Review

Human brucellosis: an overview

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Brucellosis exists worldwide. The disease mainly spreads by ingestion of unpasteurized dairy products. It is a systemic disease, and many systems can be involved. The clinical presentation may be acute or insidious. The disease mimics many illnesses and presents diagnostic difficulties. Automated blood culture systems, PCR and ELISA have proved useful as new laboratory-based diagnostic methods. Although various regimens have been used in the treatment of the disease, a combination of doxycycline and streptomycin seems to be the best current treatment for human brucellosis.

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Brucellosis is a zoonotic disease of worldwide distribution and still remains endemic in some developing countries. It affects mainly domestic animals, producing genitourinary infections leading to abortions. The illness continues to be one of the most widely distributed zoonoses, causing devastating economic losses, and commonly affects humans.[1,2] Human brucellosis has a wide clinical spectrum, and presents various diagnostic difficulties because it mimics many other diseases. There is still no optimal therapy for some particular clinical forms of brucellosis and there are difficulties with preventive measures in developing countries. The perspective of this article will be particularly on the clinical and therapeutic features of human brucellosis.

The disease, as a debilitating illness characterized by malaise, anorexia, fever, and profound muscular weakness, was described by Marston in 1860. The responsible microorganism was isolated by David Bruce in 1887. Since then, new species have been identified. At present, there are six known species of brucellae, including *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis*, and *B. neotomae*. A distinctive *Brucella* strain recently isolated was given the tentative name *Brucella maris*.[3]

Brucella species are gram-negative coccobacillary, nonmotile, aerobic microorganisms. The cells do not produce capsules, spores, or flagella. They do not grow well on media commonly used in microbiology laboratories. Growth is best on trypticase soy agar, *Brucella* agar or serum dextrose agar at 37°C, especially if a biphasic culture system is used. *B. abortus* and *B. suis* require supplementary CO_2 for growth, especially for primary isolation. This conventional culture method requires prolonged incubation (up to 3 weeks) and the use of blind subcultures.[3] Currently available methods that have been advocated include lysis–centrifugation (Isolator) blood cultures and the use of automated, continuous-monitoring blood culture instrumentation.[3–5]

EPIDEMIOLOGY

Brucella spp. can infect many species of animals and humans. Domestic animals serve as the reservoir. The host range for *Brucella* spp. is shown in Table 1. The main pathogenic species worldwide are *B. abortus*, responsible for bovine brucellosis, *B. melitensis*, the main etiologic agent of ovine and caprine brucellosis, and *B. suis*, which has a wide host range, not being confined to swine, and all strains of which are human pathogens, except for biovar 2. These three *Brucella* spp. usually cause abortion in their natural hosts, resulting in huge economic losses. They also account for most cases of human brucellosis. *B. melitensis* is the most virulent species. *B. canis* is occasionally involved in human diseases. *B. maris* has been isolated from marine mammals.[1,3,6,7] Only two cases of human infection with a marine isolate have been reported.[8]

Diseased animals excrete *Brucella* through the urine, milk, placenta and the products of miscarriages. In this way, the bacteria are disseminated and infect other animals and humans. The survival of *Brucella* outside the animal organism is variable. The duration of survival of *Brucella* in moist soil, in dung spread on the ground, has been reported to be 70–80 days. The survival of *B. melitensis* in dust varies from 15 to 40 days, depending on the ambient humidity.[2] Consequently, brucellosis is an occupational risk for farmers, veterinarians, abattoir workers and laboratory personnel.[6,7,9]

The main sources of *Brucella* are infected animals or their products, such as milk, cream, butter, fresh cheese, ice cream, urine, blood, carcasses, and abortion products. Routes of transmission of the infection to humans include direct contact with infected animals and their secretions through cuts and abrasions in the skin, by way of infected aerosols inhaled or inoculated into the conjunctival sac of the eyes, or via the ingestion of unpasteurized dairy products.[1,2,6,7,9]

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Table 1. Species of *Brucella* and animal reservoirs

Species	*Reservoir*	*Other hosts*	*Human cases (worldwide)*
B. melitensis	Goat, sheep, camel	Cattle, antelope	++++ (70% of cases)
B. abortus	Cattle, buffalo, yaks, bison	Horse	+++ (25% of cases)
B. suis	Swine	Cattle, caribou	++ (5% of cases)
B. ovis	Sheep	–	No
B. canis	Dog	–	Few
B. neotomae	Desert wood rat	–	No
B. maris	Marine mammals	–	No

Milk is the main food product serving as a vector for *Brucella*. The consumption of fresh, raw milk from animals is traditional, particularly in Saudi Arabia and other Arabic countries, which have a high incidence of brucellosis.[9] Different kinds of fresh cheese are certainly the foodstuffs responsible for human brucellosis, especially goat and ewe cheeses. *Brucella* in cheeses preserved in paste form survives, on average, for 20 days, and sometimes up to 3 months. Meat products are rarely the source of infection, because they are not usually eaten raw and the numbers of organisms in muscle tissue are low.[2,7] The control of animal brucellosis is very important for the prevention of human infection. Also important in the prevention of human infection is the use of pasteurized milk and milk products in developing countries.

Laboratory workers handling *Brucella* cultures have a high risk of acquiring brucellosis through accidents, aerosols, or inadequate laboratory precautions.[9,10] In our experience, veterinarians may also be infected by accidental inoculation percutaneously or into the conjunctival sac with live *B. abortus* strain 19 vaccine.

Human brucellosis can occur in any age group, but the majority of cases are found in young men between the ages of 20 and 40 years. This is generally related to occupational hazards in young men.[7,11–14] All persons having an underlying disease—HIV infection, chronic renal disease or liver disease—living in endemic areas have the same risk as healthy people of acquiring brucellosis.[15–17]

Human-to-human transmission is unusual. However, rare cases due to blood transfusion, bone marrow transplantation and sexual transmission have been reported.[18–21]

The true incidence of human brucellosis is unknown. The World Health Organization (WHO) points out that 500 000 cases of brucellosis are reported each year from around the world. The reported incidence and prevalence of the disease vary widely from country to country. *B. abortus* is more prevalent in the USA and northern Europe, whereas *B. melitensis* is more common in Latin America, the Mediterranean countries, and the developing countries. Some areas, such as Peru, Kuwait, and Saudi Arabia, are hyper-endemic for *Brucella* infection. The re-emergence of brucellosis in Malta and Oman has been reported.[1,5,9] Turkey is also an endemic country for brucellosis. In 1999, 11 462 cases were notified to the Ministry of Health, with the incidence rate being 17.41/100 000. The distribution of cases of human brucellosis reported between 1970 and 1999 is shown in Figure 1.[22] In a multicenter seroprevalence study in Turkey, the seropositivity rate was found to be 1.8% in the healthy population and 6% in high-risk occupational groups (veterinarians, workers in abattoirs, butchers, etc.).[23] The seropositivity rate for brucellosis in a seroprevalence study from Saudi Arabia was reported to be 15%.[9] In the USA, as an example of a developed country, brucellosis is seen sporadically, and occurred in the Mexican border region at a rate eight times the national rate (0.18/100 000 in the Mexican border region versus 0.09/100 000 and 0.02/100 000 nationally). The epidemiology has also changed in this border region, from an occupational disease associated with animal contact to a foodborne disease associated with consumption of unpasteurized dairy products.[24]

