

New developments in the diagnostic procedures for zoonotic brucellosis in humans

S. Al Dahouk ^{(1)*}, L.D. Sprague ⁽²⁾ & H. Neubauer ⁽²⁾

(1) Federal Institute for Risk Assessment, Diederdsdorfer Weg 1, D-12277 Berlin, Germany

(2) Friedrich Loeffler Institute, Institute of Bacterial Infections and Zoonoses, Naumburger Straße 96a, D-07743 Jena, Germany

*Corresponding author: sascha.al-dahouk@gmx.de

Summary

At present, laboratory diagnosis of human brucellosis is based on isolation of the bacteria from clinical samples followed by standard microbiological tube testing, detection of anti-*Brucella* antibodies using various serological tests, and the use of molecular methods for the detection of *Brucella* DNA. None of these diagnostic tools can be used on its own to reliably detect the causative agent. Cultures give a low yield and subsequent phenotypic characterisation is time consuming, meaning that the initiation of adequate antibiotic therapy is frequently delayed. Serological tests seem to be more effective but are not internationally standardised. Moreover, antibodies can remain detectable despite successful therapy, cross-reacting antibodies may occur, and variable cut-offs for different levels of endemicity are lacking. Molecular assays may reduce diagnostic delays in clinical laboratories, but diagnostic criteria for active infection have not yet been defined. This article reviews the latest microbiological methods for the diagnosis of human brucellosis and outlines developments for the future.

Keywords

Biotyping – Cultivation – Human brucellosis – Molecular technique – Serological test – Standard microbiology method.

Introduction

Human brucellosis is a zoonotic disease with a major impact on public health, even though successful eradication and control programmes for domestic animals have been established in many countries around the world. The disease primarily presents as fever of unknown origin with multiple clinical signs and symptoms. Patients regularly suffer serious focal complications such as spondylitis, neurobrucellosis or *Brucella* endocarditis (22). Chronic disease, failure of primary antibiotic treatment, and relapses are frequent. The infection is usually transmitted to humans from its animal reservoir by direct contact or indirectly via contaminated foods, e.g. unpasteurised milk or cheese.

More than 500,000 human cases are reported worldwide each year (60), but the number of undetected cases is believed to be considerably higher. This alarming situation can be attributed to the non-specific clinical picture of human brucellosis, low awareness of the disease in non-endemic countries and shortcomings in laboratory diagnosis. The number of human cases is directly correlated with the number of infected animals within a defined

region. Effective countermeasures to reduce the incidence of human brucellosis are therefore based on surveillance and control of livestock and pasteurisation or cooking of contaminated food products. Once the disease has been transmitted from its animal reservoir to humans, only early diagnosis and adequate antibiotic therapy can prevent serious sequelae in patients.

Brucellae are facultative intracellular coccobacilli belonging to the order Rhizobiales of the α -2 subgroup of Proteobacteria. The class Alphaproteobacteria includes organisms that are either mammalian or plant pathogens or symbionts (34). Within the family Brucellaceae, *Ochrobactrum* is the closest phylogenetic neighbour of *Brucella*. Historically, brucellae are differentiated by host tropism, pathogenicity and phenotypic traits. Currently, the genus consists of six 'classic' species (Table I), i.e. the *Brucella melitensis* biovars (bvs) 1–3 (mainly isolated from sheep and goats), *B. abortus* bvs 1–6 and 9 (from cattle and other bovidae), *B. suis* bvs 1–3 (from pigs), bv. 4 (from reindeer) and bv. 5 (from small rodents), *B. canis* (from dogs), *B. ovis* (from sheep) and *B. neotomae* (from desert wood rats). In the last decade, several 'new' species

Table I***Brucella* species, including 'new' atypical strains, and their pathogenicity for humans**(modified from Sprague *et al.*, 2012 (78))

