REVIEW ARTICLE

MEDICAL PROGRESS

Brucellosis

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BRUCELLOSIS, LIKE TUBERCULOSIS, IS A CHRONIC GRANULOMATOUS INfection caused by intracellular bacteria and requires combined, protracted antibiotic treatment. The disease causes much clinical morbidity as well as a considerable loss of productivity in animal husbandry in the developing world. In this era of international tourism, brucellosis has become a common imported disease in the developed world.

Brucellosis has been present for millennia[1] and has managed to elude eradication, even in most developed countries.[2,3] A high prevalence in certain geographic areas is well recognized, although largely underestimated (Table 1). The relationship between the disease and individual socioeconomic status is exemplified in the United States, where programs to eradicate brucellosis have successfully limited the annual incidence of the disease, which now occurs predominantly in California and Texas (which account for more than half of the U.S. cases), with relatively high rates of incidence in North Carolina, Illinois, Florida, Wyoming, Iowa, and Arizona. The disease usually presents in Hispanic populations and is probably related to the illegal importation of unpasteurized dairy products from neighboring Mexico, where the disease is endemic.[4,5]

THE BACTERIUM

Brucella belongs to the $\alpha 2$ subdivision of the proteobacteria, along with ochrobactrum, rhizobium, rhodobacter, agrobacterium, bartonella, and rickettsia.[6] The traditional classification of brucella species is largely based on its preferred hosts. There are six classic pathogens, of which four are recognized human zoonoses. The presence of rough or smooth lipopolysaccharide correlates with the virulence of the disease in humans. Two new brucella species, provisionally called *Brucella pinnipediae* and *B. cetaceae*, have been isolated from marine hosts within the past few years.[7,8]

Taxonomic characteristics of brucella species and biotypes[9] are summarized in Table 2. Brucella is a monospecific genus that should be termed *B. melitensis*, and all other species are subtypes, with an interspecies homology above 87 percent. The phenotypic difference and host preference can be attributed to various proteomes, as exemplified by specific outer-membrane protein markers.[10] All brucella species seem to have arisen from a common ancestor to which *B. suis* biotype 3 shares particular similarity.[11] Although the scientific accuracy of this classification cannot be disputed, its practicality has been under scrutiny.

THE *B. MELITENSIS* GENOME

The complete sequencing of the *B. melitensis* genome was achieved in 2002.[12] The complete sequencing of *B. abortus*[13] and *B. suis*[14] has recently been accomplished as well. *B. melitensis* contains two circular replicons of 1.1 and 2.2 Mb, respectively, with a 57 per-

Table 1. Annual Cases of Human Brucellosis in Various Countries, According to Year.*

Country	1996	1997	1998	1999	2000	2001	2002	2003
Albania	NA	155	376	458	220	NA	NA	NA
Algeria	4356	3,434	2,232	2,223	NA	3,200	NA	2,766
Argentina	NA	676	NA	353	507	NA	296	325
Australia	38	41	45	52	27	NA	40	17
Azerbaijan	NA	624	494	582	654	660	519	407
Bosnia-Herzegovina	NA	NA	NA	NA	NA	7	NA	48
Colombia	53	42	82	42	NA	27	NA	238
Germany	23	25	18	21	27	25	35	27
Greece	NA	254	435	543	545	405	327	222
Iran	NA	NA	NA	17,168	NA	NA	NA	17,765
Israel	235	151	197	163	131	70	56	56
Italy	1896	1,681	1,461	1,324	1,067	923	813	520
Jordan	957	NA	684	432	288	275	219	159
Kyrgyzstan	NA	NA	NA	973	1,219	1,819	1,771	NA
Lebanon	192	429	136	184	NA	NA	NA	NA
Mexico	3362	3,387	3,550	2,719	2,171	3,013	2,851	3,008
Peru	1691	NA	1,269	NA	1,072	372	991	NA
Portugal	866	1,409	816	683	500	381	206	139
Russia	656	461	NA	352	423	508	595	NA
Saudi Arabia	5997	15,933	5,781	NA	NA	NA	NA	NA
Spain	NA	878	1,520	1,519	1,104	887	886	596
Syria	NA	NA	NA	NA	6,487	4,500	NA	23,297
Tajikistan	257	NA	211	NA	851	752	1,071	1,471
Tunisia	490	291	206	355	NA	321	250	128
Turkey	9480	11,812	11,427	11,462	10,742	15,510	17,553	14,435
Turkmenistan	NA	496	NA	NA	264	246	NA	NA
United Kingdom	15	6	7	76	19	26	38	19
United States	112	98	79	82	87	136	125	93
Uzbekistan	707	459	494	480	NA	NA	408	NA