PATHOGENESIS

Brucella spp. are pathogenic bacteria of humans and animals, and are exceedingly well adapted to their hosts. They are facultative intracellular pathogens, surviving and multiplying within cells of the reticuloendothelial system and not surviving for long periods of time in open conditions. The bacteria can enter the body by ingestion, inhalation, penetration of intact skin, abrasions, or the conjunctival mucosa.[1–3,6,7,9] The low pH of gastric juice provides some protection against oral infection. Antacids or H_2 receptor blockers may increase susceptibility to brucellosis.[25] Normal human serum has moderate anti-*Brucella* activity, and complement opsonizes organisms for phagocytosis. Human neutrophils destroy some *Brucella* strains, but they lack activity against *B. melitensis*.[26,27]

Virulent *Brucella* organisms can infect both non-phagocytic and phagocytic cells. The intracellular environment of host cells sustains extensive reproduction, allowing bacterial expansion and subsequent transmission to new host cells. In contrast to other pathogenic bacteria, no classical virulence factors have been described in *Brucella* organisms. Instead of these, some molecular determinants are described for *Brucella* spp. as virulence elements, allowing them to invade, resist

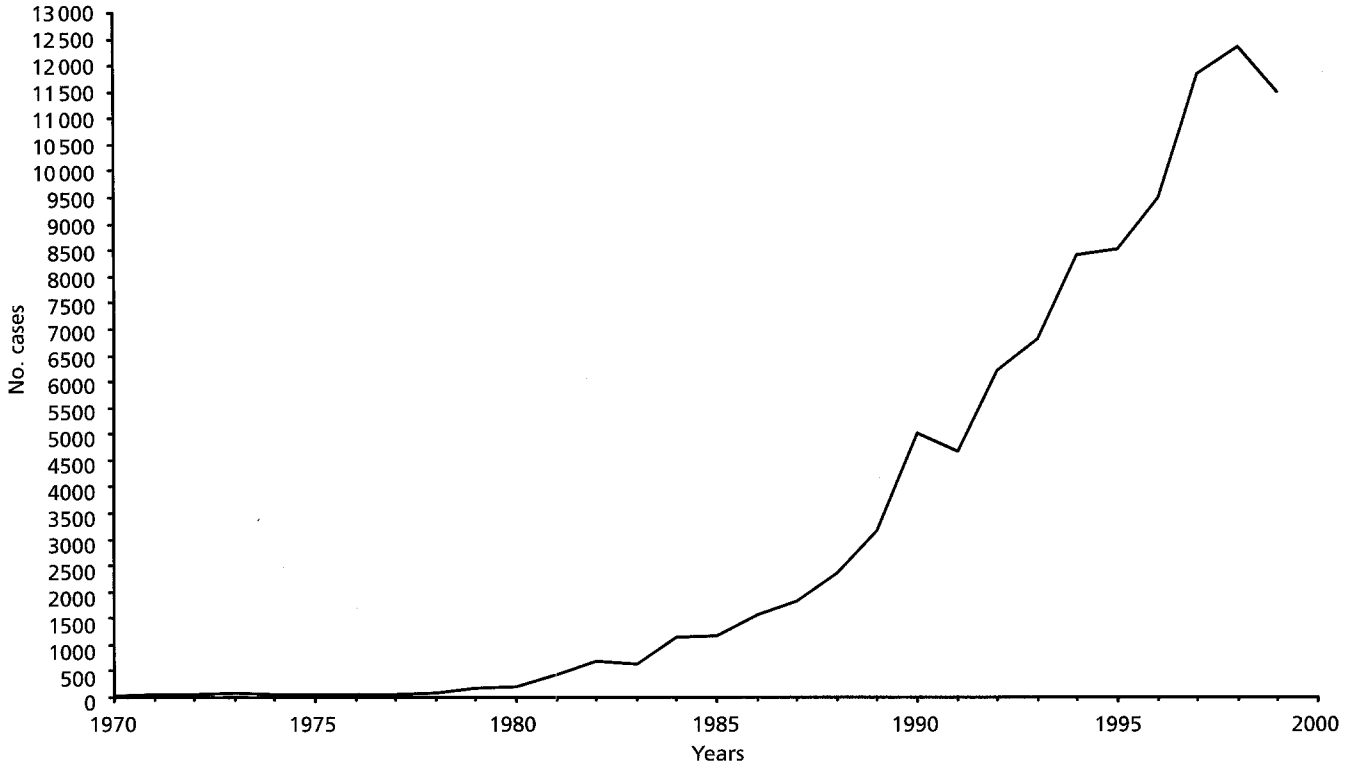

Figure 1. The distribution of human brucellosis recorded by the Ministry of Health of Turkey between 1970 and 1999.

intracellular killing, and reach their reproductive niche in professional or non-professional phagocytes.[28]

Once *Brucella* invades the mucosa, professional phagocytes lying underneath the submucosa ingest the bacterium. Macrophages and neutrophils ingest *Brucella* by zipper-like phagocytosis. Opsonized *Brucella* organisms are internalized via complement and Fc receptors in macrophages and monocytes,[28,29] whereas non-opsonized *Brucella* organisms enter via lipid rafts.[30,31] Activation of small GTPases of the Rho subfamily, such as Rho, Rac, and Cdc42, is required for *Brucella* internalization in non-professional phagocytes.[28,29]

Invasion of cells by *Brucella* is inhibited by chemicals and toxins that increase the level of cyclic AMP (dibutyl-cAMP and *Vibrio cholerae* enterotoxin), but it is stimulated by toxins and chemicals that increase the levels of cAMP (*Escherichia coli* enterotoxin A and dibutyl-cGMP). This suggests an inverse relationship between these two secondary messengers during *Brucella* infection.[28]

Rough *Brucella* attaches in higher numbers to cells than does smooth *Brucella*, although the former is less efficient at invading cells than the latter. This phenomenon suggests that O-polysaccharide and the structurally related native hapten have a role in the invasion of cells by *Brucella* and in virulence. Recently, a regulatory two-component system (*Brucella* virulence-related regulator or sensor proteins—BvrR/BvrS) has been described as playing a role in host cell invasion.[28,32]