<i>Brucella</i> species	Biovars	Animal host	Human disease
<i>B. abortus</i>	1–6, 9	Cattle, bison, buffalo, elk, yak, camels	Yes
<i>B. melitensis</i>	1–3	Sheep, goats, cows, camels	Yes
	3	Nile catfish, dogs	
<i>B. suis</i>	1	Horses	Yes (bvs 1, 3, 4)
	1–3	Pigs, wild boar	
	2	European hare	
	4	Caribou, reindeer	
	5	Rodents	
<i>B. canis</i>		Canines	Yes (rarely)
<i>B. ovis</i>		Rams	Not reported
<i>B. neotomae</i>		Rodents	Not reported
<i>B. ceti</i>		Whales, dolphins, porpoises	Yes
<i>B. pinnipedialis</i>		Seals	Not reported
<i>B. microti</i>		Common voles, red foxes, (soil)	Not reported
<i>B. inopinata</i>		Unknown	Yes
Baboon isolates		Baboons	Not reported
BO2		Unknown	Yes
Rodent isolates		Rodents	Not reported
Frog isolates		African bullfrogs	Not reported

BO: *B. inopinata*-like strain
bvs: biovars

(Table I) have been described, i.e. *B. pinnipedialis* (isolated from seals) and *B. ceti* (from whales and dolphins) (35), *B. microti* (isolated from the common vole and red foxes) (73, 74), and *B. inopinata* (isolated from a human breast implant wound and an as-yet-unknown animal reservoir) (75). An increasing number of other 'atypical' strains have been isolated, possibly representing novel species or lineages. A *B. inopinata*-like strain (BO2), for instance, was isolated from a lung biopsy sample of an Australian patient with chronic destructive pneumonia (80). Various other strains have been cultured from wild native rodents originating from North Queensland, Australia (79), from non-human primates (72), and most recently from African bullfrogs (28) (Table I).

Although the majority of reported cases are caused by *B. melitensis*, *B. abortus* and *B. suis*, in descending order of occurrence, and rarely by *B. canis*, the pathogenicity of these newly described species and atypical strains for humans is currently under investigation. Several recent reports describe infections with marine mammal strains (50, 77) and isolation of *B. inopinata* (BO1) and BO2 from humans (23, 80). *Brucella microti* is also known to be pathogenic to mammalian hosts (42). Consequently, new diagnostic tools must be developed that are capable of detecting infections caused by classic *Brucella* species and new strains. At present, the laboratory diagnosis of human brucellosis is based on the isolation of the bacteria from clinical samples followed

by standard microbiological tube testing, detection of anti-*Brucella* antibodies using various serological tests, and the use of molecular methods for the detection of *Brucella* DNA.

Isolation of brucellae from blood and tissues

The definitive diagnosis of brucellosis requires the isolation of the pathogen from blood, bone marrow or other tissues and body fluids. The number of bacteria in clinical samples may vary widely, with the isolation of *Brucella* being highly dependent on the stage of disease (acute versus chronic), antibiotic pre-treatment, the existence of an appropriate clinical specimen and the culturing methods used (7). The number of viable bacteria circulating in the blood of patients with brucellosis is assumed to be low and therefore the sample volume is critical. Moreover, the time to detection is inversely correlated with the concentration of viable organisms in the blood sample (83). Bacteraemia usually occurs early in the course of the disease, and patients with bacteraemia are more likely to suffer fever and chills than those without (43). Consequently, isolation rates are much higher during the first two weeks of symptomatic disease and in blood cultures taken during the pyrexial phase (57). Multiple blood sampling may also increase the detection rate. In acute cases, sensitivity can vary from 80% to 90%.

