borchert M, Kretzer FL, Musher DM. Phagocytosis and killing of *Brucella* by human polymorphonuclear leukocytes. *J Infect Dis*. 1985;151:682–690.

49. Corbeil LB, Blau K, Inzana TJ, et al. Killing of *Brucella abortus* by bovine serum. *Infect Immun*. 1988;56:3251–3261.

50. Montaraz JA, Winter AJ, Hunter DM, Sowa BA, Wu AM, Adams LG. Protection against *Brucella abortus* in mice with O-polysaccharide–specific monoclonal antibodies. *Infect Immun*. 1986;51:961–963.

51. Araya LN, Elzer PH, Rowe GE, Enright FM, Winter AJ. Temporal development of protective cell-mediated and humoral immunity in BALB/c mice infected with *Brucella abortus*. *J Immunol*. 1989;143:3330–3337.

52. Yingst S, Hoover DL. T cell immunity to brucellosis. *Crit Rev Microbiol*. 2003;29:313–331.

53. Giambartolomei GH, Delpino MV, Cahanovich ME, et al. Diminished production of T helper 1 cytokines correlates with T cell unresponsiveness to *Brucella* cytoplasmic proteins in chronic human brucellosis. *J Infect Dis*. 2002;186:252–259.

54. Kim S, Lee DS, Watanabe K, Furuoka H, Suzuki H, Watarai M. Interferon-gamma promotes abortion due to *Brucella* infection in pregnant mice. *BMC Microbiol*. 2005;5:22.

55. Jiang X, Baldwin CL. Effects of cytokines on intracellular growth of *Brucella abortus*. *Infect Immun*. 1993;61:124–134.

56. Bravo MJ, de Dios Colmenero J, Alonso A, Caballero A. Polymorphisms of the interferon gamma and interleukin 10 genes in human brucellosis. *Eur J Immunogenet*. 2003;30:433–435.

57. Vemulapalli R, Contreras A, Sanakkayala N, Sriranganathan N, Boyle SM, Schurig GG. Enhanced efficacy of recombinant *Brucella abortus* RB51 vaccines against *B melitensis* infection in mice. *Vet Microbiol*. 2004;102:237–245.

58. Cloeckaert A, Jacques I, Grillo MJ, et al. Development and evaluation as vaccines in mice of *Brucella melitensis* Rev.1 single and double deletion mutants of the bp26 and omp31 genes coding for antigens of diagnostic significance in ovine brucellosis. *Vaccine*. 2004;22:2827–2835.

59. Moriyon I, Grillo MJ, Monreal D, et al. Rough vaccines in animal brucellosis: structural and genetic basis and present status. *Vet Res*. 2004;35:1–38.

60. Monreal D, Grillo MJ, Gonzalez D, et al. Characterization of *Brucella abortus* O-polysaccharide and core lipopolysaccharide mutants and demonstration that a complete core is required for rough vaccines to be efficient against *Brucella abortus* and *Brucella ovis* in the mouse model. *Infect Immun*. 2003;71:3261–3271.

61. Anderson TD, Cheville NF. Ultrastructural morphometric analysis of *Brucella abortus*-infected trophoblasts in experimental placentitis: bacterial replication occurs in rough endoplasmic reticulum. *Am J Pathol*. 1986;124:226–237.

62. Anderson TD, Cheville NF, Meador VP. Pathogenesis of placentitis in the goat inoculated with *Brucella abortus*. II. Ultrastructural studies. *Vet Pathol*. 1986;23:227–239.

63. Young EJ. Human brucellosis. *Rev Infect Dis*. 1983;5:821–842.

64. Crosby E, Llosa L, Miro QM, Carrillo C, Gotuzzo E. Hematologic changes in brucellosis. *J Infect Dis*. 1984;150:419–424.

65. Hatipoglu CA, Yetkin A, Ertem GT, Tulek N. Unusual clinical presentations of brucellosis. *Scand J Infect Dis*. 2004;36:694–697.

66. Yildirmak Y, Palanduz A, Telhan L, Arapoglu M, Kayaalp N. Bone marrow hypoplasia during *Brucella* infection. *J Pediatr Hematol Oncol*. 2003;25:63–64.