* Data are from the Office International des Epizooties and various national health ministries. These numbers are believed to be a massive underestimation of the true prevalence of the disease. NA denotes not available.

cent GC content and no plasmids; 3197 open reading frames were sequenced, 2487 of which had an assigned function. *B. abortus* biovars 1 and 4 and *B. suis* biotype 1 are remarkably similar to *B. melitensis*. In contrast, *B. suis* biotypes 2 and 4 are composed of two replicons of 1.35 and 1.85 Mb, respectively, whereas *B. suis* biotype 3 is composed of a single circular replicon of 3.3 Mb.

PATHOGENETIC FEATURES

The series of host–microbe interactions that takes place in humans differs in many crucial steps from the pathogenetic mechanisms first recognized in animal models.[15] Brucella is unusual in several ways. First, the bacterium does not bear classic virulence factors, such as exotoxins or endotoxins, and its lipopolysaccharide pathogenicity is not typical. Second, it exhibits a tendency to invade and persist in the human host through inhibition of programmed cell death.[16]

Brucella invades the mucosa, after which phagocytes ingest the organisms. In so-called nonprofessional phagocytes, internalization requires the expenditure of energy, and inhibitors of energy metabolism and receptor-mediated endocytosis can

Table 2. Nomenclature and Characteristics of Brucella Species.

Species	Biotype	Animal Hosts	First Described	Human Virulence*	Species Discrimination
B. melitensis	1–3	Goats, sheep, camels	Bruce, 1887	++++	Fuchsin, positive; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive in 24 hr; CO_2 growth, negative; Tiblisi phage lysis, negative; Weybridge phage lysis, negative
B. abortus	1–6, 9	Cows, camels, yaks, buffalo	Bang, 1897	++ to +++	Fuchsin, positive (except biotype 2); thionine, negative (biotypes 1, 2, and 4); safranin inhibition, negative; H_2S production, positive (except biotype 5); urease, positive in 24 hr; CO_2 growth, positive (biotypes 1–4); Tiblisi phage lysis, positive; Weybridge phage lysis, positive
B. suis	1–5	Pigs (biotypes 1–3), wild hares (biotype 2), caribou (biotype 4), reindeer (biotype 4), wild rodents (biotype 5)	Traum, 1914	+	Fuchsin, negative (except biotype 3); thionine, positive; safranin inhibition, positive; H_2S production, positive (biotype 1); urease, positive in 15 min; CO_2 growth, negative; Tiblisi phage lysis, negative; Weybridge phage lysis, positive
B. canis	—	Canines	Carmichael and Bruner, 1968	+	Fuchsin, positive or negative; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive in 15 min; CO_2 growth, negative; Tiblisi phage lysis, negative; Weybridge phage lysis, negative
B. ovis	—	Sheep	Van Drimmelen, 1953	–	Fuchsin, negative for some strains; safranin inhibition, negative; H_2S production, negative; urease, negative; CO_2 growth, positive; Tiblisi phage lysis, negative; Weybridge phage lysis, negative
B. neotomae	—	Rodents	Stoenner and Lackman, 1957	–	Fuchsin, negative; safranin inhibition, negative; H_2S production, positive; urease, positive in 15 min; CO_2 growth, negative; Tiblisi phage lysis, positive or negative; Weybridge phage lysis, positive
B. pinnipediae and *B. cetaceae* (provisional)	—	Minke whales, dolphins, porpoises (pinnipediae), seals (cetaceae)	Ewalt and Ross, 1994	+	Fuchsin, positive; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive; CO_2 growth, negative for pinnipediae and positive for cetaceae; Tiblisi phage lysis, negative; Weybridge phage lysis, positive for pinnipediae and negative for cetaceae

* Virulence is graded on a scale from no virulence (–) to the highest degree of virulence (++++).