Once virulent *Brucella* is ingested, the bacterium redirects its intracellular trafficking through a unique pathway until reaching its place of reproduction. Phagocytes are generally prone to kill the ingested *Brucella* within phagolysomes. Only a few internalized bacteria are capable of avoiding lysosome fusion and redirecting their trafficking to their final place of reproduction, the endoplasmic reticulum.[28,29]

Brucella genomic analysis studies indicate that it has two chromosomes and that no plasmids are present. The genome contains three rRNA operons. Two are on chromosome I, and the third is on chromosome II. A set of 12 open reading frames (ORFs) located on chromosome II code for a type IV secretion system. In the biogenesis of *Brucella* in cells, the VirB operon coding for the *Brucella* type IV secretion system is required for regulation of intracellular trafficking from autophagosome-like compartments to the endoplasmic reticulum. The integrity of the lipopolysaccharide (LPS) molecule on the bacterial surface is also one of the required factors for intracellular survival, and then for virulence.[33,34]

During *Brucella* infection, several cytokines, such as interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin-2 (IL-2), IL-10 and IL-12, control the intracellular growth of *Brucella* strains within macrophages.[28]

The LPS of smooth strains probably plays a major role in intracellular survival. When the biological activity of S-LPS is compared with that of *E. coli* LPS, S-LPS has low toxicity for endotoxin-sensitive mice, rabbits and chick embryos, low toxicity for macrophages, low pyrogenity; and low hypoferremia-inducing activity.

It is also a weak inducer of IFN, TNF, nitric oxide, cyclooxygenase-2, and the chemokine monocyte chemoatractant protein-1.[1,35–37]

In humans, *Brucella* spp. infection results in the formation of granulomas consisting of epithelioid cells, polymorphonuclear leukocytes, lymphocytes, and some giant cells. The granulomatous response is characteristic of *B. abortus* infections. In *B. melitensis* infections, the granulomata are very small, but there is often toxemia. *B. suis* infection is often accompanied by chronic abscess formation in joints and the spleen.[6,7,26,27]

An infection with *Brucella* spp. induces both humoral and cellular immunity. Although humoral antibodies appear to play some role in resistance to *Brucella*, cell-mediated immunity appears to be the principal mechanism of recovery. The serum antibody response to *Brucella* infection in humans is characterized by an initial rise in antibody titers of the IgM class, followed in several weeks by a predominance of IgG antibodies. After treatment, titers gradually come down, with a faster decrease of IgG antibodies than of IgM antibodies. In some cases, low titers of IgM antibodies may persist for months or years in the absence of an active infection.[6,26,27,38]

CLINICAL MANIFESTATIONS

Brucellosis is a systemic disease in which any organ or body system can be involved. The incubation period varies between 1 and 5 weeks, and *Brucella* infection may be asymptomatic or symptomatic. The onset of symptoms is acute or insidious. According to the length and severity of symptoms, the disease is arbitrarily classified as acute (less than 8 weeks), subacute (from 8 to 52 weeks), or chronic (more than 1 year). Any organ involvement is often referred to as localized disease. It can be seen as a complication of acute brucellosis or may be the only manifestation of chronic brucellosis.[6,7,27,39]

The symptoms of brucellosis are nonspecific. The majority of patients complain of fever, sweats, malaise, anorexia, headache, arthralgia, and back-ache.[5–7,14,27,39] Nowadays, in developing countries, the clinical picture has changed, because patients with fever generally take an antibiotic on their own initiative, or at the suggestion of the pharmacist. This has lowered the rate of positive blood culture, and led to difficulties in the diagnosis of brucellosis. In our clinic between 1989 and 1998, brucellosis was diagnosed in 480 patients, of whom 67.1% had the acute form, 25.2% had the subacute form, 5% had the chronic form, and 2.7% were asymptomatic. The symptoms and findings are shown in Table 2.[14]

Table 2. Symptoms and clinical findings in 480 patients

	No. of patients	*%*
Symptoms		
Malaise	432	90
Sweating	405	84.4
Arthralgia	393	81.9
Fever	383	79.8
Back pain	281	58.5
Myalgia	236	49.2
Weight loss	213	44.4
Anorexia	198	41.3
Nausea	155	32.3
Vomiting	104	21.7
Abdominal pain	101	21
Headache	91	19
Findings		
Fever	187	39
Hepatomegaly	102	21.3
Osteoarticular involvement[a]	91	19
Splenomegaly	68	14.2
Neurological involvement[b]	31	6.5
Genitourinary involvement[c]	5	1
Endocarditis[d]	2	0.4
Peritonitis	2	0.4
Cutaneous involvement	2	0.4
Pneumonia	1	0.2

[a] Neurologic involvement in two patients.
[b] Multisystem involvement (acute respiratory distress syndrome due to pneumonia, meningitis, hepatitis, hematopoetic and cutaneous endocarditis) in one patient and osteoarticular involvement in two patients
[c] Epididymo-orchitis in four patients, prostatitis in one patient
[d] Multisystem involvement in one patient

Subclinical or asymptomatic infection

This clinical form is diagnosed by positive serology. The patients have no history or physical signs of acute or chronic illness. It has been documented more frequently in farmers, abattoir workers, and veterinarians.[6,39]

Acute form

This is the typical form of brucellosis. Almost all patients have a history of fever accompanied by weakness, malaise, headache, back-ache, anorexia, weight loss, myalgia, and arthralgia. A temperature over 38.5°C is measured in more than 85% of patients. Splenomegaly and hepatomegaly are found in about 6–35% of the cases.[5,6,14,27,40–46] Any organ involvement can be seen, but arthritis is more frequent (40–50%).[5,40–46]

Subacute form

This refers to a group of patients who have relapsed because of incomplete or partial antibiotic treatment and patients who have received inappropriate antibiotics because of incorrect diagnosis. The clinical picture is more protean and may be an important cause of fever of unknown origin. The symptoms are generally milder, and localized infection can be seen.[6,14]

Chronic form

Chronic brucellosis is similar to chronic fatigue syndrome. It is extremely rare in children, but frequent

in older people. These patients generally suffer from a psychoneurosis, sweating, and weight loss. Fever is rare. Localized infection can be seen; however, ocular manifestations, such as episcleritis and uveitis, are frequent.[6,39]

Localized infection

Localized brucellosis refers to cases in which organisms are not isolated from blood but are localized in specific tissues, such as the bone, joints, cerebrospinal fluid, liver, kidneys, spleen, or skin. Localization may be the principal manifestation of systemic infection, or may be the only manifestation of a chronic infection. Localized infection is sometimes named as a complication when it occurs as a result of systemic infection.[6,27,39] In our series, 133 (27.7%) of 480 patients had complications. In this study, we accepted any organ involvement as a complication of brucellosis.[14] The identification of any organ involvement is very important for the choice of regimen and its duration, and the prognosis.

