Human brucellosis

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Human brucellosis still presents scientists and clinicians with several challenges, such as the understanding of pathogenic mechanisms of *Brucella* spp, the identification of markers for disease severity, progression, and treatment response, and the development of improved treatment regimens. Molecular studies have shed new light on the pathogenesis of *Brucella* spp, and new technologies have permitted the development of diagnostic tools that will be useful in developing countries, where brucellosis is still a very common but often neglected disease. However, further studies are needed to establish optimum treatment regimens and local and international control programmes. This Review summarises current knowledge of the pathogenic mechanisms, new diagnostic advances, therapeutic options, and the situation of developing countries in regard to human brucellosis.

Lancet Infect Dis 2007; 7: 775–86

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Introduction

A renewed scientific interest in human brucellosis has been fuelled by its recent re-emergence and enhanced surveillance in many areas of the world,[1] and from its status as a class B bioterrorist agent.[2] The disease remains the world's most common bacterial zoonosis, with over half a million new cases annually and prevalence rates in some countries exceeding ten cases per 100 000 population.[1] Despite being endemic in many developing countries,[1,3] brucellosis remains underdiagnosed and under-reported.[4] Furthermore, since brucellosis is an important cause of veterinary morbidity and mortality, the disease can also cause important economic losses in developing countries.[5]

Although brucellosis in human beings is rarely fatal, it can be severely debilitating and disabling. Brucellosis is a febrile disease capable of masquerading as a myriad of entities, both infectious and non-infectious. The disease has a tendency towards chronicity and persistence, becoming a granulomatous disease capable of affecting any organ system.[6,7] The timely and accurate diagnosis of human brucellosis continues to challenge clinicians because of its non-specific clinical features, slow growth rate in blood cultures, and the complexity of its serodiagnosis.[8–10]

The clinical management of brucellosis is of particular concern because of high initial treatment failure and relapse rates. The availability of the complete genome sequences of the three main human brucellosis pathogens, *Brucella melitensis, Brucella suis,* and *Brucella abortus,* and the advancement of genomics and proteomics will enable scientists to better understand the disease's pathogenic mechanisms. Developments in culture and serological methods, and the availability of advanced molecular detection and typing methods have contributed to improved laboratory diagnosis. These molecular methods could serve as important alternatives to culture methods for the confirmation of the disease and may also provide valuable epidemiological tools to trace sources of infection. Some of the newer diagnostic methods are simple, robust, and affordable, and may prove to be essential in endemic areas with limited financial resources and a limited number of laboratory workers.

Pathogenicity and biology of brucellosis

Brucella species are facultative intracellular bacteria that can multiply within phagocytic cells with human beings as end hosts. Brucella may enter the host via ingestion or inhalation, or through conjunctiva or skin abrasions. After infecting the host, the pathogen becomes sequestered within cells of the reticuloendothelial system. The mechanisms by which brucella enters cells and evades intracellular killing and the host immune system are the subject of much research and debate. In depth analysis of the complete *Brucella* spp genomes has failed to identify any of the classic virulence factors such as toxins, fimbriae, and capsules, which raises the possibility that these organisms use unique and subtle mechanisms to evade host defences, penetrate host cells, alter intracellular trafficking to avoid degradation and killing in lysosomes, and modulate the intracellular environment to allow long-term intracellular survival and replication.[11] The smooth lipopolysaccharides that cover the bacterium and proteins involved in signalling, gene regulation, and transmembrane transportation are among the factors suspected to be involved in the virulence of brucella.[12]

Research suggests that the smooth, non-endotoxic lipopolysaccharides help block the development of innate and specific immunity during the early stage of infection, and protect the pathogen from the microbicidal activities of the immune system. Rough (vaccine) strains (ie, strains with lipopolysaccharide lacking the O-side chain) are less virulent because of their inability to overcome the host defence system.[13] Smooth lipopolysaccharide prevents the synthesis of immune mediators and is much less potent than *Escherichia coli* lipopolysaccharide in inducing host release of inflammatory cytokines.[14] Smooth lipopolysaccharide also has a role in cell entry and immune evasion of the infected cell. The lipopolysaccharide is thought to alter the capacity of the infected cell to present foreign antigens to the MHC class II antigen presentation system, hence preventing attack and killing of the infected cell by the immune system.[12,15] Additionally, smooth lipopolysaccharide in brucella may be involved in the inhibition of apoptosis (ie, programmed cell death) of infected cells,[16] since resistance to apoptosis of infected cells has been observed in patients with acute and chronic disease.[17] By contrast,

rough strains do not confer host cells resistance to apoptosis. Smooth brucella strains with mutations in the phosphoglucomutase gene involved in lipopolysaccharide O-chain synthesis show a profound attenuation of virulence.[12,18]

The two-component BvrR/BvrS gene sensing system that acts through a cascade of protein phosphorylation to modulate bacterial gene expression is thought to be one of the key factors involved in the modulation of cell binding and penetration. The BvrR/BvrS system of brucella has a profound effect on the expression of various cell-surface proteins including Omp25 (also known as Omp3a) and Omp22 (Omp3b).[19,20] It is thought that the altered expression of the surface proteins allows brucella to bind to and penetrate host cells while escaping from the lysosomal pathway, since mutants that are defective in this system show impaired cell penetration and increased destruction by phagolysosomes.

In other pathogenic bacteria including *Bordetella pertussis*, *Bartonella henselae*, *Helicobacter pylori*, and *Legionella pneumophila*, the type IV secretion system (VirB)—a pumping system that selectively transports proteins or other macromolecules through membranes—is essential for pathogenicity. The transported molecule in these pathogens is a classic virulence factor, such as a toxin, which is secreted into the bloodstream or injected into host cells.[21,22] In brucella, VirB is thought to be essential for intracellular survival; however, the transported effector substrate in brucella has not yet been identified and it is very unlikely that the transported molecule is a classic virulence factor. The VirB pumping system is built from a series of proteins encoded by the VirB operon. Many attenuated brucella strains show mutations within the VirB operon, indicating that an intact VirB is essential for virulence.[23] VirB seems to have a role in adherence of the bacterium to the host cell, cell entry, and it modulates the intracellular trafficking and replication of the bacterium.[24,25] After binding to macrophages, brucella is taken up by internalisation vesicles that would normally fuse with endosomes. After acidification, these endosomes lyse, destroying their contents. Acidification is thought to induce VirB expression.[25–27] The VirB system is suspected to interact with components of the endoplasmic reticulum, neutralising the pH and allowing the brucellae to undergo regulated cell division within the endoplasmic reticulum's safe environment.[22]

Heat shock protein 60 (Hsp60), a member of the GroEl family of chaperonins, is expressed on the cell surface of wild-type *Brucella* spp, but not on VirB mutants. Hsp60 seems to play a part in cell adherence by binding to a cellular prion molecule called PrPr. Since the exportation of Hsp60 is VirB-dependent, it has been postulated that Hsp60 could in fact be a virulence factor.[28]

Clinical diagnosis

Human brucellosis has a wide spectrum of clinical manifestations, earning it a place alongside syphilis and tuberculosis as one of the "great imitators".[29–33] The clinical features of brucellosis depend on the stage of the disease, and the organs and systems involved. Brucella has been reported to compromise the central and peripheral nervous system, and the gastrointestinal, hepatobiliary, genitourinary, musculoskeletal, cardiovascular, and integumentary systems. Historically, only *B melitensis*, *B abortus*, and *B suis* were considered human pathogens, but recent reports have shown that newly recognised marine mammal species have zoonotic

	Total number of patients	Fever or chills	Arthralgia or arthritis	Sweating	Constitutional symptoms*	Hepatomegaly	Splenomegaly
Memish et al (2000)[10]	160	146 (91·3%)	105 (65·6%)	30 (18·8%)	70 (43·8%)	9 (5·6%)	11 (6·9%)
Kokoglu et al (2006)[9]	138	108 (78·3%)	107 (77·5%)	100 (72·5%)	98 (71·0%)	37 (26·8%)	50 (36·2%)
Mantur et al (2006)[29]	495	417 (84·2%)	117 (23·6%)	19 (3·8%)	6 (1·2%)	56 (11·3%)	95 (19·2%)
Ruiz-Mesa et al (2005)[28]	711	702 (98·7%)	353 (49·6%)	597 (84·0%)	533 (75·0%)	250 (35·2%)	148 (20·8%)
Barroso Garcia et al (2002)[30]	565	441 (78·1%)	248 (43·9%)	483 (85·5%)	472 (83·5%)	422 (74·7%)	152 (26·9%)
Hasanjani Roushan et al (2004)[7]	469	314 (67·0%)	252 (53·7%)	357 (76·1%)	..	..	27 (5·8%)
Pappas et al (2005)[6]	100	91 (91%)	44 (44%)	..	26 (26%)	7 (7%)	16 (16%)
Troy et al (2005)[26]	28	25 (89%)	15 (54%)	..	13 (46%)	8 (29%)	5 (18%)
Andriopoulos et al (2007)[33]	144	144 (100%)	125 (86·8%)	138 (95·8%)	140 (97·2%)	..	74 (51·4%)
Giannakopoulos et al (2006)[31]†	52	42 (81%)	43 (83%)	8 (15%)	7 (13%)	..	..
Mantur et al (2004)[32]†	93	49 (53%)	19 (20%)	..	..	..	..
Tsolia et al (2002)[39]†	39	27 (69%)	27 (69%)	8 (21%)	13 (33%)	11 (28%)	15 (38%)

Data shown as number of patients with symptom (%). ..=not reported. *Constitutional symptoms comprise anorexia, malaise, asthenia, weight loss, etc. †Studies in paediatric population only.