suppress this response.[17] Brucella has a two-component system called BvrS/BvrR, which codes for a histidine kinase sensor and controls the expression of molecular determinants necessary for cell invasion.[18] After ingestion, the majority of brucellae are rapidly eliminated by phagolysosome fusion. Of those bacteria, 15 to 30 percent survive[19] in gradually evolving brucellae-containing compartments, in which rapid acidification takes place. How this unique environment is formed is incompletely understood, but it is responsible for limiting antibiotic action and explains the discrepancy between in vitro studies and in vivo events.[20] The induction of the virB operon through a type IV secretion system (a system by which macromolecules are transferred) is of paramount importance during brucella intracellular movement.[21] Replication of the bacterium takes place in the endoplasmic reticulum without affecting host-cell integrity. After replication, brucellae are released with the help of hemolysins and induced cell necrosis (Fig. 1).[22]

THE HOST RESPONSE IN HUMANS

The host response in humans reflects unique features of brucella. Smooth lipopolysaccharide does not activate the alternative complement pathway. Brucella is resistant to damage from polymorphonuclear cells owing to suppression of the myeloperoxidase–hydrogen peroxide–halide system and copper–zinc superoxide dismutase and the production of inhibitors of adenylate monophosphate and guanyl monophosphate. Impaired activity of natural killer cells and impaired macrophage generation of reactive oxygen intermediates and interferon regulatory factors have been documented.[23-25] CD4 lymphocytes play a limited role, acting either by facilitating clonal expansion of other cytolytic cells, as CD8, or by functioning as cytolytic effectors. An increase of γ/δ CD4 and CD8 lymphocytes is characteristic in brucellosis,[26] as is the importance of a Vγ9Vδ T-cell receptor.[27]

Studies using volunteers who have been vaccinated with the Rev 1 vaccine against *B. melitensis* have delineated the evolution of specific antibodies against brucellae. Class M immunoglobulins against lipopolysaccharide appeared during the first week of infection, followed by class G immunoglobulins as early as the second week. Both classes of immunoglobulin peaked during the fourth week, and the use of antibiotics was associated with a decline in both class M and class G titers. Class M titers persisted at levels that were higher than those of class G titers for more than six months, and both classes were present for almost a year. The appearance of class A immunoglobulins in conjunction with class G immunoglobulins for longer than six months was consistent with the presence of chronic disease. Antibody response in brucellosis, although extremely useful diagnostically, plays a limited part in the overall host response.

Interferon-γ has a central role in the pathogenesis of brucellosis[28,29] by activating macrophages, producing reactive oxygen species and nitrogen intermediates; by inducing apoptosis, enhancing cell differentiation and cytokine production; by converting immunoglobulin G to immunoglobulin G2a; and by increasing the expression of antigen-presenting molecules. That interferon-γ has a central role in the evolution of brucellosis is highlighted by the effect of a genetic polymorphism in interferon-γ (the +874A allele). Patients who are homozygous for the +847 allele may be relatively more susceptible to brucellosis and — in an interesting note — to tuberculosis.[30] Typically, serum interferon-γ levels in patients with brucellosis are increased.[31,32]

In contrast, the importance of tumor necrosis factor α (TNF-α) in human brucellosis is the subject of debate. Although the induction of TNF-α was noted in murine models of brucellosis, the inhibition of TNF-α in human disease is an early, crucial step in infection. This inhibition may also be involved in the impaired activation and cytotoxic function of natural killer cells owing to an active bacterial mechanism that involves outer-membrane protein 25, which has been identified as the downregulator of TNF-α.[33] Serum levels of TNF-α were undetectable in patients with active brucellosis in one study,[32] but another study reported that serum levels were increased in a linear fashion with serum levels of interferon-γ and other inflammatory markers.[31] The role of interleukin-12, mainly as a regulator of interferon-γ production, has been extensively studied in animal models and humans.[32,34]

HUMAN DISEASE

Transmission of brucellosis to humans occurs through the consumption of infected, unpasteurized animal-milk products, through direct contact with infected animal parts (such as the placenta by inoculation through ruptures of skin and mucous membranes), and through the inhalation of infected aerosolized particles. Brucellosis is an occupational

disease in shepherds, abattoir workers, veterinarians, dairy-industry professionals, and personnel in microbiologic laboratories. One important epidemiologic step in containing brucellosis in the community is the screening of household members of infected persons.[35]

Consumption of unpasteurized dairy products — especially raw milk, soft cheese, butter, and ice cream — is the most common means of transmission. Hard cheese, yogurt, and sour milk are less hazardous, since both propionic and lactic fermentation takes place. Bacterial load in animal muscle tissues is low, but consumption of undercooked traditional delicacies such as liver and spleen has been implicated in human infection.