In contrast, isolation rates are much lower in chronic cases, ranging between 30% and 70%, with the rate of successful isolation being significantly influenced by the technical approach used (30, 36). The likelihood of isolation in patients with chronic disease and focal complications can be improved by using sampling material from affected sites. If samples of contaminated tissues or excreta are to be examined, selective media such as Farrell's medium can be used (32). Owing to the presence of nalidixic acid and bacitracin in the classic selective media, growth of *B. suis* and several *B. melitensis* and *B. abortus* strains can be significantly inhibited. Hence, a new selective medium containing vancomycin, colistin, nystatin, nitrofurantoin and amphotericin B has recently been developed for veterinary samples (24). However, there have been no clinical studies evaluating its use in the diagnosis of human brucellosis. Bone marrow cultures have proven to be more sensitive than blood cultures for the detection of *Brucella* spp. at any stage of disease, and the mean time to detection is significantly reduced (39, 54). This method has also proven its usefulness in patients treated with antibiotics. As bone marrow aspiration and biopsy can be painful, the procedure should be restricted to specific cases, i.e. serologically negative patients in whom there is a strong clinical suspicion of brucellosis (39).

Most standard media, e.g. blood agar, chocolate agar, trypticase soy agar and serum dextrose agar, are suitable for culturing *Brucella* spp. Some strains may need bovine or equine serum (2%–5%) for growth, which is routinely added to the basal medium. The inoculated agar plates should be incubated at 35°C to 37°C in 5% to 10% CO₂. In primary culture from clinical specimens, it can take several days or even weeks before the punctate, non-pigmented and non-haemolytic colonies that are typical of this fastidious bacterium become visible. Colonies of smooth (S) *Brucella* strains are raised, convex, circular, translucent, and 0.5 mm to 1 mm in diameter. Colony morphology, as well as virulence, antigenic properties and phage sensitivity of the bacteria, is subject to changes after subcultivation or prolonged culture (more than four days). Thus, smooth brucellae dissociate to rough (R) forms, which grow in less convex and more opaque colonies with a dull, dry, yellowish-white granular appearance.

The recovery rate from clinical specimens can be maximised by using broth culture methods for primary enrichment, combined with blind subcultures at regular intervals. The sensitivity of culture methods has been progressively increased by various technical improvements, e.g. the biphasic Castañeda method, automated systems and yield-optimising methods such as lysis centrifugation (83). The classic Castañeda method is based on a non-selective biphasic medium. A solid and a liquid phase in the same blood culture bottle render repeated subcultures superfluous. Nevertheless, the time taken to recover brucellae from blood

can still be up to 30 days. With the launch of automated blood culture systems, such as the BACTEC™ (Becton Dickinson Diagnostic Systems, Sparks, Maryland, USA) and the BacT/Alert™ (bioMérieux Inc., Durham, North Carolina, USA), which continuously monitor the CO₂ release of potentially growing microorganisms, and the BACTEC™ Myco/F-Lytic system (Becton Dickinson Diagnostic Systems), which integrates lytic activity and automation (53), the time to detection has been significantly reduced.

Brucellae can be detected in the blood of infected patients after four days of culture or less (19). The recovery rate of bacterial pathogens, including *Brucella* spp., from sterile body fluids is also higher using automated blood culture systems (19). However, in presumptive cases it is recommended that incubation periods be prolonged to at least four weeks, with intermittent subculturing, to reliably exclude *Brucella* infection (83). The isolation rate of brucellae from blood samples can be further increased by enrichment of the bacteria using the blood clot culture technique or lysis centrifugation (30, 52). The lysis centrifugation system is the most efficient of the well-established culture methods because it is independent of disease stage. In both blood and sterile body fluids, the mean time to detection can be significantly reduced to two days (19, 30, 53). Shell vial culture is a reasonable approach when trying to isolate the facultative intracellular pathogens from clinical specimens containing very low numbers of cultivable brucellae, e.g. from pus (70).

Identification and characterisation of *Brucella* spp.