Table 1: **Most common clinical findings in patients with brucellosis**

	Total number of patients	Anaemia	Leucopenia	Leucocytosis	Thrombo-cytopenia	Thrombo-cytosis	Elevated CRP	Elevated ESR	Positive RF
Kokoglu et al (2006)[9]	138	46 (33·3%)	30 (21·7%)	10 (7·2%)	27 (19·6%)	..	72 (52·2%)	58 (42·0%)	37 (26·8%)
Hasanjani Roushan et al (2004)[7]	469	71 (15·1%)	14 (3·0%)	57 (12·2%)	16 (3·4%)	5 (1·1%)	277 (59·1%)	365 (77·8%)	40 (8·5%)
Barroso Garcia et al (2002)[30]	565	171 (30·3%)	61 (10·8%)	34 (6·0%)	..	..	..	390 (69·0%)	..
Troy et al (2005)[36]	28	21 (75%)	23 (82%)	..	5 (18%)	..	..	..	..
Tsolia et al (2002)[39]*	39	18 (46%)	..	1 (3%)	3 (8%)	..	..	..	..

Data shown as number of patients with laboratory finding (%). ..=not reported. CRP=C-reactive protein. ESR=erythrocyte sedimentation rate. RF=rheumatoid factor. *Studies in paediatric population only.

Table 2: **Most common laboratory findings in patients with brucellosis**

potential, and an apparent tendency for complicated disease in human beings.[34] Despite major ongoing controversies in the taxonomy of *Brucella* species,[35] the bulk of human disease is caused by two species: *B melitensis* and *B abortus*. Clinical differences between species are difficult to determine, since few studies have compared the clinical manifestations of sufficient cases of each species.[36] The limited data available provide conflicting evidence as to which species results in more cases of chronic or complicated disease.

Because of the protean clinical manifestations of brucellosis, the cornerstone of clinical diagnosis lies in taking a detailed history and paying careful attention to epidemiological information. Special attention must be placed on determining whether ingestion of contaminated dairy products or contact with infected animals has occurred. Detailed patient interviews are crucial for the diagnosis of human brucellosis, especially in urban and non-endemic areas, and in cases of imported brucella, in which travellers acquire the disease abroad and become ill in non-endemic settings. The diagnosis of a patient with brucellosis should prompt the clinician to consider the likely mode of transmission, and possibly to initiate screening of contacts that may have had the same exposure (eg, ingestion of contaminated dairy products or exposure to animal source).[37]

Clinical studies have shown that fever is the most common feature of brucellosis, followed by osteoarticular involvement, sweating, and constitutional symptoms (table 1). On physical examination, the most common findings are hepatomegaly and splenomegaly, which occur in about one-third of patients. Lymphadenopathy is seen in about 10% of patients. Osteoarticular manifestations (sacroiliitis, spondylitis, peripheral arthritis, and osteomyelitis) account for over half of the focal complications. Genitourinary complications (orchiepididymitis, glomerulonephritis, and renal abscesses) can be found in around 10% of patients. Neurological findings are not as uncommon as they are often portrayed; one study from Turkey reported that in a series of 305 patients with brucellosis, 20 (6·6%) patients presented with neurological involvement.[40] Neurological findings can be diverse and could include peripheral neuropathies, chorea, meningoencephalitis, transient ischaemic attacks, psychiatric manifestations, and cranial nerve compromise. Mucocutaneous manifestations include erythematous papular lesions, purpura, dermal cysts, and Stevens-Johnson syndrome. Pulmonary manifestations, including pleural effusions and pneumonias, can be found in up to 16% of complicated cases of brucellosis.[41] Leucocytosis is observed in about 9% of patients and if found, focal complications should be excluded (table 2). Leucopenia (11% of patients) and thrombocytopenia (10% of patients) are seen in similar frequencies. Anaemia is seen more frequently, affecting 26% of patients. Common disease findings, however, may vary between different areas and populations. Endocarditis—with the aortic valve being the most commonly affected structure and multiple valve involvement being common within this subset of patients[42]—is the most serious complication, accounting for most of the 5% total mortality rate of human brucellosis.

In brucellosis with focal complications, imaging studies can provide useful anatomic and topographic confirmation of suspected lesions to enable adequate planning of medical and surgical treatment. Advances in the imaging field have produced mixed results in the diagnosis of brucellosis: attempts at improving bone scintigraphy have failed to produce breakthroughs,[43,44] whereas improved computed tomography (such as diffusion/perfusion studies), and magnetic resonance imaging (such as fluid-attenuated inversion recovery [FLAIR] modalities) have enabled improved sensitivity in detecting both bone and soft-tissue lesions (figure 1).[45,46] Digital subtraction angiography and transoesophageal echocardiography have also proved remarkably useful in detecting vascular and valvular insults in neurobrucellosis and brucellar endocarditis.[42,45,47]

Childhood brucellosis deserves special mention, since the condition is easily overlooked in infants.[39] The most common osteoarticular finding in children is monoarticular arthritis (usually of the knees and hips), whereas in adults, sacroiliitis is most frequent. Limited data support vertical transmission of human brucellosis[48,49] and transmission via breastmilk.[50,51] Reports of transmission of human brucellosis via blood transfusion have also been published,[52,53] and some authors have even suggested that sexual transmission can occur.[54] Further study is needed to substantiate these

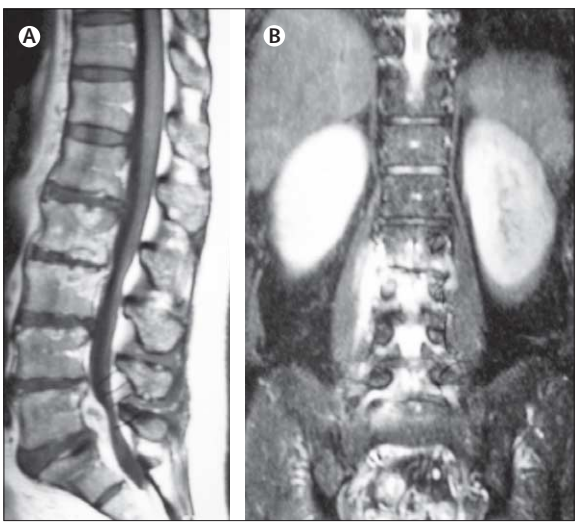

***Figure 1*: Magnetic resonance imaging (MRI) has become a powerful aid in investigating bone and soft-tissue lesions in brucellosis patients**
(A) T1 and (B) T2-weighted MRI images of a 63-year-old male with spinal osteomyelitis, sacroiliitis, and psoas abscess caused by brucella infection.

	Incubation time	Requires blind sub-cultures	Sensitivity (disease stage)	References
Ruiz-Castañeda	7–21 days	Yes	70–80% (acute); <50% (chronic)	32, 39, 43, 48, 49, 53
Lysis centrifugation	2–4 days	No	>90% (acute); 70% (chronic)	48, 55, 56, 57
Bone marrow culture	4–7 days	Depends on method and media used	97% (acute); 90% (subacute); 50% (chronic)	49, 54, 58, 59

***Table 3*: Characteristics and sensitivities of culture methods in clinical specimens**

claims, although intrauterine transmission, transmission during delivery, and transmission through breastmilk are indeed among the main routes of transmission in the mammalian reservoirs.

Culture

Blood culture is the gold standard in the diagnosis of bacterial infections, including brucellosis (table 3). Although the biphasic Ruiz-Castañeda system (figure 2) is the traditional method for the isolation of *Brucella* spp from clinical samples,[60–62] it has now largely been replaced by automated culture systems—such as the lysis centrifugation method[58,59]—with increased sensitivity and reduced culture times.[55–57,63]

The sensitivity of blood culture depends on several factors, particularly the phase of the disease and previous use of antibiotics.[64,65] For instance, in acute cases, the sensitivities of the Ruiz-Castañeda method and lysis centrifugation have been reported as high as 80% and 90%, respectively, but as low as 30% and 70%, respectively, in chronic cases.[59,64,66] Bone marrow cultures may provide a higher sensitivity, yield faster culture times, and may be superior to blood cultures when evaluating patients with

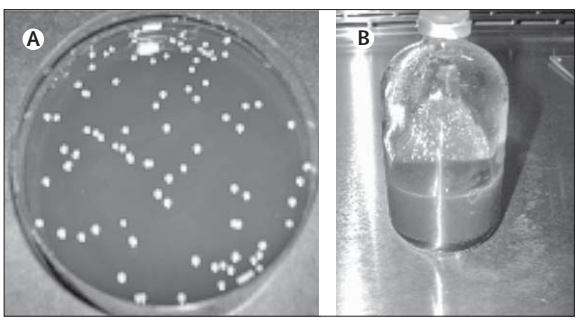

***Figure 2*: Blood culture is the gold standard in the diagnosis of brucellosis**
(A) The lysis centrifugation method has a better sensitivity and provides quicker results than (B) the biphasic Ruiz-Castañeda method.

previous antibiotic use.[55,64–67] Brucella can also be cultured from pus, tissue samples, and cerebrospinal, pleural, joint, or ascitic fluid.[68,69] Since brucellosis constitutes one of the most common laboratory-acquired infections, special care should be taken when using the lysis centrifugation method to avoid infection from contaminated aerosols.[70–73]

So far, there is no evidence to suggest that drug resistance has an important part in treatment failure and relapse.[74,75] An early study by Ariza and colleagues[76] showed that brucella strains recovered from relapsed patients had antibiotic sensitivity profiles identical to the original strains recovered during the initial infection. Resistant *Brucella* spp isolates have rarely been reported. Since brucella does not contain any plasmids and human beings are end hosts, these factors may contribute to the absence of any pronounced degree of antibiotic resistance.