67. Gurkan E, Baslamisli F, Guvenc B, Bozkurt B, Unsal C. Immune thrombocytopenic purpura associated with *Brucella* and *Toxoplasma* infections. *Am J Hematol*. 2003;74:52–54.

68. Mazokopakis E, Christias E, Kofteridis D. Acute brucellosis presenting with erythema nodosum. *Eur J Epidemiol*. 2003;18:913–915.

69. Gotuzzo E, Alarcon GS, Bocanegra TS, et al. Articular involvement in human brucellosis: a retrospective analysis of 304 cases. *Semin Arthritis Rheum*. 1982;2:245–255.

70. Alarcon GS, Bocanegra TS, Gotuzzo E, Espinoza LR. The arthritis of brucellosis: a perspective one hundred years after Bruce's discovery. *J Rheumatol*. 1987;14:1083–1085.

71. 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.

72. Howard CB, Alkrinawi S, Gadalia A, Mozes M. Bone infection resembling phalangeal microgeodic syndrome in children: a case report. *J Hand Surg* [Br]. 1993;18:491–493.

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

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

75. Kelalis PP, Greene LF, Weed LA. Brucellosis of the urogenital tract: a mimic of tuberculosis. *J Urol*. 1962;88:347–353.

76. Peled N, David Y, Yagupsky P. Bartholin's gland abscess caused by *Brucella melitensis*. *J Clin Microbiol*. 2004;42:917–918.

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

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

79. Solera J, Martinez-Alfaro E, Espinosa A. Recognition and optimum treatment of brucellosis. *Drugs*. 1997;53:245–256.

80. Gursoy S, Baskol M, Ozbakir O, Guven K, Patiroglu T, Yucesoy M. Spontaneous bacterial peritonitis due to *Brucella* infection. *Turk J Gastroenterol*. 2003;14:145–147.

81. Refik MM, Isik AT, Doruk H, Comert B. *Brucella*: a rare causative agent of spontaneous bacterial peritonitis. *Indian J Gastroenterol*. 2003;22:190.

82. Peery TM, Belter LF. Brucellosis and heart disease. II. Fatal brucellosis: a review of the literature and report of new cases. *Am J Pathol*. 1960;36:673–697.

83. Reguera JM, Alarcon A, Miralles F, Pachon J, Juarez C, Colmenero JD. Brucella endocarditis: clinical, diagnostic, and therapeutic approach. *Eur J Clin Microbiol Infect Dis*. 2003;22:647–650.

84. Namiduru M, Karaoglan I, Yilmaz M. Guillain-Barre syndrome associated with acute neurobrucellosis. *Int J Clin Pract*. 2003;57:919–920.

85. Goktepe AS, Alaca R, Mohur H, Coskun U. Neurobrucellosis and a demonstration of its involvement in spinal roots via magnetic resonance imaging. *Spinal Cord*. 2003;41:574–576.

86. Centers for Disease Control and Prevention. Case definitions of infectious conditions under public health surveillance. *MMWR Morb Mortal Wkly Rpt*. 1997;46(RR10):1–55.

87. Harrington JM, Shannon HS. Incidence of tuberculosis, hepatitis, brucellosis, and shigellosis in British medical laboratory workers. *Br Med J*. 1976;1:759–762.

88. Martin-Mazuelos E, Nogales MC, Florez C, Gomez-Mateos JM, Lozano F, Sanchez A. Outbreak of *Brucella melitensis* among microbiology laboratory workers. *J Clin Microbiol*. 1994;32:2035–2036.

89. Yagupsky P, Peled N, Riesenberg K, Banai M. Exposure of hospital personnel to *Brucella melitensis* and occurrence of laboratory-acquired disease in an epidemic area. *Scand J Infect Dis*. 2000;32:31–35.

90. Noviello S, Gallo R, Kelly M, et al. Laboratory-acquired brucellosis. *Emerg Infect Dis*. 2004;10:1848–1850.

91. Al Dahouk SA, Tomaso H, Nockler K, Neubauer H. The detection of *Brucella* spp. using PCR-ELISA and real-time PCR assays. *Clin Lab*. 2004;50:387–394.

92. 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.

93. Buchanan TM, Faber LC. 2-mercaptoethanol *Brucella* agglutination test: usefulness for predicting recovery from brucellosis. *J Clin Microbiol*. 1980;11:691–693.