In human brucellosis, detection of the agent is paramount as early onset of antibiotic treatment prevents chronicity and focal complications (4). Brucellae are very small, faintly stained Gram-negative coccobacilli resembling 'fine sand' when viewed under the microscope. Oxidase- and urease-positive bacteria must raise the suspicion of *Brucella*, and the viable pathogen has to be handled in a biosafety level 3 laboratory. The diagnostic tool of choice for initial and rapid confirmation of suspicious colonies is the slide agglutination test using undiluted polyvalent *Brucella* antiserum (anti-smooth serum) mixed with a saline suspension of the bacteria. A direct urease test on positive blood cultures suggestive of *Brucella* spp. (68) or fluorescence *in situ* hybridisation (FISH) using *Brucella*-specific probes (82) can also be used to reduce the time to the presumptive diagnosis of human brucellosis.

The identification of *Brucella* species and biovars is based on a toolbox of elaborate microbiological methods testing for CO₂ requirement, H₂S production, urease activity, agglutination with monospecific sera (A and M), selective

inhibition of growth on media containing dyes such as thionin or basic fuchsin, and phage typing (7, 9). These methods are time consuming, hazardous and subject to variable interpretation, and are therefore not suited to routine clinical microbiology laboratories. Commercially available biochemical identification systems such as the API 20 NE® (bioMérieux, Nürtingen, Germany) must be used with caution, owing to the potential misidentification of *Brucella* spp. as *Psychrobacter phenylpyruvicus* (formerly *Moraxella phenylpyruvica*) (14) or *Ochrobactrum anthropi* (29). A valuable alternative to the standard microbiology methods is the semi-automated metabolic biotyping system (Micronaut™), based on a selection of 93 different substrates (6). This novel technology not only reduces hands-on time but also minimises the risk of laboratory-acquired infections. *Brucella* can be identified and differentiated up to the species and biovar level within 48 hours. This method offers a feasible alternative to the present time-consuming tube testing.

In the last decade, matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS) has emerged as a powerful tool for bacterial identification in clinical microbiology laboratories. In comparison with conventional phenotypic techniques or molecular methods, it is rapid, precise and cost effective. MALDI-TOF MS is able to identify *Brucella* spp. directly, from both culture plates and blood culture bottles (33, 45), but is not yet routinely used because access to comprehensive protein profile databases is limited. The generation of *Brucella*-specific databases is severely hampered by the classification of the microorganism as a category B bioterrorism agent. Susceptibility testing of clinical isolates has no added value and is not necessary, because the antimicrobial resistance patterns of brucellae do not change after primary treatment (2, 11). A repeated course of the standard antibiotic therapy in relapsed cases usually results in complete elimination of the infection.

Serological diagnosis of human brucellosis

In contrast to bacterial culture, serological testing is fast, non-hazardous and more sensitive and therefore preferred in routine clinical practice. However, serological tests can only indirectly prove *Brucella* infections by high or rising titres of specific antibodies. Agglutination titres $\geq 1:160$ or a fourfold rise of titres in follow-up sera are considered to be indicative of active infection. Diagnostic titres, however, can be detected months or even years after acute infection despite therapeutic success and negative blood cultures (12). In addition, a high proportion of the population in endemic regions may have persistent antibody titres due to ongoing exposure to *Brucella*. It is therefore essential to

consider this background prevalence in healthy individuals when determining reliable cut-off values for serological assays. Hence, the significance of antibody detection without clinical signs and symptoms or a history of potential exposure remains questionable (4).

Serological tests are used for the initial diagnosis of human brucellosis as well as during treatment follow-up. Serological tests can be negative, especially early in the course of the disease, and laboratory testing should be repeated after one to two weeks in clinically suspicious cases. Sequential serological testing also allows the monitoring of treatment response. In the first week of infection, immunoglobulin (Ig) M isotype antibodies predominate, followed by a shift to IgG in the second week (8). Titres of both subtypes rise continuously and reach a peak within four weeks. Effective antibiotic therapy is usually accompanied by a rapid decline in antibody titres, whereas persisting high IgG titres can be an indication of treatment failure (15, 17). Relapse is often characterised by a second peak of anti-*Brucella* IgG and IgA, but not IgM, immunoglobulins.