Skeletal system

Osteoarticular complications are common in brucellosis, and clinical manifestations include arthralgias, arthritis, spondylitis, sacroiliitis, osteomyelitis, tenosynovitis, and bursitis. Arthralgia can present in up to 85% of patients with brucellosis.[6,11,41–43] Many studies have reported the clinical manifestations of osteoarticular infection in 17–37% in patients. *B. melitensis* is responsible for almost all cases of osteoarticular involvement.[6,14,41–45] The sites of osteoarticular involvement obtained from some selected studies are shown in Table 3. The most commonly affected sites are the sacroiliac joints.[14,40–42]

Spondylitis is one of the most serious complications of brucellosis. It is reported at a rate of 2–58% in patients with brucellosis. This complication is usually seen in elderly patients, and affects the lumbar vertebrae. Back or neck pain, fever and constitutional symptoms are the most common symptoms. Paravertebral abscesses, spinal epidural abscesses and neurologic complications can be seen with the extension of infection (Figure 2).[40,44–47]

Nervous system

Nervous system involvement is seen in about 2–6.5% of brucellosis cases. Neurologic complications may occur at any stage of the disease. Clinical syndromes include meningitis, encephalitis, meningoencephalitis, radiculitis, myelitis, and neuritis.[14,26,27,48–50] In our series, nervous system involvement was observed in 31 (6.5%) of 480 patients with brucellosis, of whom 17 (54.8%) had meningitis, 4 (12.9%) encephalitis, 2 (6.5%) myelitis, 1 (3.2%) meningoencephalitis, 1 (3.2%) meningomyelitis, 1 (3.2%) polyradiculoneuritis, 2 (6.5%) peripheral neuritis, 2 (6.5%) meningitis plus peripheral neuritis, and 1 (3.2 %) cerebral infarct.[14]

Meningitis is the most frequent central nervous system complication, clinically presenting as a subacute or chronic disease. Cerebrospinal fluid analysis reveals a lymphocytic pleocytosis, elevated protein, and low glucose levels.[48–50]

Cardiovascular system

Endocarditis is the most serious clinical form, and may lead to death; however, it occurs in less than 2% of cases. The clinical presentation is similar to that of other bacterial endocarditis, except that there are difficulties in the isolation of *Brucella* from blood cultures. The aortic valve or mitral valve is generally infected.[26,27,51–53] Myocarditis, pericarditis and infected aortic aneurisms have been noted.[26,27] Multisystem involvement, including meningitis, pneumonia, hepatitis, skin lesions, nephritis, and peritonitis with endocarditis, have been reported.[53,54]

Gastrointestinal system

Gastrointestinal system symptoms, including anorexia, nausea, vomiting, diarrhea, abdominal pain, and gastrointestinal bleeding, can be seen in brucellosis.[6,14,26,27,55,56] Liver and spleen enlargement with mild nonspecific elevation of liver enzyme levels can be detected in approximately 50% of patients with brucellosis. However, diffuse hepatitis or granulomatous hepatitis is also

Table 3. Anatomic distribution of osteoarticular involvement of brucellosis in selected studies (%).

Site	*Colmenero et al.*[40] *n = 65*	*Mousa et al.*[41] *n = 169*	*Taşova et al.*[42] *n = 87*	*Aygen et al.*[14] *n = 91*
Sacroiliitis	45	20	60.9	53.8
Spondylitis	58	6	13.8	9.9
Knee	2	36	8	26.3
Hip	3	53	–	–
Ankle	6	15	5.7	2.1
Elbow	–	5.3	–	–
Shoulder	–	5	–	1
Wrist	–	3.5	–	–
Sternoclavicular	2	1.8	–	–
Foot interphalangial	–	–	–	1
Bursitis	3	1.2	5.7	2.2
Polyarthricular	22	Not stated	5.7	2.1

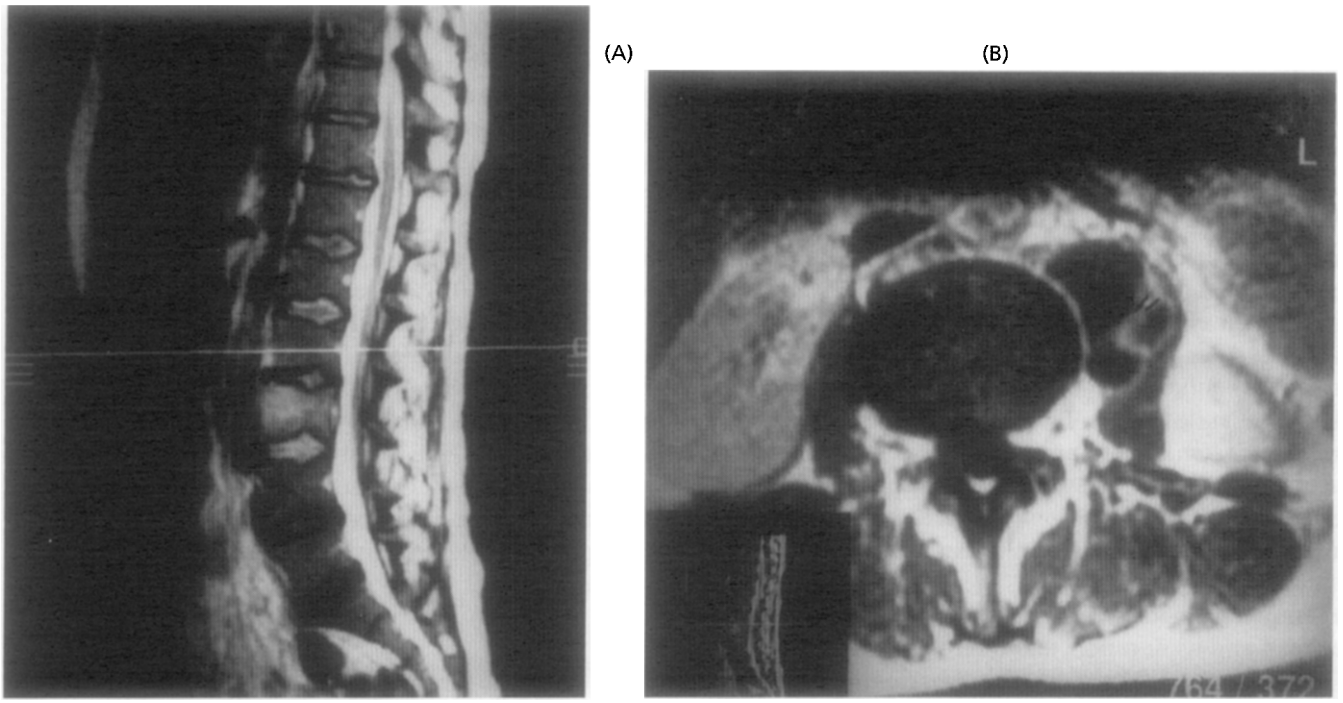

Figure 2. Psoas and epidural abscesses secondary to brucellar spondylitis. (A) The saggital T_1-weighted image revealing an epidural abscess and involvement of the body of L3. (B) An axial T_2-weighted image revealing a left paravertebral abscess at L3.

seen during the course of brucellosis.[26,27,57–59] Abscesses in liver and spleen[57,58] and spontaneous peritonitis in cirrhotic patients[16,17] have also been reported as rare complications of brucellosis.