Airborne transmission of brucellosis has been studied in the context of using brucella as a biologic weapon. In fact, *B. suis* was the first agent contemplated by the U.S. Army as a potential biologic weapon[36] and is still considered in that category. In a hypothetical attack scenario, it was estimated that release of an aerosolized form of brucella under optimal circumstances for dispersion would cause 82,500 cases of brucellosis and 413 fatalities.[37] Cases of laboratory-acquired brucellosis are the perfect examples of airborne spreading of the disease.[38]

After entering the human body and being taken up by local tissue lymphocytes, brucellae are transferred through regional lymph nodes into the circulation and are subsequently seeded throughout the body, with tropism for the reticuloendothelial system. The period of inoculation usually ranges from two to four weeks.

The classic categorization of brucellosis as acute, subacute, or chronic is subjective and of limited clinical interest. Four species of brucella can cause

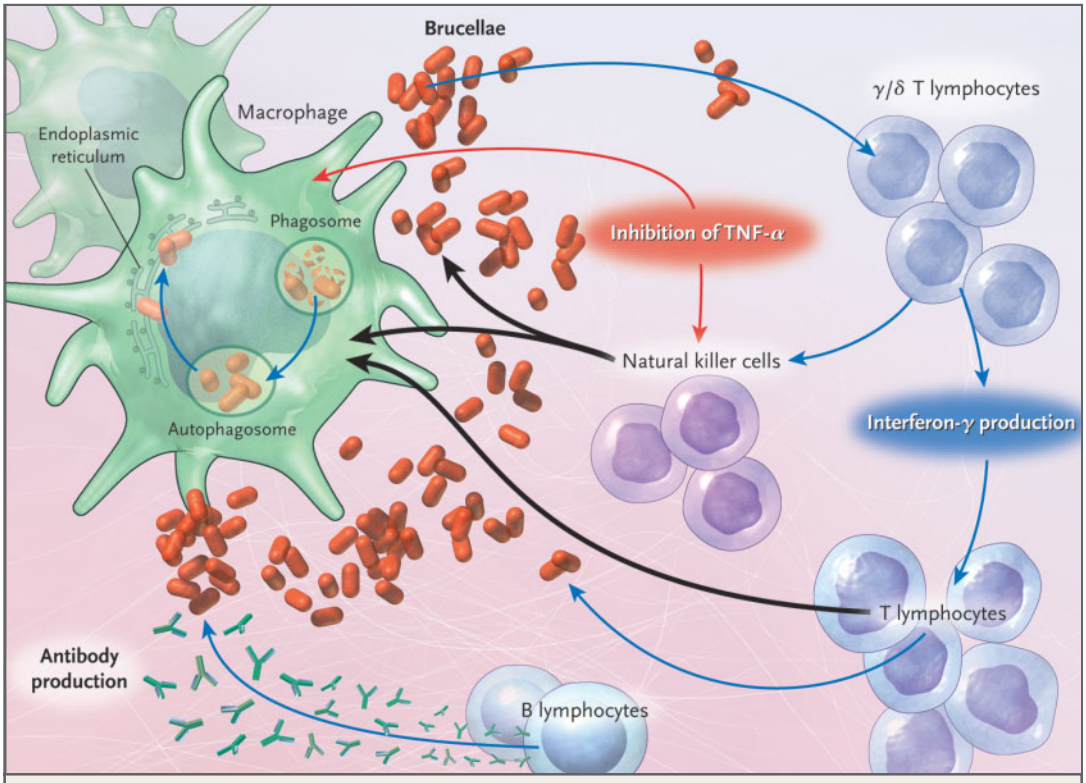

Figure 1. Schematic Representation of Major Events in the Pathogenesis of Brucellosis and the Host Immune Response.
Brucellae enter the macrophages, where the minority of the bacteria survive in specialized evolving compartments and multiply in the endoplasmic reticulum. The inhibition of tumor necrosis factor α (TNF-α) by the bacteria disrupts the bactericidal effect of natural killer cells and macrophages. Interferon-γ production induces a bactericidal effect by natural killer cells and T lymphocytes directly and through macrophage induction. Antibody production by B lymphocytes is also induced but plays a minor role in the immune response. T lymphocytes include both helper and suppressor cells, depending on the stage of the disease. Red arrows indicate negative effect, blue arrows positive effect, and black arrows killing effect.