Serodiagnosis

Agglutination tests

In the absence of culture facilities, the diagnosis of brucellosis traditionally relies on serological testing with a variety of agglutination tests such as the Rose Bengal test, the serum agglutination test, and the antiglobulin or Coombs' test. In general, the Rose Bengal test is used as a screening test, and positive results are confirmed by the serum agglutination test.[38,77] These agglutination tests are based on the reactivity of antibodies against smooth lipopolysaccharide. These antibodies tend to persist in patients long after recovery; therefore, in endemic areas, high background values could occur that may affect the diagnostic value of the test.[78] Furthermore, the brucella smooth lipopolysaccharide antigen tends to show cross-reactivity with other Gram-negative bacteria such as *Yersinia enterocolitica* 0:9, *Vibrio cholerae*, *Escherichia coli* O:157, and *Francisella tularensis*, increasing the possibility of false-positive results.[79] The sensitivity of the Rose Bengal test is very high, however, and false-negative results are rarely observed.[38,80] The specificity of the assay is also fairly high, and in unexposed populations, false-positive results are rare. Differences in the quality of the reagent used and disagreement in the interpretation of results might add to variability of test results.[81,82]

As with other serological tests, the sensitivity and specificity of the confirmatory agglutination tests for brucellosis depend on the cut-off value used, and on the background level of reactive antibodies in the population. By doing the test on a serial dilution of the samples with results judged positive above a certain titre, the specificity and positive predictive value of a positive test result could be increased by selecting a higher cut-off value for areas where brucellosis is endemic. However, by selecting a higher cut-off value the sensitivity decreases and because patients with acute brucellosis and those with persisting and relapsing disease may present with low antibody levels, the interpretation of test results and diagnosis of these patients by serological testing might not be straightforward. In a recent study, Mantecon and co-workers[83] calculated a sensitivity for the serum agglutination test of 84·6% at any titre for patients with culture-confirmed acute brucellosis. However, in the same study it was noted that the sensitivity at the generally accepted cut-off value of a serum dilution of 1/160 was 64·7%, while at a cut-off value of 1/320 the sensitivity was just 47·1%. The latter cut-off value is often recommended when the assay is used in endemic areas. If collection of a follow-up sample is feasible, the sensitivity of the test could be increased by testing paired serum samples and looking for seroconversion, or a four-fold increase in titre. Demonstration of seroconversion or a significant increase in antibody titre provides strong supportive evidence for the infection and this may be observed by testing a follow-up sample collected a few weeks to several months after the initial diagnosis.

To exclude the possibility of cross-reactive IgM antibodies, the 2-mercaptoethanol test for measuring specific agglutinating IgG antibodies is sometimes used; results are compared with the serum agglutination test titre and reactivity in the 2-mercaptoethanol test is taken as evidence for the presence of specific IgG antibodies. However, many patients have low levels of agglutinating IgG antibodies and results can easily be misinterpreted. Coombs' test may be more suitable for confirmation of brucellosis in relapsing patients or patients with persisting disease, but few laboratories have the expertise and equipment to do this very sensitive but complex and demanding technique.

ELISA

ELISA has become increasingly popular as a well-standardised assay for brucellosis. The sensitivity of ELISAs prepared in the laboratory may be high, especially when the detection of specific IgM antibodies is complemented with the detection of specific IgG antibodies.[83] The specificity of ELISA, however, seems to be less than that of the agglutination tests. Since ELISA for brucella is based on the detection of antibodies against smooth lipopolysaccharide, the cut-off value may need adjustment to optimise specificity when used in endemic areas, and this may influence sensitivity.[78] Curiously, test performance of commercial ELISAs, as described in their package inserts, is based on comparison with commercial ELISAs of other brands, and not culture. Manufacturers further overlook the fact that cut-off values should be established based on local epidemiological conditions. Furthermore, the only study on this topic, by Araj and colleagues,[84] used healthy individuals as negative controls, thereby possibly overestimating test specificity, and did not investigate patients who presented with clinical suspicion of brucellosis but had a different final diagnosis, and who may have had cross-reactive antibodies. Commercial ELISAs also have not been evaluated under different epidemiological conditions and results should be interpreted with care. Studies testing the more recently developed Brucellacapt (Vircell SL, Granada, Spain) assay in an endemic region in Spain showed that at a cut-off value of 1/80, a sensitivity of 98% for culture-confirmed patients and a specificity of 96% for samples collected from healthy individuals was obtained; however, a specificity of 63% was found when testing patients who had conditions other than brucellosis.[85] At a higher cut-off value, the specificity improved but the sensitivity dropped.

Serological testing with agglutination tests[40] and ELISA[86] has been applied in the diagnosis of central nervous system brucellosis with varying success, and further research is aimed to improve the diagnosis of this condition.

Rapid point-of-care assays

The serum agglutination test, Coombs' test, ELISA, and Brucellacapt all require a well-equipped laboratory, a facility often lacking in health centres of resource-poor countries where the disease is endemic. Rapid tests such as the fluorescent polarisation immunoassay (FPA) for brucellosis and the immunochromatographic brucella IgM/IgG lateral flow assay (LFA; figure 3), a simplified version of ELISA, have great potential as point-of-care tests.[87,88] The FPA test is done by incubation of a serum sample with brucella O-polysaccharide antigen linked to a fluorescent probe.[87] The sensitivity of this test at the selected cut-off value is 96% for culture-confirmed brucellosis, and the specificity was determined to be 98% for samples from healthy blood donors. The LFA uses a drop of blood obtained by fingerprick, does not require specific training, is easy to interpret, and can be used at the bedside. The components are stabilised and do not require refrigeration for transportation or storage.[89] The sensitivity and specificity of LFA are high (more than 95%), and the test can be used at all stages of disease.

Further studies will be needed to confirm the usefulness of these new point-of-care tests in different clinical settings in endemic areas, with particular attention to the diagnosis of patients with acute and relapsing brucellosis. The requirement of specific equipment and reagents might make the FPA test too

expensive, but a simple test such as the LFA could be ideal for field testing risk groups during outbreaks.[88] Another useful application for these tests is to screen the contacts of brucellosis patients.[90–92]

Treatment monitoring

Currently, tests to predict treatment outcome and relapse are not available. The 2-mercaptoethanol test is sometimes used to monitor response to treatment.[93] It was observed that a low 2-mercaptoethanol titre measured 12 months after treatment is consistent with cure, whereas a substantial proportion of patients continuing to show elevated 2-mercaptoethanol titres are symptomatic. The predictive value of the 2-mercaptoethanol test is, however, debated. In view of the risk of relapse and treatment failure in human brucellosis, more accurate markers for predicting the outcome of treatment are needed. Serological changes are more easily observed in ELISA than in the classic agglutination tests, with relapsing patients showing a rise in IgG antibody levels.[78] The predictive value of serum antibodies against smooth lipopolysaccharide as measured in ELISA for persisting disease seems to be low because 12 months after therapy, 25% of cured patients have specific IgM antibodies and almost 90% have specific IgG antibodies. Two-dimensional gel electrophoresis has identified several immunogenic brucella proteins. Some of these proteins that are expressed during infection may well be of diagnostic importance in evaluating response to treatment.[94]