Hematopoietic system

Hematologic abnormalities, such as anemia, leukopenia, and thrombocytopenia, are common in the course of brucellosis. Leukopenia with lymphocytosis is characteristic of brucellosis.[14,27,41,55,56] In our series, anemia was found in 54.6% of the cases, leukocytosis in 6.5%, leukopenia in 7.7%, lymphopenia in 63%, and thrombocytopenia in 13.7%.[14] Petechial or purpuric skin and mucosal lesions, epistaxis, hemoptysis and gastrointestinal or vaginal bleeding can be seen, and these complications are significantly associated with clotting abnormalities (low platelet count, low fibrinogen level, and prolongation of thrombin clotting time).[27,56,60] Occasionally, thrombocytopenia may be severe enough to cause bleeding and result in death. Forty-three cases of brucellosis complicated by thrombocytopenic purpura, with a mortality rate of 9.3%, have been reported in the world literature. Possible mechanisms for thrombocytopenia are hypersplenism, reactive hemophagocytosis, and immune destruction of platelets.[60]

Genitourinary system

Genitourinary involvement occurs in 1–20% of patients with brucellosis. Unilateral epididymo-orchitis is the usual manifestation. Pyelonephritis, interstitial nephritis, renal abscess, cystitis and prostatitis are rare complications. The onset of symptoms is generally acute (78%), and less frequently (22%) subacute or chronic. The clinical presentation includes genitourinary symptoms and systemic symptoms.[61–64]

In pregnant animals, *Brucella* organisms preferentially reproduce in placental trophoblasts during the middle and late stages of gestation. Infected trophoblasts produce cortisol, which is not normally generated by the placenta. Erythritol is a key sugar produced by the pregnant host, and *Brucella* can utilize it. Erythritol is not present in human placenta.[28,33] *Brucella* can infect human chorioamniotic tissue during pregnancy, and brucellosis can cause abortion, premature delivery, and intrauterine infection with fetal death. It is well known that the effect of *Brucella* infection on pregnancy is no more than those of other bacterial infections.[27,39] However, a retrospective study from Saudi Arabia reported that the incidence of spontaneous abortion in the first and second trimesters was 43%, and that of intrauterine fetal death in the third trimester was 2%.[65] This result needs to be evaluated with a prospective and controlled clinical study.

Other systems

A variety of skin lesions have been described in brucellosis, including maculopapular lesions, papules, petechiae, purpura, impetiginous and psoriform lesions,[66,67] ocular lesions; uveitis, choroiditis and scleritis,[26,27] respiratory system involvement, pneumonia, acute respiratory distress syndrome, empyema, and mediastinitis.[54,68,69]

Diagnosis

The diagnosis of brucellosis requires the isolation of *Brucella* from blood or body tissues, or the combination of suggestive clinical presentation and positive serology. A definitive diagnosis of brucellosis is based on the culture of *Brucella* from different samples, mainly blood. In the case of focal complications, culture material, if possible, should be taken from the affected places, such as liver, lymph node, abscess, synovial fluid, prostatic fluid, or cerebrospinal fluid.[3,5,6,26,27] The bacteria grow slowly in vitro, and most blood cultures are positive between the 7th and 21st day using classic biphasic Castaneda flasks. The rate of positive blood cultures in brucellosis ranges from 15% to 80%.[3,5,6,14,70] The number of positive results is usually greater in acute brucellosis; however, this figure is notably reduced in the case of focal complications and chronic illness. In our study, positive blood cultures were detected in 45% of the patients with brucellosis.[14] Currently, automated blood culture systems (BacT/Alert, BACTEC 9000 series, Vital, ESP) have replaced conventional blood culture systems in many laboratories. Automated blood culture systems seem to shorten the time (mean time 3 days) needed to detect these organisms from blood and other body fluids.[3–6]

A study showed that bone marrow cultures were significantly more sensitive than cultures of blood (92% versus 70%, respectively), and the time to detection was shorter for bone marrow cultures than for blood cultures.[70] Bone marrow culture is recommended for patients with fever of unknown origin, negative serology, unexplained hematologic involvement, and suspicion of chronic brucellosis.

Amplification of DNA by polymerase chain reaction (PCR) is currently used to diagnose brucellosis. For PCR, peripheral blood or non-blood samples can be used.[71,72] It was reported that the sensitivity of PCR was 100% and the specificity 98.3% in patients with brucellosis of bacteremic, non-bacteremic and focal complications.[71] PCR appears to be a very useful technique for the initial diagnosis and the early detection of relapses.[73] However, it is not yet routinely used.

In the absence of bacterial isolation, the diagnosis can be made serologically. Several serologic tests have been developed to measure antibodies against brucellae: the tube agglutination test (TAT), the rose Bengal test, the anti-*Brucella* Coombs test, and ELISA. The TAT is widely used, and a single titer of ≥160 or a fourfold rise in titer is considered significant.[3,38,74] A modification of the assay involving the use of 2-mercaptoethanol allows the measurement of only IgG. After cure, the IgG may be present for as long as 1 year, and the titer may increase in patients who relapse.[3,38] Detection of antibodies to *Brucella* cytoplasmic proteins by ELISA and Western blot in cerebrospinal fluid is another diagnostic approach in neurobrucellosis.[75]

A serologic cross-reaction can be seen between *Brucella* infection and *Yersinia enterocolitica* 0:9 infection. The S-LPS of smooth *Brucella* spp. contain two distinct epitopes, designated A and M. The A-epitope is identical to the already mentioned a-1,2-linked polymers of the pentasaccharide *N*-acetyl-4-amino-4,6-dideoxy-a-D-mannose, and comprises four or five sugar units. This epitope is present in *Yersinia enterocolitica* 0:9 and in *Brucella* spp., which explain this serologic cross-reactivity. The M-epitope seems to be specific for *Brucella* strains.[76]