human disease: *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*. Disease from marine species has also emerged.[39] The vast majority of cases worldwide are attributed to *B. melitensis*. A recent study did not report any clinical differences between cases caused by *B. melitensis* and those caused by *B. abortus*.[40] Sufficient data on virulence and clinical presentation of biotypes of *B. melitensis* are lacking, although separate biotypes that predominate in various regions — for example type 2 in northwestern Greece, type 3 in Turkey,[41] and type 1 in Spain[42] — may account for variations in clinical presentation (Table 3).

Human brucellosis is traditionally described as a disease of protean manifestations. However, fever is invariable and can be spiking and accompanied by rigors, if bacteremia is present, or may be relapsing, mild, or protracted. Malodorous perspiration is almost pathognomonic. Constitutional symptoms are generally present. Physical examination is generally nonspecific, though lymphadenopathy, hepatomegaly, or splenomegaly is often present.

Osteoarticular disease is universally the most common complication of brucellosis, and three distinct forms exist — peripheral arthritis, sacroiliitis, and spondylitis. Peripheral arthritis is the most common and is nonerosive, since it usually involves the knees, hips,[43] ankles, and wrists in the context of acute infection. Prosthetic joints can also be affected in peripheral arthritis. Brucellosis has also been proposed as a cause of reactive arthritis. A second form, characterized by sacroiliitis, is readily diagnosed, also usually in the context of acute brucellosis.[44] On the other hand, a third form of osteoarticular disease, spondylitis, remains notoriously difficult to treat and often seems to result in residual damage.[45] The lumbar spine is the usual site of involvement. Spondylitis can be easily diagnosed with plain radiography, in which the characteristic Pons sign (a steplike erosion of the anterosuperior vertebral margin) can be identified, or with scintigraphy and magnetic resonance imaging. The latter imaging technique is popular and produces impressive scans but is costly and not always available. Osteoarticular complications are sometimes linked to a genetic predisposition, with recent data suggesting an association with HLA-B39.[46]

The reproductive system is the second most common site of focal brucellosis. Brucellosis can present as epididymoorchitis in men and is often difficult to differentiate from other local disease.[47] The effect of the local inflammation on subsequent testicular function has not been adequately studied. Brucellosis in pregnancy poses a substantial risk of spontaneous abortion.[48]

Table 3. Clinical Presentation of Human Brucellosis.*

Features	Percentage of Cases
Signs and symptoms	
Fever	91
Constitutive symptoms (e.g., malaise, arthralgias)	26
Hepatomegaly	17
Splenomegaly	16
Lymphadenopathy	7
Complications	
Peripheral arthritis	22 (8 in hips, 7 in knees, 4 in elbows, 4 in wrists, 4 in other locations)†
Sacroiliitis	3
Spondylitis	19 (15 lumbar, 3 dorsal, 1 cervical)
Central nervous system disorders	3
Epididymoorchitis	5.7‡
Vomiting and diarrhea	3
Respiratory disorders	6
Rashes	3
Cardiovascular disorders	0
Laboratory findings	
Hematologic	49 (40 relative lymphocytosis, 5 isolated thrombocytopenia, 2 isolated leukopenia, 2 pancytopenia)
Transaminasemia	24
Positive blood cultures	16
Rate of relapse	4

* Data are from the most recent 100 patients who received the diagnosis of brucellosis at the University Hospital of Ioannina and whose cases were followed for at least a year.
† Some of the patients had polyarthritis.
‡ Data are for 70 male patients.