Currently, there is no standardised reference antigen for serological tests. This is unfortunate as antigen preparation can significantly influence the serological diagnosis of human brucellosis. The diagnostic antigen of classic serological tests is usually made from whole-cell extracts containing large amounts of smooth lipopolysaccharides (S-LPS). As the humoral immune response during natural infection is mainly mediated by antibodies directed against S-LPS, these assays reliably detect agglutinating and/or non-agglutinating antibodies. However, because of cross-reactivity with various other clinically relevant bacteria, the specificity of LPS-based assays can be low. The immunodominant epitope of the *Brucella* O-polysaccharide resembles the corresponding epitopes of *Yersinia enterocolitica* O:9, *Salmonella urbana* group N, *Vibrio cholerae*, *Francisella tularensis*, *Escherichia coli* O157 and *Stenotrophomonas maltophilia* (8). In contrast, the S-LPS antigen is not shared by all *Brucella* spp., thus explaining why canine brucellosis cannot be diagnosed by standard serological methods based on smooth *Brucella* antigens (48).

Serum agglutination test, Rose Bengal test and lateral flow assay

For the serological diagnosis of human brucellosis, the serum agglutination test (SAT) is still considered to be the reference method (8). The labour-intensive and time-consuming classic tube agglutination test (Wright test) has been successively replaced by more practicable formats such as slide, plate and card agglutination for routine clinical laboratories. The Rose Bengal test (RBT) is an example of such a card test. It is based on an 8% antigen suspension (pH 3.65 ± 0.05) of the *B. abortus* strain 1119-3 (United

States Department of Agriculture) stained with Rose Bengal dye. In endemic countries, the RBT is traditionally used for rapid screening in emergency departments, although its performance is poor in patients formerly and/or repeatedly exposed to the agent (71). In order to exclude false-positive RBT results, diagnostic titres have to be confirmed by more specific serological tests. In high-risk populations, testing of diluted sera using the RBT might be a reasonable alternative, as this would reduce the need for a considerable number of confirmatory tests (27).

In general, the likelihood of a true-positive serological test result in suspected cases can be increased by a high pre-test probability based on clinical signs and symptoms of the disease. Although the definite cure of *Brucella* infections is usually associated with lower SAT titres, significant titres can be found in 3% to 5% of patients up to two years after successful antibiotic treatment (69). The significance of diagnostic titres in follow-up sera from patients with brucellosis can therefore be assessed only within the context of a compatible clinical picture (4). The lateral flow assay is another easy-to-perform technique suitable for rapid field or bedside testing in poor endemic areas where well-equipped laboratories are not available. In complicated and chronic cases, this test has proven to be somewhat more sensitive than the SAT (84).

The classic Coombs' test (CT) can be used as a complement to the SAT to detect incomplete, blocking or non-agglutinating antibodies. Particularly in chronic courses and during relapse, when SAT results are either negative or inconclusive, the CT has proven to be the diagnostic tool of choice (17). However, the CT and SAT are labour intensive and time consuming. An alternative is the Brucellacapt® (Vircell, Santa Fé, Granada, Spain), a single-step immunocapture assay for the detection of total anti-*Brucella* antibodies. Brucellacapt titres are a good marker of infection activity independent of disease stage. Brucellacapt titres decrease slowly in patients who have relapsed and show a more distinct decline after successful antibiotic treatment than the corresponding SAT titres (17, 51).