94. Al Dahouk S, Tomaso H, Nockler K, Neubauer H, Frangoulidis D. Laboratory-based diagnosis of brucellosis—a review of the literature. Part I: techniques for direct detection and identification of *Brucella* spp. *Clin Lab*. 2003;49:487–505.

95. Al Dahouk S, Tomaso H, Nockler K, Neubauer H, Frangoulidis D. Laboratory-based diagnosis of brucellosis–a review of the literature. Part II: serological tests for brucellosis. *Clin Lab*. 2003;49:577–589.

96. ASM (American Society for Microbiology) Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism. *Brucella* species, revised October 15, 2004. Available at: http://www.asm.org/ASM/files/LeftMarginHeaderList/DOWNLOADFILENAME/000000000523/Brucella101504.pdf#xml=http://search.asm.org/texis/search/pdfhi.txt?query=brucellosis+sentinel+guidelines&pr=ASM+Site&prox=page&rorder=500&rprox=500&rdfreq=500&rwfreq=500&rlead=500&sufs=0&order=dd&mode=&opts=&cq=&id=4552ca9010. Accessed May 14, 2006.

97. 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.

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

99. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing*. Ninth Informational Supplement M11-2S. Wayne, Pa: NCCLS; 1999.

100. 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.

101. Ariza J, Gudiol F, Pallares R, et al. Treatment of human brucellosis with doxycycline plus rifampin or doxycycline plus streptomycin. A randomized, double-blind study. *Ann Intern Med*. 1992;117:25–30.

102. Joint FAO/WHO expert committee on brucellosis. *World Health Organ Tech Rep Ser*. 1986;740:1–132.

103. Ariza J, Gudiol F, Pallares R, et al. Treatment of human brucellosis with doxycycline plus rifampin or doxycycline plus streptomycin: a randomized, double-blind study. *Ann Intern Med*. 1992;117:25–30.

104. Montejo JM, Alberola I, Glez-Zarate P, et al. Open, randomized therapeutic trial of six antimicrobial regimens in the treatment of human brucellosis. *Clin Infect Dis*. 1993;16:671–676.

105. Solera J, Rodriguez-Zapata M, Geijo P, et al. Doxycycline-rifampin versus doxycycline-streptomycin in treatment of human brucellosis due to *Brucella melitensis*. The GECMEI Group. *Antimicrob Agents Chemother*. 1995;39:2061–2067.

106. Akova M, Uzun O, Akalin HE, Hayran M, Unal S, Gur D. Quinolones in treatment of human brucellosis: comparative trial of ofloxacin-rifampin versus doxycycline-rifampin. *Antimicrob Agents Chemother*. 1993;37:1831–1834.

107. Agalar C, Usubutun S, Turkyilmaz R. Ciprofloxacin and rifampicin versus doxycycline and rifampicin in treatment of brucellosis. *Eur J Clin Microbiol Infect Dis*. 1999;18:535–538.

108. Karabay O, Sencan I, Kayas D, Sahin I. Ofloxacin plus rifampicin versus doxycycline plus rifampicin in the treatment of brucellosis: a randomized clinical trial [ISRCTN11871179]. *BMC Infect Dis*. 2004;4:18–23.

109. Colmenero JD, Fernandez-Gallardo LC, Agundez JAG, Sedeno J, Benitez J, Valverde E. Possible implications of doxycycline-rifampin interaction for treatment of brucellosis. *Antimicrob Agents Chemother*. 1994;38:2798–2802.

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

111. Mert A, Kocak F, Ozaras R, et al. The role of antibiotic treatment alone for the management of *Brucella* endocarditis in adults: a case report and literature review. *Ann Thorac Cardiovasc Surg*. 2002;8:381–385.

112. Lubani MM, Dudin KI, Sharda DC, et al. A multicenter therapeutic study of 1,100 children with brucellosis. *Pediatr Infect Dis J*. 1989;8:75–78.

113. US Army Medical Research Institute of Infectious Diseases. *USAMRIID's Medical Management of Biological Casualties Handbook*. 4th ed. Fort Detrick, Md: USAMRIID; 2001. Available at: http://www.usamriid.army.mil/education/bluebook.html. Accessed October 30, 2006.