Hepatitis is common, usually manifesting as mild transaminasemia. Liver abscess and jaundice are rare.[49] Granulomas can be present in liver-biopsy specimens in cases of both *B. melitensis* and *B. abortus*.[50] Ascites is often present, either as a temporary exacerbation of preexisting hepatic disease or as frank peritonitis.[51]

The central nervous system is involved in 5 to 7 percent of cases in most studies, and such com-

plications often have an ominous prognosis. Meningitis, encephalitis, meningoencephalitis, meningovascular disease, brain abscesses, and demyelinating syndromes have all been reported.[52]

Endocarditis remains the principal cause of mortality in the course of brucellosis. It usually involves the aortic valve and typically requires immediate surgical valve replacement. Early recognition, adequate antibiotic treatment, and the absence of signs of heart failure can guide the practitioner toward prolonged, conservative treatment.[53]

Respiratory complications of brucellosis are considered rare. A recent multinational review of cases with respiratory complications indicated that approximately 16 percent of cases had pulmonary involvement that included lobar pneumonia and pleural effusions.[54]

In sum, practically every organ and system of the human body can be affected in brucellosis — a fact that underscores the importance of including brucellosis in the differential diagnosis in areas of endemic disease, even if clinical features are not entirely compatible.

The blood count is often characterized by mild leukopenia and relative lymphocytosis, along with mild anemia and thrombocytopenia. Pancytopenia in brucellosis is multifactorial and is attributed to hypersplenism and bone marrow involvement. Rarely, marked pancytopenia or isolated deficits can be attributed to diffuse intravascular coagulation, hemophagocytosis, or immunologically mediated cellular destruction.[55,56]

SPECIAL SITUATIONS

Relapses, at a rate of about 10 percent, usually occur in the first year after infection,[57] are often milder in severity than the initial disease, and can be treated with a repeated course of the usual antibiotic regimens. Most cases of relapse are caused by inadequate treatment or are associated with characteristics of the initial infection that include a duration of less than 10 days, male sex, bacteremia, and thrombocytopenia.[58] Childhood brucellosis generally exhibits a more benign course in terms of the rate and severity of complications and the response to treatment.[59]

Although the relationship between brucellosis and T-cell–mediated immunity has been well described, brucellosis is not an opportunistic infection in patients who are infected with the human immunodeficiency virus (HIV) or who have AIDS, even in areas of endemic disease. Most patients with HIV infection and brucellosis have a benign clinical course in the early stages of HIV infection, according to the number of CD4+ T lymphocytes.[60]

DIAGNOSIS

The development of a definitive diagnostic test for brucellosis remains an elusive target. Ever since the development of the first serologic test for brucellosis by Bruce more than a century ago, a definitive diagnostic technique has been actively pursued.

The absolute diagnosis of brucellosis requires isolation of the bacterium from blood or tissue samples. The sensitivity of blood culture varies, depending on individual laboratory practices and how actively the obtaining of cultures is pursued. The percentage of cases with positive cultures ranges from 15 to 70 percent.[61] Brucellae are cultured in standard biphasic (solid and liquid) mode or with the Castaneda bottle, which incorporates both solid and liquid mediums in the same container. Automated systems are also reliable in isolating brucella.[62] Blood-culture sensitivity may be improved by a lysis-centrifugation technique.[63] Even with automated systems, subcultures should be performed for at least four weeks. Brucellae are small, gram-negative and oxidase- and urease-positive coccobacilli that resemble fine grains of sand. Catalase tests, which can have positive results for brucella, should not be performed because the technique can cause the nebulization of particles. Species identification is performed on the basis of particular characteristics (Table 2).

Bone marrow cultures are considered the gold standard for the diagnosis of brucellosis, since the relatively high concentration of brucella in the reticuloendothelial system makes it easier to detect the organism. Furthermore, bacterial elimination from the bone marrow is equivalent to microbial eradication.[64] However, harvesting bone marrow for culture remains an invasive, painful technique, and results have not been universally reproducible.

There are two broad categories of serologic methods for diagnosing brucellosis: those based on antibody production against lipopolysaccharide and those based on antibody production against other bacterial antigens. Developed by Bruce, the serum agglutination test remains the most popular diagnostic tool for brucellosis. Titers above 1:160 are considered diagnostic in conjunction with a compatible clinical presentation. However, in areas