Although SAT is still the gold standard assay in the serological diagnosis of human brucellosis, enzyme-linked immunosorbent assays (ELISAs) are widely used in routine clinical laboratories, predominantly in non-endemic countries. Commercial ELISA kits reliably detect anti-*Brucella* antibodies and the results are consistent with the SAT and CT (10, 61). Although the SAT is considerably cheaper than ELISA, turnaround time is significantly longer (31). In chronic and convalescent cases, ELISA is more sensitive than the SAT, whereas both assays show similar results in acute cases. The detection of the IgG antibody class by ELISA is more sensitive than IgM detection (31). Testing for IgM may give false-negative results in the presence of an excess of IgG or false-positive results in the

presence of rheumatoid factor, which should be routinely eliminated by absorption prior to testing for anti-*Brucella* IgM antibodies (26, 76). In many patients, false-negative results are due to the quantification of only a single subgroup of immunoglobulins (38). Combined evaluation of IgM and IgG results can increase the sensitivity of ELISA and enable correct staging of the disease (31, 55).

Molecular detection of brucellae in clinical samples

Polymerase chain reaction (PCR) assays can be used to detect *Brucella* DNA in pure cultures and in clinical specimens, i.e. serum, whole-blood and urine samples, various tissues, cerebrospinal, synovial or pleural fluid, and pus (20, 21, 25, 62, 65, 66). Direct detection of *Brucella* DNA in brucellosis patients is a challenge because of the small number of bacteria present in clinical samples and inhibitory effects arising from matrix components (64). Basic sample preparation methods should diminish inhibitory effects and concentrate the bacterial DNA template. Residual PCR inhibition in complex matrices can be unmasked by the use of an internal amplification control (5). The QIAamp™ DNA Mini Kit (Qiagen Inc., Valencia, California, USA) and the UltraClean™ DNA BloodSpin Kit (MO BIO Laboratories Inc., Carlsbad, California, USA) are among the many commercial kits that have been successfully used to extract *Brucella* DNA from whole-blood, serum and tissue samples (49, 67, 81).

The advantages of PCR are numerous. Independent of the disease stage, it is more sensitive than blood cultures and more specific than serological tests. Various PCR assays targeting different gene loci have been successfully used for the diagnosis of human brucellosis (3, 58). By increasing the number of molecular markers, both sensitivity and specificity can be increased accordingly. Molecular assays targeting the *IS711* insertion element, which is found in multiple copies within *Brucella* chromosomes, also improve analytical sensitivity (16, 40). The analytical sensitivity can be further increased by using real-time PCR assays, which can detect as few as five bacteria per reaction (5, 59). Moreover, real-time PCR enables high-throughput screening of clinical samples and delivers results within a few hours.

Genus-specific PCR assays are generally adequate for the molecular diagnosis of human brucellosis (4). The *bcsp31* gene, coding for a 31-kDa immunogenic outer membrane protein conserved among all *Brucella* spp., is the most common molecular target in clinical applications (13). Such a genus-specific PCR can help to avoid false-negative results in patients infected with unusual species and biovars.

However, a primary molecular diagnosis must always be confirmed using a second gene target (5). For confirmation and distinction from closely related microorganisms, 16S rRNA gene sequencing can be used (37). Differential PCR assays, e.g. the conventional Bruce-ladder PCR (46, 47, 56) or species-specific real-time PCR assays (5), can also be used for confirmatory identification and differentiation of *Brucella* species.

Subtyping at the strain level can be useful for differentiating re-exposure from relapse (2). A multiple locus VNTR (variable number of tandem repeats) analysis assay based on 16 markers (MLVA-16) has proven its usefulness for diagnostic purposes in human brucellosis (1, 41, 44). A relapse or primary treatment failure can be confirmed by assessing identical MLVA-16 genotypes of *Brucella* strains isolated from the same patient before and after first-line therapy, and, as a consequence, antibiotic therapy should be prolonged. Re-infection is usually characterised by divergent genotypes of *Brucella* isolates, and standard therapy can be repeated without substantial loss of efficacy.