Treatment

Appropriate antibiotics should have in vitro activity and adequate intracellular concentrations. Previous in vitro studies have shown that tetracycline, doxycycline, rifampicin, streptomycin and trimethoprim–sulfamethoxazole are active against *B. melitensis*.[3,26,27] In our study including 52 isolates of *B. melitensis*, all the isolates were susceptible to tetracycline, doxycycline, streptomycin, rifampicin, ceftriaxone, ceftizoxime, ciprofloxacin, and ofloxacin, but 15 isolates were inhibited at high concentrations of trimethoprim–sulfamethoxazole (MIC_{90} 4/76 mg/L). Erythromycin did not show good activity in vitro (MIC_{90} 32 mg/L).[77] *Brucella* species are extremely sensitive to many antimicrobials; however, the results of in vitro susceptibilty tests do not always predict clinical efficacy. Successful treatment of brucellosis requires prolonged chemotherapy with a combination of agents. Currently, monotherapy is unacceptable. The duration of therapy should be individualized according to any organ involvement, particularly nervous system, cardiovascular system and osteoarticular involvement.

The tetracyclines are, at present, the antibiotics of choice in brucellosis. A combination of doxycycline (100 mg twice daily for 6 weeks) and streptomycin (1 g/day for 2 weeks) has been used widely and successfully, with an associated relapse rate of 5%.[11,13,26,27,39] Combinations of gentamicin or netilmicin with doxycycline have also proven to be effective, and could cause fewer adverse reactions.[78,79]

Previously, the WHO suggested a combination of 500 mg of oral tetracycline four times per day for 6 weeks and intramuscular streptomycin at a daily dose of 1 g for the first 3 weeks. The current suggestion of the WHO is the combination of doxycycline (200 mg daily) and rifampicin (600–900 mg daily) for 6 weeks.[80] Recently, Solera et al,[13] in a large multicenter study of 194 patients, showed better results with doxycycline–streptomycin than with the doxycycline–rifampicin combination (7% versus 24% of failures, $P < 0.01$). The doxycycline–streptomycin regimen could prove to be more effective than the doxycycline–rifampicin regimen in patients with spondylitis.[11] Various regimens were given to 480 patients with brucellosis in our study, and 26 of these (5.4%) relapsed. One hundred and twenty-

five patients with no osteoarticular or nervous system involvement were given a combination of doxycycline and rifampicin. No therapeutic failure was observed, and relapse was determined in three (2.4%) patients in this group. The relapse rate in patients with osteoarticular involvement (12.1%) was higher than that in other patient groups (4.2%).[14]

Quinolones, especially ofloxacin and ciprofloxacin, have good activity in vitro.[77] Monotherapy with quinolones is unacceptable, as the relapse rate is over 21%.[81] In a small comparative clinical study, the ofloxacin–rifampicin combination was as effective as the doxycycline–rifampicin combination given over a 6-week period.[12]

Although brucellae are moderately sensitive to trimethoprim-sulfamethoxazole. It can be used as an alternative, particularly in children and pregnant women.[26,27,65] Azithromycin has also shown in vitro activity against *Brucella*, but a clinical trial with an azithromycin–gentamicin combination showed a high rate of therapeutic failure and relapse.[82]

The therapy for any organ involvement or complications is the same as for brucellosis without focal disease. A few localized forms may require surgery. Mortality due to *Brucella* endocarditis has been reduced significantly by a medical–surgical approach to treatment.[51] The duration of therapy must be individualized. In cases of osteoarticular involvement, particularly spondylitis, the duration of therapy should be at least 8 weeks (8–12 weeks).[6,14,41,43–47]

The preferred regimen in neurobrucellosis is a combination of doxycycline and rifampicin. Our initial suggestion is the addition of a third-generation cephalosporin (ceftriaxone for 2–3 weeks) to the combination. In our clinical study, in 24 cases with neurobrucellosis, no therapeutic failure or relapse were observed with the combination doxycyline–rifampicin–ceftriaxone. The duration of therapy in neurobrucellosis should be 8–12 weeks or longer.[14,48]

All regimens currently used have abated symptoms and reduced complications, but therapeutic failure or relapse has been seen. Those patients may require a second cure. Mortality is low in brucellosis. Three deaths (0.6%) (two due to neurobrucellosis, and one due to endocarditis) were seen among our cases.[14]

REFERENCES

1. Corbel MJ. Brucellosis: an overview. Emerg Infect Dis 1997; 3:213–221.
2. Roux J. Public health importance of brucellosis. In: Tümbay E, Hilmi S, Anğ Ö, eds. Brucella and brucellosis in man and animals. Yzmir: Ege Üniversity Press, 1991:3–10.
3. Shapiro DS, Wong JD. Brucella. In: Murray PR, Baron EJ, Phaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology, 7th edn. Washington, DC: American Society for Microbiology, 1999:625–631.
4. Sümerkan B, Gökahmetoğlu S, Eşel D. Brucella detection in blood: comparison of the Bac T/Alert Standard aerobic bottle, Bact T/Alert FAN aerobic bottle and BacT/Alert enhanced FAN aerobic bottle in simulated blood culture. Clin Microbiol Infect 2001; 7:369–372.
5. Memish Z, Mah MW, Al Mahmoud S, Al Shaalan M, Khan Y. Brucella bacteraemia: clinical and laboratory observation in 160 patients. J Infect 2000; 40:59–63.
6. Trujillo IZ, Zavala AN, Caceres JG, Miranda CQ. Brucellosis. Infect Dis Clin North Am 1994; 8:225–241.
7. Hall WH. Brucellosis. In: Evans AS, Brachman PS, eds. Bacterial infections of humans, epidemiology and control, 2nd edn. New York: Plenum Medical Book Co., 1991:133–149.
8. Sohn AH, Probert WS, Glaser CA, et al. Human neurobrucellosis with intracerebral granuloma caused by marine mammal Brucella spp. Emerg Infect Dis 2003; 9(4):485–488.
9. Memish Z. Brucellosis control in Saudi Arabia: prospects and challenges. J Chemother 2001; 13(suppl 1):11–17.
10. Fiori PL, Mastrandrea S, Rappelli R, Cappuccinelli P. Brucella abortus infection acquired in microbiology laboratories. J Clin Microbiol 2000; 38:2005–2006.
11. Ariza J, Gudiol F, Pallares R, et al. Treatment of human brucellosis with doxycycline plus rifampin or doxycycline plus streptomycin. Ann Intern Med 1992; 117:25–30.
12. Akova M, Uzun Ö, Akalın HE, Hayran M, Ünal S, Gür D. Quinolones in treatment of human brucellosis: comparative trial of ofloxacin–rifampin versus doxycycline–rifampin. Antimicrob Agents Chemother 1993; 37:1831–1834.
13. Solera J, Rodriques-Zapata M, Geijo P, et al. Doxycycline–rifampin versus doxycycline–streptomycin in treatment of human brucellosis due to Brucella melitensis. Antimicrob Agents Chemother 1995; 39:2061–2067.
14. Aygen B, Doganay M, Sümerkan B, Yıldız O, Kayabaş Ü. Clinical manifestations, complications and treatment of brucellosis: an evaluation of 480 patients. Med Mal Infect 2002; 32:485–493.
15. Moreno S, Ariza J, Espinosa FJ, et al. Brucellosis in patients infected with the human immunodeficiency virus. Eur J Clin Microbiol Infect Dis 1998; 17:319–326.
16. Doganay M, Aygen B, İnan M, Özbakır Ö. Brucella peritonitis in a cirrhotic patients with ascites. Eur J Med 1993; 2:441–442.
17. Demirkan F, Akalın HE, Şimşek H, Özyılkan E, Telatar H. Spontaneous peritonitis due to Brucella melitensis in a patient with cirrhosis. Eur J Clin Microbiol Infect Dis 1993; 12:66–67.
18. Ruben B, Band JD, Wong P, Colville J. Person-to-person transmission of Brucella melitensis. Lancet 1991; 337:14–15.
19. Goossens H, Marcelis L, Dekeyser P, Butzler JP. Brucella melitensis: person to person transmission. Lancet 1983; 1:773.
20. Naparstek E, Block CS, Slavin S. Transmission of brucellosis by bone marrow transplantation. Lancet 1982; 1: 574.
21. Doganay M, Aygen B, Eşel D. Brucellosis due to blood transfusion. J Hosp Infect 2001;49:151–152.
22. T.C. Sağlık Bakanlığı Temel Sağlık Hizmetleri Genel Müdürlüğü. Temel Sağlık Hizmetleri Genel Müdürlüğü Çalışma Yılığı 1996–1999. Ankara: Turkish Ministry of Health, 2001.
23. Çetin ET, Çoral B, Bilgiç A, ve ark. Türkiye'de insanda