of endemic disease, using a titer of 1:320 as diagnostic may be more specific. Seroconversion and evolution of the titers can also be used in diagnosis. Drawbacks of the serum agglutination test include the inability to diagnose *B. canis* infections; the appearance of cross-reactions of class M immunoglobulins with *Francisella tularensis*, *Escherichia coli* O116 and O157, *Salmonella urbana*, *Yersinia enterocolitica* O:9, *Vibrio cholerae*, *Xanthomonas maltophilia*, and *Afipia clevelandensis*; and the percentage of cases in which seroconversion does not occur. Lack of seroconversion can be attributed to the performance of tests early in the course of infection, the presence of blocking antibodies, or the so-called "prozone" phenomenon (i.e., the inhibition of agglutination at low dilutions due to an excess of antibodies or to nonspecific serum factors).[65] Some of these shortcomings can be overcome by modifications such as the addition of EDTA, 2-mercaptoethanol, or antihuman globulin. Other variations of agglutination tests[66] have not proven superior. A new dipstick test, however, offers a rapid and reliable diagnostic alternative in acute brucellosis.[67] The superiority of most of the other agglutination tests over the serum agglutination test has not been consistently proven. Serum agglutination tests have a major drawback in that they are not suitable for patient follow-up, since titers can remain high for a prolonged period.[68]

Indirect enzyme-linked immunosorbent assays (ELISAs) typically use cytoplasmic proteins as antigens. ELISA measures class M, G, and A immunoglobulins, which allows for a better interpretation of the clinical situation and overcomes some of the shortcomings of the serum agglutination test. A comparison with the serum agglutination test yields higher sensitivity and specificity.[69] In patients with neurobrucellosis, ELISA offers significant diagnostic advantages over conventional agglutination methods.[70]

All told, antibody profiles do not have specific clinical correlations, and titers often remain high for a protracted period.[71] The asymptomatic patient with an isolated positive titer of class G and A immunoglobulins, or A immunoglobulins only, has not been adequately studied. Variations of ELISA exist, such as competitive ELISA and sandwich ELISA, which may prove useful as a follow-up tool.

The development of a specific polymerase chain reaction (PCR) is a recent advance. PCR is fast, can be performed on any body tissue, and can yield positive results as soon as 10 days after inoculation. It was first developed for brucellosis in 1990, using a 635-bp fragment of *B. abortus* strain 19.[72] Subsequently, two major gene sequences have been used as targets: the 16S rRNA gene sequence,[73] which presents total genus-specific homology and has been satisfactory in clinical settings,[74] and the *BCSP31* gene, which encodes an immunogenic protein of the external membrane of *B. abortus*[75] and has been extensively studied in clinical practice.[76] Cross-reactivity with ochrobactrum is noticed sporadically with both techniques. A comparison of the two techniques showed superiority of the 16S rRNA target in terms of sensitivity.[77]

Nested PCR has proved to have superior specificity and sensitivity, although it is more prone to contamination.[78] Real-time PCR is most likely the diagnostic tool of the future, offering the possibility of results in 30 minutes.[79-81] PCR ELISA is another new promising variation.[82,83] Other variations of PCR exist, such as arbitrarily primed PCR, PCR with random amplification of polymorphic DNA, and a specific multiplex PCR that can concomitantly diagnose brucellosis, Q fever, plague, and anthrax and was developed for purposes of biowarfare defense.[84] Although PCR is very promising, standardization of extraction methods and set-up is lacking, and a better understanding of the clinical significance of the results is still needed.[85]

TREATMENT

Treatment of human brucellosis should involve antibiotics that can penetrate macrophages and can act in the acidic intracellular environment. There is a general need for combined treatment, since all monotherapies are characterized by unacceptably high relapse rates. Practitioners must weigh such questions as the optimal duration of treatment,[86] cost-effective and conveniently administered regimens, favorable pharmacokinetics and pharmacodynamics, and attention to local virulence factors.[87]

The general discrepancy between in vitro findings and in vivo observations precludes the study of resistance patterns of brucellosis or in vitro evaluation of the efficacy of individual antibiotics. Table 4 summarizes information about the various antibiotics that are used to treat brucellosis.

In 1986, the World Health Organization issued guidelines for the treatment of human brucellosis. The guidelines discuss two regimens, both using doxycycline for a period of six weeks, in combination with either streptomycin for two to three weeks

Table 4. Antibiotics Used in the Treatment of Brucellosis in Humans.