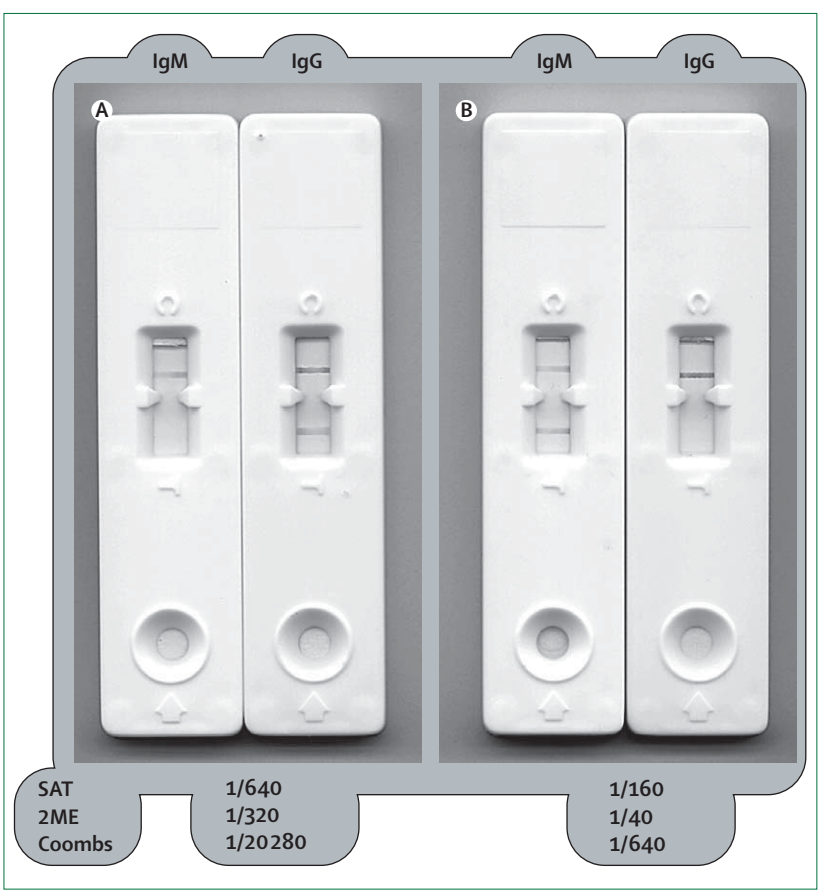

Figure 3: **The brucella IgM/IgG lateral flow assay, a point-of-care test for the confirmation of brucellosis**
The brucella IgM/IgG lateral flow assay is an example of a simple and rapid test for the diagnosis of brucellosis that can be done at the bedside using a drop of blood collected by fingerprick. The results for two serum samples, one containing (A) specific IgG antibodies and (B) one containing specific IgM antibodies, are shown. Ratios indicate the serum dilution at which the specified reference tests reacted for the two serum samples. SAT=serum agglutination test. 2ME=2-mercaptoethanol test.

Molecular detection

Applications in the diagnosis of brucellosis

PCR is a convenient tool for the diagnosis of human brucellosis that may improve sensitivity compared with culture.[95] Several genus-specific PCR systems using primer pairs that target 16S RNA sequences and the genes of different outer membrane proteins have been developed.[96–101] Each of these PCR systems produces a discrete DNA product, whose length is identical for and specific to all *Brucella* species. Queipo-Ortuno and co-workers[102] found 100% sensitivity and 98·3% specificity using the B4/B5 primer pair amplifying a 223-bp fragment of the *bcsp31* gene, compared with 70% sensitivity for blood culture. Adequate comparisons of the different PCR systems and primers are still lacking, and results may presumably depend on the nature of the clinical specimen, the sample preparation procedure, and the duration and stage of illness. For example, Zerva and colleagues[103] reported that the sensitivity of the B4/B5 primer pair improved from 61% to 94% when serum instead of whole blood samples was used. However, using a modified detection system, Vrioni and co-workers[104] found no improvement in the detection rate by testing whole blood samples.

The incorporation of a robust DNA extraction method, such as the diatom-guanidinium isothiocyanate method, which effectively removes inhibitors commonly present in a variety of clinical specimens, may improve sensitivity and reproducibility.[105] Indeed, using a commercially available sample preparation kit, based on guanidinium isothiocyanate for sample lysis and DNA extraction, and a solid matrix to bind and isolate the purified DNA, Mitka and colleagues[106] found an almost 100% sensitivity for each of four PCR systems by testing either serum, buffy coat, or whole blood samples from 200 patients with acute brucellosis of whom 74% were culture-confirmed.

PCR could be particularly useful in patients with specific complications such as neurobrucellosis, or other localised infections, since serological testing often fails in such patients.[107–109] However, because these PCR systems are complex, time consuming, and have a high risk of contamination, they are less suitable for routine diagnostic purposes; real-time PCR systems have been developed that are faster and less prone to contamination and are thus more clinically useful.[109–115] Comparative analysis of the various real-time PCRs is needed to assess their diagnostic value. However, the high costs of these assays will restrict their use.

Applications in the assessment of brucellosis treatment

Relapsing brucellosis is another diagnostic challenge in which PCR could prove to be useful.[116] PCR was recently used to assess treatment efficacy.[117] PCR showed the persistence of low levels of brucella DNA in the serum of treated patients, with seven (35%) patients testing positive 1–2 years after the end of treatment. Of these seven patients, four relapsed and three did not. In another study, follow-up samples collected after treatment completion showed that two out of 20 patients tested PCR positive, and these PCR results were confirmed by culture.[118] These results strongly suggest that the bacterium may persist in the blood of treated patients for a long time, likely inside macrophages, which could account for the high treatment failure and relapse rates.[106,119] Indeed, one study has shown that persistence of brucella DNA correlates with ineffective treatment and relapse.[106] Therefore, PCR may be used as an alternative to culture in the confirmation of brucellosis, to monitor treatment efficacy, and to diagnose relapsing patients.

Other applications of PCR

PCR also appears to be useful in species differentiation and biotyping of isolates. Short nucleotide repeat sequences present at different loci in the brucella genome show a wide variation in the number of repeats between species and isolates.[120,121] PCR amplification of these variable repeats is more robust than classic typing methods for species and biovar identification, and is more powerful than other molecular tools such as outer membrane protein typing,[122,123] IS711 typing,[124] or typing by amplified fragment length polymorphism.[125] PCR amplification of a set of loci containing these repeats has allowed the characterisation of individual and unique isolates even from within the same region.[126,127] This application could be applied epidemiologically to trace infections to specific flocks or dairy producers, and the emergence of cases with identical isolates could perhaps be taken as evidence for the presence of a focus of intensified transmission. Furthermore, the method could prove useful in discriminating reinfection and relapse.[127]

Treatment

WHO has not updated its recommended treatment regimens for brucellosis in more than 20 years,[128] despite treatment failure and relapse rates ranging from 4·6% to 24% for the oral regimen and 5% to 8% for the oral/parenteral regimen.[129,130] The cause for such high failure rates remains unclear. Since antibiotic resistance can be discounted as a major factor, it would seem logical to assume that patient compliance is an important factor, especially when one considers the length of treatment and the frequency of adverse reactions. Another possibility is suggested by the observations of persistent PCR positivity despite "successful" or optimum therapy.[106,118,119] Treatment failure results in increased medical costs, increased patient suffering, and hence more effective regimens are urgently needed.

Some attempts at predicting relapses have been made, but these require large-scale substantiation to determine their true clinical effectiveness. Ariza and colleagues[131] determined the following independent risk factors for relapse from a prospective cohort of 530 adults: "less-effective" antibiotic therapy, positive blood cultures during initial disease, disease duration of 10 days or less before start of treatment, male sex, and a platelet count of 150x10³ per µL or less.[131] Solera and colleagues[132] have proposed a multivariate model to stratify a patient's risk of relapsing into groups of low (4·5%), medium (32%), and high (67%) probability of relapse within 12 months. The independent predictors of relapse were temperatures of 38·3°C or higher, positive blood cultures at baseline, and a duration of symptoms before treatment of less than 10 days.

The WHO oral regimen consists of 200 mg doxycycline plus 600–900 mg rifampicin daily for a minimum of 6 weeks, whereas the alternate oral/parenteral scheme replaces rifampicin with 15 mg/kg streptomycin daily for the first 2–3 weeks of treatment only. Although these regimens are based on the results of many clinical and in-vitro studies, much controversy remains regarding the ideal treatment. An in-vitro study has shown that doxycycline and rifampicin retain adequate activity at a pH of 5 (the pH of brucella-destroying phagolysosomes), and that rifampicin increases its activity at low pH.[75]

A meta-analysis has shown that the classic streptomycin plus tetracycline treatment regimen results in a higher cure rate and in fewer relapses than the WHO oral regimen.[133] The search for an oral-only monotherapy has so far failed, but has resulted in the evaluation of many candidates including macrolides and quinolones. A recent study evaluating minocycline in combination with rifampicin showed a very low relapse rate.[134] Concerns about treatment failure and relapse, and fear of emerging rifampicin resistance in areas endemic to tuberculosis have led to the pursuit of triple-drug combinations with some encouraging initial results.[135,136] Solera and co-workers[137] have suggested the addition of gentamycin for the first 7 or preferably 10 days to the standard oral regimen, and Mantur and colleagues[32] did not observe any relapse among 93 patients treated with a gentamycin-doxycycline-rifampicin triple therapy. Extending the antibiotic treatment also appears to have a positive effect on relapse and failure rates in all treatment regimens. Improved antibiotic delivery methods—for example, through antibiotic-loaded microspheres—could shorten duration, decrease toxicities, and improve the efficacy of treatment; however, more research in this field is still needed.[138]