bruselloz insidansının saptanması. Turkish J Med Sci 1990; 14:324–334.
24. Doyle TJ, Bryan RT. Infectious disease morbidity in the US region bordering Mexico, 1990–1998. J Infect Dis 2000; 182:1503–1510.
25. Steffen R. Antacids: a risk factor in travellers brucellosis? Scand J Infect Dis 1977; 9:311–312.
26. Young EJ. Brucellosis. In: Emmerson AM, Hawkey PM, Gillespie SH, eds. Principles and practice of clinical bacteriology. Chichester: Wiley, 1997:337–348.
27. Young EJ. Brucella species. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and practice of infectious diseases, Vol. 2. Philadelphia: Churchill Livingstone, 2000:2386–2392.
28. Gorvel JP, Moreno E. Brucella intracellular life: from invasion to intracellular replication. Vet Microbiol 2002; 90:281–297.
29. Pizarro-Cerda J, Moreno E, Gorvel JP. Invasion and intracellular trafficking of Brucella abortus in nonphagocytic cells. Microb Infect 2000; 2:829–835.
30. Naroeni A, Porte F. Role of cholesterol and ganglioside GM(1) in entry and short term survival of Brucella suis in murine macrophages. Infect Immun 2002; 70:1640–1644.
31. Watarai M, Makino S, Fujii Y, Okamoto K, Shirahata T. Modulation of Brucella-induced macropinocytosis by lipid rafts mediates intracellular replication. Cell Microbiol 2002; 4:341–355.
32. Lopez-Goni I, Guzman-Verri C, Manterola L, Sola-Landa A, Moriyon I, Moreno E. Regulation of Brucella virulence by the two-component system BvrR/BvrS. Vet Microbiol 2002; 90:329–339.
33. DelVecchio VG, Kapatral V, Elzer P, Patra G, Mujer CS. The genome of Brucella melitensis. Vet Microbiol 2002; 90:587–592.
34. Boschiroli ML, Ouahrani-Bettache S, Foulongne V, et al. Type IV secretion and Brucella virulence. Vet Microbiol 2002; 90:341–348.
35. Lopez-Urrutia L, Alonso A, Bayon Y, Nieto ML, Orduna A, Crespo MS. Brucella lipopolysaccharides induce cyclooxygenase-2 expression in monocytic cells. Biochem Biophys Res Commun 2001; 289:372–375.
36. Caron E, Peyrard T, Köhler S, Cabane S, Liautard J-P, Dornand J. Live Brucella spp. fail to induce tumor necrosis factor alpha excretion upon infection of U937-derived phagocytes. Infect Immun 1994; 62:5267–5274.
37. Lopez-Urrutia L, Alonso A, Nieto ML, Bayon Y, Orduna A, Crespo MS. Lipopolysaccharides of Brucella abortus and Brucella melitensis induce nitric oxide synthesis in rat peritoneal macrophages. Infect Immun 2000; 68:1740–1745.
38. Ariza J, Pellicer T, Pallares R, Foz A, Gudiol F. Specific antibody profile in human brucellosis. Clin Infect Dis 1992; 14:131–140.
39. Young EJ. Human brucellosis. Rev Infect Dis 1983; 5:821–842.
40. Colmenero JD, Reguera JM, Fernandez-Nebro A, Cabrero-Franquela F. Osteoarticular complications of brucellosis. Ann Rheum Dis 1991; 50:23–26.
41. Mousa ARM, Muhtaseb SA, Almudallal DS, Khodeir SM, Marafie AA. Osteoarticular complications of brucellosis: a study of 169 cases. Rev Infect Dis 1987; 9: 531–543.
42. Taşova Y, Saltoğlu N, Şahin G, Aksu HZS. Osteoarticular involvement of brucellosis in Turkey. Clin Rheumatol 1999; 18:214–219.
43. Ariza J, Pujol M, Valverde J, et al. Brucellar sacroiliitis: findings in 63 episodes and current relevance. Clin Infect Dis 1993; 16:761–765.
44. Tekkök IH, Berker M, Özcan OE, Özgen T, Akalin E. Brucellosis of spine. Neurosurgery 1993; 33:838–844.
45. Solera J, Lozano E, Martinez-Alfaro E, Espinosa A, Castillejos ML, Abad L. Brucella spondylitis: review of 35 cases and literature survey. Clin Infect Dis 1999; 29:1440–1449.
46. Mousa AM, Bahar RH, Araj GF, et al. Neurological complications of brucella spondylitis. Acta Neurol Scand 1990; 81:16–23.
47. Aygen B, Eşel D, Erdoğan N, Yildiz O, Sümerkan B, Doganay M. Psoas abscess secondary to brucellar spondylitis. Turkish J Med Sci 2002; 32:81–83.
48. Aygen B, Sümerkan B, Mirza M, Doganay M, Arman F. Treatment of neurobrucellosis with a combination of ceftriaxone, rifampicin and doxycycline. Med Mal Infect 1996; 26:1199–1201.
49. Bouza E, Torre MG, Parras F, Guerrero A, Rodriguez-Creixems M, Gobernado J. Brucellar meningitis. Rev Infect Dis 1987; 9:810–822.
50. McLean DR, Russel N, Khan MY. Neurobrucellosis: clinical and therapeutic features. Clin Infect Dis 1992; 15:582–590.
51. Hadjinikolaou L, Triposkiadis F, Zairis M, Chlapoutakis E, Spyrau P. Succesful management of Brucella melitensis endocarditis with combined medical and surgical approach. Eur J Cardiothorac Surg 2001; 19:806–810.
52. Al-Kasab S, Al-Fagih MR, Al-Yousef S, et al. Brucella infective endocarditis. J Thorac Cardiovasc Surg 1988; 95:862–867.
53. Al-Mudallal DS, Mousa ARM, Marafie AA. Apyrexic Brucella melitensis aortic valve endocarditis. Trop Geogr Med 1989; 41:372–376.
54. Aygen B, Sümerkan B, Yıldız O, Doganay M. An unusual manifestation of brucellosis: a case with multisystemic involvement and adult respiratory distress syndrome. Probl Infect Parasitic Dis 2001; 29:10–11.
55. Aygen B, Sümerkan B, Kardaş Y, Doganay M, İnan M. Brucelloz: 183 olgunun değerlendirilmesi. Klimik Dergisi 1995; 8:13–16.
56. Crosby E, Llosa L, Quesada MM, Carrillo C, Gotuzzo E. Hematologic changes in brucellosis. J Infect Dis 1984; 150:419–424.
57. Paton NI, Tee NWS, Vu CKF, Teo TP. Visceral abscesses due to Brucella suis infection in a retired pig farmer. Clin Infect Dis 2001; 32:129–130.
58. Ariza J, Pigrau C, Canas C, et al. Current understanding and management of chronic hepatosplenic suppurative brucellosis. Clin Infect Dis 2001; 32:1024–1033.
59. Young EJ. Brucella melitensis hepatitis: the absence of granulomas. Ann Intern Med 1979; 91:414–415.
60. Young EJ, Tarry A, Genta RM, Ayden N, Gotuzzo E. Thrombocytopenic purpura associated with brucellosis: report of 2 cases and literature review. Clin Infect Dis 2000; 31:904–909.
61. İbrahim AIA, Awad R, Shetty SD, Soad M, Bilal ME. Genito-urinary complications of brucellosis. Br J Urol 1988; 61:294–298.
62. Memish ZA, Venkatesh S. Brucella epididymo-orchitis in Saudi Arabia: a retrospective study of 26 cases and review of the literature. BJU Int 2001; 88:72–76.