Antibiotic	Minimum Inhibitory Concentration (μg/ml)	Dose	Combinations
Doxycycline	0.06–1	100 mg twice daily for 6 wk	Doxycycline combined with streptomycin, with rifampin, with gentamicin, or with ciprofloxacin; doxycycline and streptomycin combined with rifampin or trimethoprim–sulfamethoxazole; doxycycline combined with rifampin and trimethoprim–sulfamethoxazole
Streptomycin	0.25–16	15 mg/kg of body weight intramuscularly for 2–3 wk	Streptomycin and doxycycline; streptomycin and doxycycline combined with rifampin or trimethoprim–sulfamethoxazole
Rifampin	0.1–2	600–1200 mg/day for 6 wk	Rifampin and doxycycline; rifampin and doxycycline combined with streptomycin or trimethoprim–sulfamethoxazole; rifampin and ofloxacin; rifampin and ciprofloxacin
Gentamicin	0.25–2	5 mg/kg/day in 3 divided intravenous doses for 5–7 days	Gentamicin and doxycycline
Trimethoprim–sulfamethoxazole	0.38–8	960 mg twice daily for 6 wk	Trimethoprim–sulfamethoxazole combined with doxycycline, with rifampin, or with streptomycin; trimethoprim–sulfamethoxazole and doxycycline combined with streptomycin or with rifampin
Ofloxacin	0.1–2	400 mg twice daily for 6 wk	Ofloxacin and rifampin
Ciprofloxacin	0.25–1	500 mg twice daily for 6 wk	Ciprofloxacin with doxycycline or rifampin

or rifampin for six weeks. Both combinations are the most popular treatments worldwide, although they are not used universally. The streptomycin-containing regimen is slightly more efficacious in preventing relapse.[88] This may be related to the fact that rifampin down-regulates serum doxycycline levels.[89] However, parenteral administration of streptomycin mandates either hospital admission or the existence of an adequate health care network — both of which are often absent in areas of endemic disease. On the other hand, the use of rifampin in areas in which brucellosis is endemic, where tuberculosis is also usually endemic, raises concern about the development of community resistance to rifampin.

Alternative drug combinations have been used, including other aminoglycosides (e.g., gentamicin and netilmicin).[90] Trimethoprim–sulfamethoxazole is a popular compound in many areas, usually used in triple regimens. Quinolones are an alternative. Various combinations that incorporate ciprofloxacin and ofloxacin have been tried clinically, yielding similar efficacy to that of the classic regimens.[91] Only in vitro observations exist for moxifloxacin and levofloxacin.[92] Although quinolones have been used and will continue to be used, the cost of this approach remains a major drawback. The action of macrolides is attenuated in the acidic phagolysosomal environment, and thus these agents are not useful in brucellosis.[93]

Most complications of brucellosis can be adequately treated with standard regimens. The protracted administration of triple regimens is used for neurobrucellosis. The addition of steroids in neurobrucellosis has not proved to be consistently beneficial.[94] A recent meta-analysis of the efficacy of various combinations for spondylitis advocated a duration of treatment of at least three months; the superiority of any particular regimen could not be proved.[95] Quinolones may prove cost-effective in spondylitis, according to preliminary results.[95]

Rifampin is the mainstay of treatment in cases of brucellosis during pregnancy, in various combinations. Brucellosis in children is treated with combinations that are based on rifampin and trimethoprim–sulfamethoxazole and with aminoglycosides.[96]

A human vaccine has not been developed for brucellosis. Although there are adequate scientific and financial tools for such development in some quarters, knowledge is still incomplete about the molecular pathogenesis of brucellosis. Numerous vaccines have been tested in the past, but none have gained wide acceptance.[97] Vaccines derived from the *B. abortus* strain 19 have been used in the former Soviet Union, and strains of *B. abortus* 104M have been used in China. A phenol-insoluble peptidoglycan fraction of *B. melitensis* strain M15 was used in France.[98] Theoretical vaccine targets for the future might use *rfbK* mutations of *B. melitensis*, outer-

membrane protein 25, and the cytoplasmic protein BP26.[99]

THE FUTURE

Eradication of brucellosis depends largely on socioeconomic and political circumstances. Progress in understanding the molecular pathogenesis of the disease, vaccine engineering, and postgenomic approaches may lead to new preventive interventions. Furthermore, the discovery of new pathways in modifying the acidic intracellular environment in which the microbe moves might be used in adjuvant pharmacotherapy. Determination of microbial load might modify treatment planning and the potential for complications.

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