Evaluation of immunomodulation with levamisole plus conventional therapy in the management of chronic brucellosis has shown mixed results.[139–141] Although initial reports of the addition of interferon alfa-2a to standard therapy in anergic patients seemed somewhat promising, this has not led to any practical application.[142] Currently,

extended treatment with standard drug combinations should be given to those patients with persisting signs and symptoms of recurrent disease. Additionally, when treating focal infections, careful attention must be given to the penetration and activity of the drug in the particular tissue involved, and the choice and duration of therapy must be individualised, with prolonged treatment in cases with specific complications such as endocarditis or central nervous system involvement. The more effective doxycycline-streptomycin combination is preferred in patients with more severe disease, such as spinal involvement, and the duration of therapy may be prolonged.[143] Abscesses and specific focalised forms of brucellosis including endocarditis, cerebral, epidural, or splenic abscess might require surgical interventions since these forms are resistant to antibiotics. Finally, the treatment of brucellosis in children and pregnant and lactating women requires special attention and insufficient data are available to give specific recommendations.[49,128]

Special considerations for developing countries

Most of the programmes that have been successful in controlling brucellosis took place in developed nations, with developing countries continuing to bear the burden of this disease.[65] Notably, most cases of human brucellosis in non-endemic developed countries result from dairy products imported from endemic areas[36,144–146] or from patients who import the disease.[144,147] Despite the existence of effective vaccines for cattle (S19) and goats (Rev 1), control efforts in economically poor endemic areas have failed as a result of inconsistent infrastructure and lack of funding.[148,149] A major issue is that control measures should continue for a long period of time and be complemented with a monitoring system that may be hard to keep in place once the number of cases begins to decrease. A case in point is the current situation of many former Soviet republics, where the collapse of the USSR and its administrative, economic, and public-health infrastructure has led to the re-emergence of brucellosis.[150]

Data on the yearly economic impact of brucellosis in the developing world associated with disease in livestock have generally been hard to assess, especially in Africa.[3] In countries such as Argentina and Mexico, which depend heavily on the sale of livestock products for both domestic and international markets, these annual costs are estimated to be US$60 million and $200 million respectively.[151,152] Studies done in developing countries by the United Nations highlight the need for effective control programmes, which have an obvious benefit to the health of both human beings and livestock. If the costs of control programmes are shared between the public and private sectors and include international aid, they are likely to be profitable and cost effective.[153]

The economic impact in terms of human disease has been even harder to gauge; not only must the cost of treatment and diagnosis be considered, but also the cost in terms of disability-adjusted life years. Regardless of the measures used, the economic burden of human brucellosis in endemic areas is high and justifies widespread and sustained control efforts.[5,153]

Disease control in nomadic and migratory populations has proven especially challenging. Patients may not have access to medical services, and staff at local health-care centres may not be able to make or confirm the diagnosis. The difficulty in obtaining cheap, accurate, and timely diagnostic tools for human brucellosis is a major problem in urban and rural settings in the developing world. New diagnostic methods, particularly point-of-care tests that do not require laboratory facilities, will contribute substantially to early diagnosis and patient management, and help to create awareness. Adequate brucellosis control will probably have the biggest impact on high-risk shepherding communities who depend on their herds to satisfy their economic and nutritional needs. Although rural shepherding communities and abattoir and veterinary workers have traditionally been regarded as the main risk groups, it is important to recognise that large urban populations in developing countries are also at risk of acquiring the disease by consuming unpasteurised dairy products, as shown by a brucella antibody prevalence rate of 12·3% in milk supplies sampled at 219 consumer households in urban Kenya.[154]

Conclusions

Developing countries could benefit from the renewed interest in brucellosis shown by the scientific community. Molecular detection methods such as PCR amplification and genotyping will be powerful epidemiological tools for confirmation of the disease and for identification of sources of infection. These methods do not carry the risk of laboratory-acquired infection that culture techniques do. Additionally, rapid point-of-care assays will enable fast and accessible diagnostic capabilities even in remote areas. The search for new and improved treatment regimens will hopefully provide strategies to effectively cure even the most complex cases of brucellosis often seen in endemic areas.

New insights into the pathogenesis of *Brucella* spp have not yet led to new developments in the treatment of patients as might have been hoped. The fact that brucella does not use any of the classic virulence mechanisms has perhaps made investigation in this area slower, but certainly more exciting. Since brucellosis poses a severe health threat and restricts export of livestock and animal products from endemic areas, it hampers much-needed economic development in these areas. Effective vaccines are currently available and it is important to find means and resources for their effective use in resource-poor countries in conjunction with sustained control efforts that incorporate local farming practices, dietary habits, and traditional beliefs. Brucellosis is routinely overlooked, misdiagnosed, or at best diagnosed

Search strategy and selection criteria

We searched Medline for relevant studies published from 1997 to 2007 using the term "brucell*" with specific keywords such as "PCR", "treatment", "sero*", "diagnos*", "clinical", and "epidemiolog*". Only English and Spanish language papers were reviewed. Additionally, publications from the authors' personal collections were used.

incidentally; therefore, physicians in both endemic and non-endemic areas must become aware of and consider brucellosis in their differential diagnosis of febrile diseases with peculiar musculoskeletal or other focal findings. Clinicians' recognition and reporting of the disease is essential for the allocation of resources and efforts for the development of sustained control measures.

Conflicts of interest
We declare that we have no conflicts of interest.

Acknowledgments
We thank Gavin Jackson for editorial support.

References

1 Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. *Lancet Infect Dis* 2006; **6:** 91–99.
2 Greenfield RA, Drevets DA, Machado LJ, et al. Bacterial pathogens as biological weapons and agents of bioterrorism. *Am J Med Sci* 2002; **323:** 299–315.
3 McDermott JJ, Arimi SM. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Vet Microbiol* 2002; **90:** 111–34.
4 Godfroid J, Cloeckaert A, Liautard JP, et al. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. *Vet Res* 2005; **36:** 313–26.
5 Colmenero Castillo JD, Cabrera Franquelo FP, Hernandez Marquez S, et al. Socioeconomic effects of human brucellosis. *Rev Clin Esp* 1989; **185:** 459–63.
6 Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. *N Engl J Med* 2005; **352:** 2325–36.
7 Hasanjani Roushan MR, Mohrez M, Smailnejad Gangi SM, Soleimani Amiri MJ, Hajiahmadi M. Epidemiological features and clinical manifestations in 469 adult patients with brucellosis in Babol, Northern Iran. *Epidemiol Infect* 2004; **132:** 1109–14.
8 Colmenero JD, Reguera JM, Cabrera FP, et al. Serology, clinical manifestations and treatment of brucellosis in different age groups. *Infection* 1990; **18:** 152–56.
9 Kokoglu OF, Hosoglu S, Geyik MF, et al. Clinical and laboratory features of brucellosis in two university hospitals in southeast Turkey. *Trop Doct* 2006; **36:** 49–51.
10 Memish Z, Mah MW, Al Mahmoud S, Al Shaalan M, Khan MY. Brucella bacteraemia: clinical and laboratory observations in 160 patients. *J Infect* 2000; **40:** 59–63.
11 Delrue RM, Lestrate P, Tibor A, Letesson JJ, De Bolle X. Brucella pathogenesis, genes identified from random large-scale screens. *FEMS Microbiol Lett* 2004; **231:** 1–12.
12 Lapaque N, Moriyon I, Moreno E, Gorvel JP. Brucella lipopolysaccharide acts as a virulence factor. *Curr Opin Microbiol* 2005; **8:** 60–66.
13 Porte F, Naroeni A, Ouahrani-Bettache S, Liautard JP. Role of the *Brucella suis* lipopolysaccharide O antigen in phagosomal genesis and in inhibition of phagosome-lysosome fusion in murine macrophages. *Infect Immun* 2003; **71:** 1481–90.
14 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:** 1385–89.
15 Forestier C, Moreno E, Pizarro-Cerda J, Gorvel JP. Lysosomal accumulation and recycling of lipopolysaccharide to the cell surface of murine macrophages, an in vitro and in vivo study. *J Immunol* 1999; **162:** 6784–91.
16 Gross A, Terraza A, Ouahrani-Bettache S, Liautard JP, Dornand J. In vitro *Brucella suis* infection prevents the programmed cell death of human monocytic cells. *Infect Immun* 2000; **8:** 342–51.
17 Tolomeo M, Di Carlo P, Abbadessa V, et al. Monocyte and lymphocyte apoptosis resistance in acute and chronic brucellosis and its possible implications in clinical management. *Clin Infect Dis* 2003; **36:** 1533–38.
18 Ugalde JE, Comerci DJ, Leguizamon MS, Ugalde RA. Evaluation of *Brucella abortus* phosphoglucomutase (pgm) mutant as a new live rough-phenotype vaccine. *Infect Immun* 2003; **71:** 6264–69.
19 Guzman-Verri C, Manterola L, Sola-Landa A, et al. The two-component system BvrR/BvrS essential for *Brucella abortus* virulence regulates the expression of outer membrane proteins with counterparts in members of the Rhizobiaceae. *Proc Natl Acad Sci USA* 2002; **99:** 12375–80.
20 Lopez-Goni I, Guzman-Verri C, Manterola L, et al. Regulation of brucella virulence by the two-component system BvrR/BvrS. *Vet Microbiol* 2002; **90:** 329–39.
21 O'Callaghan D, Cazevieille C, Allardet-Servent A, et al. A homologue of the *Agrobacterium tumefaciens* VirB and *Bordetella pertussis* Ptl type IV secretion systems is essential for intracellular survival of *Brucella suis*. *Mol Microbiol* 1999; **33:** 1210–20.
22 Boschiroli ML, Ouahrani-Bettache S, Foulongne V, et al. Type IV secretion and brucella virulence. *Vet Microbiol* 2002; **90:** 341–48.
23 Celli J, Salcedo SP, Gorvel JP. Brucella coopts the small GTPase Sar1 for intracellular replication. *Proc Natl Acad Sci USA* 2005; **102:** 1673–78.
24 Boschiroli ML, Ouahrani-Bettache S, Foulongne V, et al. The *Brucella suis* virB operon is induced intracellularly in macrophages. *Proc Natl Acad Sci USA* 2002; **99:** 1544–49.
25 Arenas GN, Staskevich AS, Aballay A, Mayorga LS. Intracellular trafficking of *Brucella abortus* in J774 macrophages. *Infect Immun* 2000; **68:** 4255–63.
26 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:** 5711–24.
27 Porte F, Liautard JP, Kohler S. Early acidification of phagosomes containing *Brucella suis* is essential for intracellular survival in murine macrophages. *Infect Immun* 1999; **67:** 4041–47.
28 Watarai M. Interaction between *Brucella abortus* and cellular prion protein in lipid raft microdomains. *Microbes Infect* 2004; **6:** 93–100.
29 Mantur BG, Biradar MS, Bidri RC, et al. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. *J Med Microbiol* 2006; **55:** 897–903.
30 Barroso Garcia P, Rodriguez-Contreras Pelayo R, Gil Extremera B, et al. Study of 1595 brucellosis cases in the Almeria province (1972–1998) based on epidemiological data from disease reporting. *Rev Clin Esp* 2002; **202:** 577–82.
31 Giannakopoulos I, Nikolakopoulou NM, Eliopoulou M, et al. Presentation of childhood brucellosis in western Greece. *Jpn J Infect Dis* 2006; **59:** 160–63.
32 Mantur BG, Akki AS, Mangalgi SS, et al. Childhood brucellosis—a microbiological, epidemiological and clinical study. *J Trop Pediatr* 2004; **50:** 153–57.
33 Andriopoulos P, Tsironi M, Deftereos S, Aessopos A, Assimakopoulos G. Acute brucellosis: presentation, diagnosis, and treatment of 144 cases. *Int J Infect Dis* 2007; **11:** 52–57.
34 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:** 485–88.
35 Whatmore AM, Perrett LL, Macmillan AP. Characterisation of the genetic diversity of *Brucella* by multilocus sequencing. *BMC Microbiol* 2007; **7:** 34.
36 Troy SB, Rickman LS, Davis CE. Brucellosis in San Diego: epidemiology and species-related differences in acute clinical presentations. *Medicine (Baltimore)* 2005; **84:** 174–87.