63. Navarro-Martinez A, Solera J, Corredoira J, et al. Epididymoorchitis due to Brucella melitensis: a retrospective study of 59 patients. Clin Infect Dis 2001; 33:2017–2022.
64. Aygen B, Sümerkan B, Doğanay M, Sehmen E. Prostatitis and hepatitis due to Brucella melitensis: a case report. J Infect 1998; 36:111–127.
65. Khan MY, Mah MW, Memish ZA. Brucellosis in pregnant women. Clin Infect Dis 2001; 32:1172–1177.
66. FrancoVicoria R, Balpardo J, Santamaria JM, et al. Cutaneous vasculitis in a patient with acute brucellosis. Dermatologica 1985; 171:126–128.
67. Berger TG, Guill MA, Goette DK. Cutaneous lesions in brucellosis. Arch Dermatol 1981; 117:40–42.
68. Mili M, Auckenthaler R, Micod LP. Chronic brucella empyema. Chest 1993; 103:620–621.
69. Gelfand MS, Kaiser AB, Dale WA. Localized brucellosis: popliteal artery aneurysm, mediastinitis, dementia and pneumonia. Rev Infect Dis 1989; 11:783–788.
70. 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.
71. Queipo-Ortuno MI, Morata P, Ocan P, Manchado P, Calmenero JD. Rapid diagnosis of human brucellosis by peripheral blood PCR assay. J Clin Microbiol 1997; 35:2927–2930.
72. Morata P, Queipo-Ortuno MI, Reguera JM, Miralles F, Loper-Gonzalez JJ, Colmenero JD. Diagnostic yield of a PCR assay in focal complications of brucellosis. J Clin Microbiol 2001; 39:3743–3746.
73. Morata P, Queipo-Ortuna MI, Requera JM, Garcia-Ordonez MA, Pichardo C, Colmenero JD. Posttreatment follow-up of brucellosis by PCR assay. J Clin Microbiol 1999; 37:4163–4166.
74. 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.
75. Baldi PC, Araj GF, Racaro GC, Wallach JC, Fossati CA. Detection of antibodies to Brucella cytoplasmic proteins in the cerebrospinal fluid of patients with neurobrucellosis. Clin Diagn Lab Immunol 1999; 6:756–759.
76. Kittelberger R, Bundesen PG, Cloeckaert A, Greiser-Wilke I, Letesson JJ. Serological cross-reactivity between Brucella abortus and Yersinia enterocolitica 0:9: IV. Evaluation of the M- and C-epitope antibody response for the specific detection of B. abortus infection. Vet Microbiol 1998; 60:45–57.
77. Sümerkan B, Doganay M, Bakışkan V, Fazlı ŞA, Aygen B. Antimicrobial susceptibility of clinical isolates of Brucella melitensis. Turkish J Med Sci 1993; 18:17–22.
78. Solera J, Espinosa A, Geijo P, Martinez-Alfaro E, Saez L. Treatment of human brucellosis with netilmicin and doxycycline. Clin Infect Dis 1996; 22:441–445.
79. Solera J, Espinosa A, Martinez-Alfaro E, et al. Treatment of human brucellosis with doxycycline and gentamicin. Antimicrob Agents Chemother 1997; 41:80–84.
80. Joint FAO/WHO Expert Committee on Brucellosis (sixth report). WHO technical report series no. 740. Geneva: World Health Organization, 1986:56–57.
81. Doganay M, Aygen B. Use of ciprofloxacin in the treatment of brucellosis. Eur J Clin Microbiol Infect Dis 1992; 11:74–75.
82. Solera J, Beato JL, Martinez-Alfaro E, et al. Azithromycin and gentamycin therapy for the treatment of humans with brucellosis. Clin Infect Dis 2001; 32:506–509.