The clinical significance of *Brucella* DNA detection in the course of the disease is not clear as *Brucella* DNA-negative patients can also relapse (59). Molecular detection of *Brucella* DNA can be a sign of acute or chronic brucellosis and can also be detected in asymptomatic subjects with a history of brucellosis (18). Despite successful antibiotic therapy, *Brucella* DNA remains detectable in the majority of brucellosis patients throughout treatment and treatment follow-up, as well as years after clinical cure and in the absence of any symptoms indicative of chronic disease or relapse (49, 59, 81). This phenomenon might be explained by the survival and persistence of brucellae in human macrophages and the higher diagnostic yield of modern real-time PCR assays capable of detecting non-viable or phagocytosed microorganisms. Quantitative real-time PCR could be the tool to differentiate active from past *Brucella* infections (63) and to unravel the mystery of DNA persistence in brucellosis patients.

Novel approaches

Despite the availability of modern and suitable laboratory methodology, brucellosis is frequently underdiagnosed in developed, non-endemic countries. This is mainly because of a lack of awareness of this infection among physicians. In poor, endemic countries, the diagnosis is frequently missed because laboratory facilities are poorly equipped, and 'modern' diagnostic means such as ELISA and PCR are considered too expensive. In view of these two epidemiological settings (non-endemic versus endemic), the authors will try to provide solutions and recommendations for translational research regarding the development of novel diagnostics.

In non-endemic countries, in order to avoid delayed onset of therapy ultimately leading to chronicity of the disease and focal complications, diagnosis of brucellosis should rely on three criteria: clinical presentation, sojourn in an endemic region and/or consumption of unprocessed animal products, and corresponding laboratory findings. The clinical presentation should be based on the classic symptoms of brucellosis, i.e. undulant fever accompanied by weight loss, chills, drenching sweats, and swelling of the liver and spleen, or its sequelae. Precise medical history-taking is paramount, i.e. the physician must find out if the patient has recently travelled to an endemic region or consumed unprocessed milk or meat products. Finally, sound laboratory findings are required to confirm the clinical picture/diagnosis. Laboratory diagnosis should rely either on a positive culture from blood or an organ sample or on a positive PCR result from blood, serum or organ samples or positive IgM and IgG ELISA results, preferably demonstrating rising titres. In rare cases, clinical suspicion without laboratory confirmation can justify therapy.

In endemic countries with limited financial means, diagnosis of brucellosis should rely on two criteria: clinical presentation and laboratory diagnosis. This diagnosis should be based either on a positive culture from blood or organ samples or on positive serological results, preferably demonstrating rising titres. Serum agglutination can be used provided that a reasonable sensitivity at a cut-off of greater than 1:160 is given. The test has to be confirmed with either the CT or a comparable immunocapture assay to detect incomplete blocking or non-agglutinating antibodies. In rare cases, clinical diagnosis without laboratory confirmation can justify therapy.

In order to improve laboratory-based diagnosis of brucellosis, the following issues ought to be dealt with. Public health authorities need to increase awareness of brucellosis among practising physicians and improve the microbiological education of medical students. Moreover, serological and microbiological methods standardised with regard to nomenclature are needed to obtain comparable results. This also applies to the definition of guidelines for the handling of diagnostic and therapeutic regimes. Owing to the lack of laboratory criteria describing 'cured status', persistent antibody titres in the course of follow-up are difficult to interpret. The detection of anti-*Brucella* antibodies is not in itself sufficient evidence for the presence of the pathogen. Criteria describing the success of treatment or predicting relapse are also lacking. A single agglutination titre $\leq 1:160$ is a useless piece of information as many patients are seronegative in the acute phase of the disease. This necessitates the serological testing of paired sera or performance of more than one serological test.