37 Almuneef MA, Memish ZA, Balkhy HH, et al. Importance of screening household members of acute brucellosis cases in endemic areas. *Epidemiol Infect* 2004; **132:** 533–40.

38 Ruiz-Mesa JD, Sanchez-Gonzalez J, Reguera JM, Martin L, Lopez-Palmero S, Colmenero JD. Rose Bengal test: diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. *Clin Microbiol Infect* 2005; **11:** 221–25.

39 Tsolia M, Drakonaki S, Messaritaki A, et al. Clinical features, complications and treatment outcome of childhood brucellosis in central Greece. *J Infect* 2002; **44:** 257–62.

40 Yetkin MA, Bulut C, Erdinc FS, Oral B, Tulek N. Evaluation of the clinical presentations in neurobrucellosis. *Int J Infect Dis* 2006; **10:** 446–52.

41 Kerem E, Diav O, Navon P, Branski D. Pleural fluid characteristics in pulmonary brucellosis. *Thorax* 1994; **49:** 89–90.

42 Uddin MJ, Sanyal SC, Mustafa AS, et al. The role of aggressive medical therapy along with early surgical intervention in the cure of *Brucella endocarditis*. *Ann Thorac Cardiovasc Surg* 1998; **4:** 209–13.

43 Aydin M, Fuat Yapar A, Savas L, et al. Scintigraphic findings in osteoarticular brucellosis. *Nucl Med Commun* 2005; **6:** 639–47.

44 Cascio A, Iaria C, Campenni A, Blandino A, Baldari S. Use of sulesomab in the diagnosis of brucellar spondylitis. *Clin Microbiol Infect* 2004; **10:** 1020–22.

45 Adaletli I, Albayram S, Gurses B, et al. Vasculopathic changes in the cerebral arterial system with neurobrucellosis. *AJNR Am J Neuroradiol* 2006; **27:** 384–86.

46 Al-Sous MW, Bohlega S, Al-Kawi MZ, Alwatban J, McLean DR. Neurobrucellosis: clinical and neuroimaging correlation. *AJNR Am J Neuroradiol* 2004; **25:** 395–401.

47 Kula S, Erer D, Buyukates M, et al. *Brucella endocarditis*: case report and review of the literature. *J Heart Valve Dis* 2001; **10:** 486–88.

48 Giannacopoulos I, Eliopoulou MI, Ziambaras T, Papanastasiou DA. Transplacentally transmitted congenital brucellosis due to *Brucella abortus*. *J Infect* 2002; **45:** 209–10.

49 Palanduz A, Palanduz S, Guler K, Guler N. Brucellosis in a mother and her young infant: probable transmission by breast milk. *Int J Infect Dis* 2000; **4:** 55–56.

50 Barroso Espadero D, Arroyo Carrera I, Lopez Rodriguez MJ, Lozano Rodriguez JA, Lopez Lafuente A. The transmission of brucellosis via breast feeding. A report of 2 cases. *An Esp Pediatr* 1998; **48:** 60–62.

51 Celebi G, Kulah C, Kilic S, Ustundag G. Asymptomatic *Brucella bacteraemia* and isolation of *Brucella melitensis* biovar 3 from human breast milk. *Scand J Infect Dis* 2007; **39:** 205–08.

52 Al-Kharfy TM. Neonatal brucellosis and blood transfusion: case report and review of the literature. *Ann Trop Paediatr* 2001; **21:** 349–52.

53 Doganay M, Aygen B, Esel D. Brucellosis due to blood transfusion. *J Hosp Infect* 2001; **49:** 151–52.

54 Ruben B, Band JD, Wong P, Colville J. Person-to-person transmission of *Brucella melitensis*. *Lancet* 1991; **337:** 14–15.

55 Ozturk R, Mert A, Kocak F, et al. The diagnosis of brucellosis by use of BACTEC 9240 blood culture system. *Diagn Microbiol Infect Dis* 2002; **44:** 133–35.

56 Durmaz G, Us T, Aydinli A, et al. Optimum detection times for bacteria and yeast species with the BACTEC 9120 aerobic blood culture system: evaluation for a 5-year period in a Turkish university hospital. *J Clin Microbiol* 2003; **41:** 819–21.

57 Navas E, Guerrero A, Cobo J, Loza E. Faster isolation of *Brucella* spp from blood by isolator compared with BACTEC NR. *Diagn Microbiol Infect Dis* 1993; **16:** 79–81.

58 Cockerill FR 3rd, Wilson JW, Vetter EA, et al. Optimal testing parameters for blood cultures. *Clin Infect Dis* 2004; **38:** 1724–30.

59 Mantur BG, Mangalgi SS. Evaluation of conventional castaneda and lysis centrifugation blood culture techniques for diagnosis of human brucellosis. *J Clin Microbiol* 2004; **42:** 4327–28.

60 Yagupsky P. Detection of brucellae in blood cultures. *J Clin Microbiol* 1999; **37:** 3437–42.

61 Ruiz-Castañeda M. A practical method for routine blood cultures in brucellosis. *Proc Soc Exp Biol Med* 1954; **86:** 154–55.

62 Ganado W, Bannister W. Bacteraemia in human brucellosis. *BMJ* 1960; **1:** 601–03.

63 Bannatyne RM, Jackson MC, Memish Z. Rapid diagnosis of brucella bacteremia by using the BACTEC 9240 system. *J Clin Microbiol* 1997; **35:** 2673–74.

64 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:** 122–25.

65 Doganay M, Aygen B. Human brucellosis: an overview. *Int J Infect Dis* 2003; **7:** 173–82.

66 Gaviria-Ruiz MM, Cardona-Castro NM. Evaluation and comparison of different blood culture techniques for bacteriological isolation of *Salmonella typhi* and *Brucella abortus*. *J Clin Microbiol* 1995; **33:** 868–71.

67 Deepak S, Bronson SG, Sibi, Joseph W, Thomas M. Brucella isolated from bone marrow. *J Assoc Physicians India* 2003; **51:** 717–18.

68 Etemadi H, Raissadat A, Pickett MJ, Zafari Y, Vahedifar P. Isolation of *Brucella* spp from clinical specimens. *J Clin Microbiol* 1984; **20:** 586.

69 Doern GV. Detection of selected fastidious bacteria. *Clin Infect Dis* 2000; **30:** 166–73.

70 Robichaud S, Libman M, Behr M, Rubin E. Prevention of laboratory-acquired brucellosis. *Clin Infect Dis* 2004; **38:** e119–22.

71 Noviello S, Gallo R, Kelly M, et al. Laboratory-acquired brucellosis. *Emerg Infect Dis* 2004; **10:** 1848–50.

72 Yagupsky P, Baron EJ. Laboratory exposures to brucellae and implications for bioterrorism. *Emerg Infect Dis* 2005; **11:** 1180–85.

73 Miller CD, Songer JR, Sullivan JF. A twenty-five year review of laboratory-acquired human infections at the National Animal Disease Center. *Am Ind Hyg Assoc J* 1987; **48:** 271–75.

74 Turkmani A, Ioannidis A, Christidou A, et al. In vitro susceptibilities of *Brucella melitensis* isolates to eleven antibiotics. *Ann Clin Microbiol Antimicrob* 2006; **5:** 24.

75 Akova M, Gur D, Livermore DM, Kocagoz T, Akalin HE. In vitro activities of antibiotics alone and in combination against *Brucella melitensis* at neutral and acidic pHs. *Antimicrob Agents Chemother* 1999; **43:** 1298–300.

76 Ariza J, Bosch J, Gudiol F, Liñares J, Viladrich PF, Martín R. Relevance of in vitro antimicrobial susceptibility of *Brucella melitensis* to relapse rate in human brucellosis. *Antimicrob Agents Chemother* 1986; **30:** 958–60.

77 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:** 577–89.

78 Ariza J, Pellicer T, Pallares R, Foz A, Gudiol F. Specific antibody profile in human brucellosis. *Clin Infect Dis* 1992; **14:** 131–40.

79 Nielsen K, Smith P, Widdison J, et al. Serological relationship between cattle exposed to *Brucella abortus*, *Yersinia enterocolitica* O:9 and *Escherichia coli* O157:H7. *Vet Microbiol* 2004; **100:** 25–30.

80 Serra J, Vinas M. Laboratory diagnosis of brucellosis in a rural endemic area in northeastern Spain. *Int Microbiol* 2004; **7:** 53–58.

81 Maichomo MW, McDermott JJ, Arimi SM, Gathura PB. Assessment of the Rose-Bengal plate test for the diagnosis of human brucellosis in health facilities in Narok district, Kenya. *East Afr Med J* 1998; **75:** 219–22.

82 Blasco JM, Garin-Bastuji B, Marin CM, et al. Efficacy of different Rose Bengal and complement fixation antigens for the diagnosis of *Brucella melitensis* infection in sheep and goats. *Vet Rec* 1994; **134:** 415–20.

83 Mantecon MA, Gutierrez P, del Pilar Zarzosa M, et al. Utility of an immunocapture-agglutination test and an enzyme-linked immunosorbent assay test against cytosolic proteins from *Brucella melitensis* B115 in the diagnosis and follow-up of human acute brucellosis. *Diagn Microbiol Infect Dis* 2006; **55:** 27–35.

84 Araj GF, Kattar MM, Fattouh LG, Bajakian KO, Kobeissi SA. Evaluation of the PANBIO brucella immunoglobulin G (IgG) and IgM enzyme-linked immunosorbent assays for diagnosis of human brucellosis. *Clin Diagn Lab Immunol* 2005; **12:** 1334–35.

85 Orduna A, Almaraz A, Prado A, et al. Evaluation of an immunocapture-agglutination test (Brucellacapt) for serodiagnosis of human brucellosis. *J Clin Microbiol* 2000; **38:** 4000–05.

86 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:** 756–59.

87 Lucero NE, Escobar GI, Ayala SM, Silva Paulo P, Nielsen K. Fluorescence polarization assay for diagnosis of human brucellosis. *J Med Microbiol* 2003; **52:** 883–87.

88 Irmak H, Buzgan T, Evirgen O, et al. Use of a new, simple and rapid diagnostic test, the brucella-IgM and IgG flow assays in the serodiagnosis of human brucellosis in an endemic area in eastern Turkey. *Am J Trop Med Hyg* 2004; **70:** 688–94.

89 Hasanjani-Roushan MR, Soleimani-Amin MJ, Abdoel TH, Smits HL. Application of a rapid and simple brucella-specific IgM and IgG antibody test for the serodiagnosis of brucellosis in a hospital in Iran. *Trans R Soc Trop Med Hyg* 2005; **99:** 744–50.

90 Abramson O, Rosenvasser Z, Block C, Dagan R. Detection and treatment of brucellosis by screening a population at risk. *Ped Infect Dis J* 1991; **10:** 434–38.

91 Almuneef MA, Memish ZA, Balkhy HH, et al. Importance of screening household members of acute brucellosis cases in endemic areas. *Epidemiol Infect* 2004; **132:** 533–40.

92 Alsubaie S, Almuneef M, Alshaalan M et al. Acute brucellosis in Saudi families: relationship between brucella serology and clinical symptoms. *Int J Infect Dis* 2005; **9:** 218–24.

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

94 Nockler K, Scholz HC, Tomaso H, Bogumil R, Neubauer H. Immunoproteomic characterization of *Brucella abortus* 1119-3 preparations used for the serodiagnosis of brucella infections. *J Immunol Methods* 2006; **309:** 34–47.

95 Bricker BJ. PCR as a diagnostic tool for brucellosis. *Vet Microbiol* 2002; **90:** 435–46.

96 Baily GG, Krahn JB, Drasar BS, Stoker NG. Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. *J Trop Med Hyg* 1992; **95:** 271–75.

97 Bardenstein S, Mandelboim M, Ficht TA, Baum M, Banai M. Identification of the *Brucella melitensis* vaccine strain Rev1 in animals and humans in Israel by PCR analysis of the PstI site polymorphism of its omp2 gene. *J Clin Microbiol* 2002; **40:** 1475–80.

98 Cloeckaert A, Grayon M, Grepinet O. An IS711 element downstream of the bp26 gene is a specific marker of *Brucella* spp isolated from marine mammals. *Clin Diagn Lab Immunol* 2000; **7:** 835–39.

99 Leal-Klevezas DS, Martinez-Vazquez IO, Lopez-Merino A, Martinez-Soriano JP. Single-step PCR for detection of *Brucella* spp from blood and milk of infected animals. *J Clin Microbiol* 1995; **33:** 3087–90.

100 Herman L, De Ridder H. Identification of *Brucella* spp by using the polymerase chain reaction. *Appl Environ Microbiol* 1992; **58:** 2099–101.

101 Fekete A, Bantle JA, Halling SM, Sanborn MR. Preliminary development of a diagnostic test for brucella using polymerase chain reaction. *J Appl Bacteriol* 1990; **69:** 216–27.

102 Queipo-Ortuño MI, Morata P, Ocon P, Manchado P, Colmenero JD. Rapid diagnosis of human brucellosis by peripheral-blood PCR assay. *J Clin Microbiol* 1997; **35:** 2927–30.

103 Zerva L, Bourantas K, Mitka S, Kansouzidou A, Legakis NJ. Serum is the preferred clinical specimen for diagnosis of human brucellosis by PCR. *J Clin Microbiol* 2001; **39:** 1661–64.

104 Vrioni G, Gartzonika C, Kostoula A, et al. Application of a polymerase chain reaction enzyme immunoassay in peripheral whole blood and serum specimens for diagnosis of acute human brucellosis. *Eur J Clin Microbiol Infect Dis* 2004; **23:** 194–99.

105 Boom R, Sol CJ, Salimans MM, et al. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990; **28:** 495–503.

106 Mitka S, Anetakis C, Souliou E, Diza E, Kansouzidou A. Evaluation of different PCR assays for early detection of acute and relapsing brucellosis in humans in comparison with conventional methods. *J Clin Microbiol* 2007; **45:** 1211–18.

107 Colmenero J, Queipo-Ortuno MI, Maria Reguera J, et al. Chronic hepatosplenic abscesses in brucellosis. Clinico-therapeutic features and molecular diagnostic approach. *Diagn Microbiol Infect Dis* 2002; **42:** 159–67.

108 Morata P, Queipo-Ortuño MI, Reguera JM, et al. Diagnostic yield of a PCR assay in focal complications of brucellosis. *J Clin Microbiol* 2001; **39:** 3743–46.

109 Colmenero JD, Queipo-Ortuno MI, Reguera JM, et al. Real time polymerase chain reaction: a new powerful tool for the diagnosis of neurobrucellosis. *J Neurol Neurosurg Psychiatry* 2005; **76:** 1025–27.

110 Queipo-Ortuno MI, Colmenero JD, Baeza G, Morata P. Comparison between LightCycler real-time polymerase chain reaction (PCR) assay with serum and PCR-enzyme-linked immunosorbent assay with whole blood samples for the diagnosis of human brucellosis. *Clin Infect Dis* 2005; **40:** 260–64.

111 Debeaumont C, Falconnet PA, Maurin M. Real-time PCR for detection of *Brucella* spp DNA in human serum samples. *Eur J Clin Microbiol Infect Dis* 2005; **24:** 842–45.