However, molecular assays and techniques also need to be evaluated. This applies especially to the use of reverse

transcription real-time PCR for the diagnosis of an active infection. FISH technology targeting bacterial rRNA should enable speedier diagnosis and institution of therapy. The use of bacterial proteins secreted during active (and/or chronic) infection needs to be investigated in view of their potential use in diagnostic assays. This also applies to brucellosis-specific biomarkers. The applicability of specific antigens in the lymphocyte stimulation test as well as the test's sensitivity and specificity in acute and chronic and in silent and active infection needs to be evaluated. Finally, it is essential to conduct meta-analyses comparing the various tests in terms of their specificity, sensitivity and analytical sensitivity. Threshold values for local

populations, e.g. southern versus northern Europeans or endemic versus non-endemic areas within a territorial state, must be defined. International reference material must be made available by non-governmental organisations and the World Health Organization. So, let us begin to close the gap between the status quo and our desired future.

Nouveaux développements dans les procédures de diagnostic de la brucellose zoonotique chez l'homme

S. Al Dahouk, L.D. Sprague & H. Neubauer

Résumé

À l'heure actuelle, le diagnostic de la brucellose humaine au laboratoire est basé sur l'isolement de la bactérie à partir d'échantillons cliniques suivi de l'analyse microbiologique normalisée en tubes à essai, la détection sérologique des anticorps dirigés contre *Brucella* et le recours à des méthodes de détection moléculaire ciblant l'ADN de *Brucella*. Aucun de ces outils diagnostiques ne suffit à détecter l'agent causal de manière autonome et fiable. Les cultures présentent des concentrations faibles, de sorte que la caractérisation du phénotype s'avère longue et difficile, retardant le démarrage d'une antibiothérapie appropriée. Les épreuves sérologiques semblent plus efficaces mais n'ont pas encore fait l'objet d'une normalisation internationale. De plus, on constate la persistance d'anticorps, même après la réussite d'un traitement, ainsi qu'une possibilité de réactions croisées, et les variations de seuils limite correspondant à différents degrés d'endémicité n'ont pas encore été établies. Les épreuves moléculaires réduisent les délais du diagnostic dans les laboratoires cliniques, mais les paramètres diagnostiques révélant une infection active restent à définir. Les auteurs font le point sur les méthodes microbiologiques récentes utilisées pour détecter la brucellose humaine et tracent les perspectives d'avenir dans ce domaine.

Mots-clés

Brucellose humaine – Caractérisation de biotypes – Culture – Épreuve sérologique – Méthode microbiologique normalisée – Technique moléculaire.

Novedades en los procedimientos de diagnóstico de la brucelosis zoonótica en el ser humano

S. Al Dahouk, L.D. Sprague & H. Neubauer

Resumen

Actualmente, el diagnóstico de laboratorio de la brucelosis humana reposa en el aislamiento de la bacteria a partir de muestras clínicas, seguido de un clásico análisis microbiológico en tubo, la detección de anticuerpos anti-*Brucella* mediante diversas pruebas serológicas y el uso de métodos moleculares para detectar el ADN brucélico. Ninguno de estos procedimientos de diagnóstico sirve por sí solo para detectar de forma fiable el agente causal de una infección. Los cultivos son poco productivos, y la subsiguiente caracterización fenotípica lleva mucho tiempo, lo que a menudo retrasa el inicio de la adecuada terapia antibiótica. Las pruebas serológicas parecen ser más eficaces, pero no están normalizadas a escala internacional. Además, a veces puede ocurrir que se sigan detectando anticuerpos aun después de un tratamiento antibiótico eficaz o que haya anticuerpos que ofrezcan reacciones cruzadas, sin olvidar que no se dispone de valores umbral variables para distintos niveles de endemidad. Las técnicas moleculares pueden reducir los plazos de diagnóstico en laboratorio clínico, pero todavía no se han definido criterios de diagnóstico de la infección activa. Los autores examinan los más recientes métodos microbiológicos para diagnosticar la brucelosis humana y explican a grandes líneas la evolución que se vislumbra de cara al futuro.

Palabras clave

Brucelosis humana – Cultivo – Método microbiológico clásico – Prueba serológica – Técnica molecular – Tipificación biológica.



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