112 Redkar R, Rose S, Bricker B, DelVecchio V. Real-time detection of *Brucella abortus*, *Brucella melitensis* and *Brucella suis*. *Mol Cell Probes* 2001; **15:** 43–52.

113 Newby DT, Hadfield TL, Roberto FF. Real-time PCR detection of *Brucella abortus*: a comparative study of SYBR green I, 5'-exonuclease, and hybridization probe assays. *Appl Environ Microbiol* 2003; **69:** 4753–59.

114 Probert WS, Schrader KN, Khuong NY, Bystrom SL, Graves MH. Real-time multiplex PCR assay for detection of *Brucella* spp, *B abortus*, and *B melitensis*. *J Clin Microbiol* 2004; **42:** 1290–93.

115 Bogdanovich T, Skurnik M, Lubeck PS, Ahrens P, Hoorfar J. Validated 5' nuclease PCR assay for rapid identification of the genus brucella. *J Clin Microbiol* 2004; **42:** 2261–63.

116 Nimri LF. Diagnosis of recent and relapsed cases of human brucellosis by PCR assay. *BMC Infect Dis* 2003; **3:** 5.

117 Navarro E, Segura JC, Castano MJ, Solera J, Use of real-time quantitative polymerase chain reaction to monitor the evoluation of *Brucella melitensis* DNA load during therapy and post-treatment follow-up in patients with brucellosis. *Clin Infect Dis* 2006; **42:** 1266–73.

118 Morata P, Queipo-Ortuño MI, Reguera JM, et al. Posttreatment follow-up of brucellosis by PCR assay. *J Clin Microbiol* 1999; **37:** 4163–66.

119 Maas KS, Mendez M, Zavaleta M, et al. Evaluation of brucellosis by PCR and persistence after treatment in patients returning to the hospital for follow-up. *Am J Trop Med Hyg* 2007; **76:** 698–702.

120 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.

121 Whatmore AM, Shankster SJ, Perrett LL, et al. Identification and characterization of variable-number tandem-repeat markers for typing of *Brucella* spp. *J Clin Microbiol* 2006; **44:** 1982–93.

122 Vizcaino N, Caro-Hernandez P, Cloeckaert A, Fernandez-Lago L. DNA polymorphism in the omp25/omp31 family of *Brucella* spp: identification of a 1.7-kb inversion in *Brucella cetaceae* and of a 15·1-kb genomic island, absent from *Brucella ovis*, related to the synthesis of smooth lipopolysaccharide. *Microbes Infect* 2004; **6:** 821–34.

123 Cloeckaert A, Verger JM, Grayon M, et al. Classification of *Brucella* spp isolated from marine mammals by DNA polymorphism at the omp2 locus. *Microbes Infect* 2001; **3:** 729–38.

124 Cloeckaert A, Grayon M, Grepinet O. An IS711 element downstream of the bp26 gene is a specific marker of *Brucella* spp isolated from marine mammals. *Clin Diagn Lab Immunol* 2000; **7:** 835–39.

125 Whatmore AM, Murphy TJ, Shankster S, et al. Use of amplified fragment length polymorphism to identify and type brucella isolates of medical and veterinary interest. *J Clin Microbiol* 2005; **43:** 761–69.

126 Le Fleche P, Jacques I, Grayon M, et al. Evaluation and selection of tandem repeat loci for a brucella MLVA typing assay. *BMC Microbiol* 2006; **6:** 9.

127 Al Dahouk S, Fleche PL, Nockler K, et al. Evaluation of brucella MLVA typing for human brucellosis. *J Microbiol Methods* 2007; **69:** 137–45.

128 Joint Food and Agriculture Organization (FAO), World Health Organization (WHO). FAO-WHO expert committee on brucellosis. 6th report. WHO technical report series, no 740. Geneva: World Health Organization, 1986: 56–57.

129 Hasanjani Roushan MR, Mohraz M, Hajiahmadi M, Ramzani A, Valayati AA. Efficacy of gentamicin plus doxycycline versus streptomycin plus doxycycline in the treatment of brucellosis in humans. *Clin Infect Dis* 2006; **42:** 1075–80.

130 Falagas ME, Bliziotis IA. Quinolones for treatment of human brucellosis: critical review of the evidence from microbiological and clinical studies. *Antimicrob Agents Chemother* 2006; **50:** 22–33.

131 Ariza J, Corredoira J, Pallares R, et al. Characteristics of and risk factors for relapse of brucellosis in humans. *Clin Infect Dis* 1995; **20:** 1241–49.

132 Solera J, Martinez-Alfaro E, Espinosa A, Castillejos ML, Geijo P, Rodriguez-Zapata M. Multivariate model for predicting relapse in human brucellosis. *J Infect* 1998; **36:** 85–92.

133 Solera J, Martinez-Alfaro E, Saez L. Meta-analysis of the efficacy of the combination of rifampicin and doxycycline in the treatment of human brucellosis. *Med Clin (Barc)* 1994; **102:** 731–38.

134 Cascio A, Scarlata F, Giordano S, et al. Treatment of human brucellosis with rifampin plus minocycline. *J Chemother* 2003; **15:** 248–52.

135 Haddadi A, Mohraz M, Marefat M. Study of brucellosis treatment regimens in brucellosis patients in Imam Khomeini Hospital of Tehran. *Iranian J Infect Dis Trop Med* 2003; **20:** 29–32.

136 Ranjbar M, Keramat F, Mamani M, et al. Comparison between doxycycline-rifampin-amikacin and doxycycline-rifampin regimens in the treatment of brucellosis. *Int J Infect Dis* 2007; **111:** 152–56.

137 Solera J, Espinosa A, Martínez-Alfaro E, et al. Treatment of human brucellosis with doxycycline and gentamicin. *Antimicrob Agents Chemother* 1997; **41:** 80–84.

138 Lecaroz C, Blanco-Prieto MJ, Burrell MA, Gamazo C. Intracellular killing of *Brucella melitensis* in human macrophages with microsphere-encapsulated gentamicin. *J Antimicrob Chemother* 2006; **58:** 549–56.

139 Dizer U, Hayat L, Beker CM, et al. The effect of the doxycycline-rifampicin and levamisole combination on lymphocyte subgroups and functions of phagocytic cells in patients with chronic brucellosis. *Chemotherapy* 2005; **51:** 27–31.

140 Irmak H, Buzgan T, Karahocagil MK, et al. The effect of levamisole combined with the classical treatment in chronic brucellosis. *Tohoku J Exp Med* 2003; **201:** 221–28.

141 Printzis S, Raptopoulou-Gigi M, Orphanou-Koumerkeridou H, Lagre F, Goulis G. Immunotherapy in chronic brucellosis. Effect of levamisole and interferon; mechanisms of action and clinical value. *Immunopharmacol Immunotoxicol* 1994; **16:** 679–93.

142 Boura P, Tsapas G, Kountouras J, Zaharioudaki E. Interferon-A 2a administration in chronic anergic brucellosis. *Hepatogastroenterology* 1995; **42:** 919–22.

143 Alp E, Koc RK, Durak AC, et al. Doxycycline plus streptomycin versus ciprofloxacin plus rifampicin in spinal brucellosis. *BMC Infect Dis* 2006; **6:** 72–81.

144 Doyle TJ, Bryan RT. Infectious disease morbidity in the US region bordering Mexico, 1990–1998. *J Infect Dis* 2000; **182:** 1503–10.

145 Thapar MK, Young EJ. Urban outbreak of goat cheese brucellosis. *Pediatr Infect Dis* 1986; **5:** 640–43.

146 Al Dahouk S, Nockler K, Hensel A, et al. Human brucellosis in a nonendemic country: a report from Germany, 2002 and 2003. *Eur J Clin Microbiol Infect Dis* 2005; **24:** 450–56.

147 Luzzi GA, Brindle R, Sockett PN, et al. Brucellosis: imported and laboratory-acquired cases, and an overview of treatment trials. *Trans R Soc Trop Med Hyg* 1993; **87:** 138–41.

148 Schurig GG, Sriranganathan N, Corbel MJ. Brucellosis vaccines: past, present and future. *Vet Microbiol* 2002; **90:** 479–96.

149 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.

150 Lundervold M, Milner-Gulland EJ, O'Callaghan CJ, et al. A serological survey of ruminant livestock in Kazakhstan during post-Soviet transitions in farming and disease control. *Acta Vet Scand* 2004; **45:** 211–24.

151 Samartino LE. Brucellosis in Argentina. *Vet Microbiol* 2002; **90:** 71–80.

152 Luna-Martinez JE, Mejia-Teran C. Brucellosis in Mexico: current status and trends. *Vet Microbiol* 2002; **90:** 19–30.

153 Roth F, Zinsstag J, Orkhon D, et al Human health benefits from livestock vaccination for brucellosis: case study. *Bull World Health Organ* 2003; **81:** 867–76.

154 Arimi SM, Koroti E, Kang'ethe EK, Omore AO, McDermott JJ. Risk of infection with *Brucella abortus* and *Escherichia coli* O157:H7 associated with marketing of unpasteurized milk in Kenya. *Acta Trop* 2005; **96:** 1–8.