

CLINICAL ALERT

Human brucellosis in South Africa: Public health and diagnostic pitfalls

J M Wojno,^{1,2} MB BCh, FCPATH (SA), MMed Path (Microbiol); C Moodley,^{1,2} BSc Hons, PhD (Mol Cell Biol); J Pienaar,³ BVSc; N Beylis,^{1,2} MB BCh, FCPATH (Microbiol); L Jacobsz,⁴ MB ChB, MMed (Clin Path); M P Nicol,^{1,2} MB BCh, MMed Path (Microbiol), DTM&H, FCPATH (Microbiol), PhD; J Rossouw,⁵ BSc Hons, MSc, PhD (Microbiol Plant Pathol); C Bamford,^{1,2} MB ChB, FCPATH (Microbiol), MMed Path (Microbiol)

¹ National Health Laboratory Service, Microbiology Laboratory, Groote Schuur Hospital, Cape Town, South Africa

² Division of Medical Microbiology, Department of Pathology, Faculty of Health Sciences, University of Cape Town, South Africa

³ Department of Agriculture, Forestry and Fisheries (Animal Health: Beaufort West), Western Cape, South Africa

⁴ National Health Laboratory Service, George Hospital, Western Cape, South Africa

⁵ Special Bacterial Pathogens Reference Laboratory, Centre for Emerging and Zoonotic Diseases, National Institute for Communicable Diseases, Sandringham, Johannesburg, South Africa

Corresponding author: J M Wojno (tina.wojno@gmail.com)

Human brucellosis in South Africa (SA) is under-diagnosed and under-reported. This is because many clinicians have little or no experience in managing affected patients, and in part because of the nonspecific and insidious nature of the disease. A case of human brucellosis caused by *Brucella melitensis* in a patient from the Western Cape Province of SA is described, and the resulting exposure of staff members at two medical microbiology laboratories, as well as the public health investigation that was conducted, are discussed. The objective of this article is to highlight the need for strengthening integration between public health, medical and veterinary services and exposing deficiencies in public health, veterinary and laboratory practices.

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Brucellosis is a zoonotic infection mainly affecting farm animals such as cattle, sheep and goats. *Brucella* species primarily infect the reproductive tract, causing spontaneous abortions and infertility in livestock. Brucellosis has a worldwide distribution, but is most prevalent in areas with poorly established domestic animal and public health programmes.^[1,2] According to the US Centers for Disease Control and Prevention, Africa is among the areas most commonly affected.

Human brucellosis is thought to be considerably under-diagnosed, and although it is a notifiable disease in South Africa (SA), as in many parts of the world, it is probably under-reported.^[1] Fig. 1 indicates the estimated seroprevalence of *B. abortus* in animals in SA. No formal *B. melitensis* surveillance occurs in SA.

Humans are infected with *Brucella* by contact with sick animals or infected animal products. Organisms can be inoculated via cuts and/or abrasions when handling infected animal carcasses or products of conception, or inhaled as aerosols during these and other procedures. Ingestion of unpasteurised milk/dairy products or undercooked meat can also lead to infection. High-risk individuals include farmers, veterinarians, abattoir workers and meat handlers.^[2] Laboratory workers are also at risk if inadvertently exposed to live cultures of *Brucella*.

Most cases of human brucellosis are caused by *B. melitensis*, which is the species associated with more severe disease. The usual animal reservoirs for *Brucella* are sheep and goats (*B. melitensis*), cattle (*B. abortus*), and pigs (*B. suis*). Disease in humans may have an acute or subacute onset, with the illness having a propensity to become chronic and relapsing. Brucellosis is a systemic disease, usually presenting as a febrile illness with constitutional symptoms such as anorexia, malaise, headaches and chills. The patient may complain of arthralgia and back pain, as well as abdominal pain. Clinical findings include lymphadenopathy and hepatosplenomegaly. Up to 40% of

patients with brucellosis have osteoarticular complications.^[2] Chronic brucellosis is difficult to treat, requiring prolonged antibiotics and in some cases surgery.

A definitive diagnosis of *Brucella* infection is established by isolating the organism from blood, bone marrow, cerebrospinal fluid, tissue, pus or other relevant samples.^[2] A presumptive identification of the cultured isolate can be made using basic biochemical tests and colonial morphology. Cultured isolates should be referred to a specialised facility for further confirmatory testing and speciation, which can be done using molecular methods such as the *Brucella*-specific, Bruce-ladder multiplex polymerase chain reaction (PCR) and biotyping.^[1,3,4]

Case report

A 27-year-old man from a town in the Great Karoo, Western Cape Province, SA, initially presented to a local district hospital in November 2014 with a history of lower back pain and fever with new onset of vomiting and diarrhoea. On examination he appeared chronically unwell and had abdominal tenderness. Initial blood results showed pancytopenia, a normocytic, normochromic anaemia, and mildly elevated transaminases and canalicular enzymes. An abdominal ultrasound scan revealed diffuse hepatosplenomegaly with no granulomas or other focal lesions. One of two blood cultures sent by the regional hospital was positive for Gram-negative bacilli after 3 days of incubation. This provisional result was communicated to the treating clinician, who commenced ceftriaxone empirically. The bone marrow report showed prominent serous atrophy and listed common causes for this, which included chronic illnesses such as carcinoma, tuberculosis and other chronic infections. No granulomas were observed. On provisional testing of the cultured isolate, *Brucella* was suspected and the national and provincial departments of health were notified,

prompting a veterinary investigation. An in-depth history revealed that the patient had regularly fed his dog with cattle, sheep and goat abattoir waste that was disposed of at an open-access municipal waste site in the local town. There was no history of consumption of unpasteurised milk.

The cultured isolate was subsequently confirmed to be *B. melitensis* by the Department of Veterinary Tropical Disease at the University of Pretoria, using Bruce-ladder multiplex PCR.^[3] Biotyping performed by the Agricultural Research Council-Onderstepoort Veterinary Institute identified the isolate as *B. melitensis* biovar 1.^[4]

Laboratory exposure

By the time *Brucella* was suspected as the infecting agent, staff at two medical microbiology laboratories involved had potentially been exposed. Management of staff, which included post-exposure prophylaxis, varied depending on the risk category.^[5] The exposed laboratory workers were followed up by occupational health and safety staff for 24 weeks.

A total of 75 laboratory staff/visitors were identified as having been exposed to the organism. To make a serological diagnosis of *Brucella*, paired sera are collected at least 2 - 4 weeks apart looking for a four-fold or greater rise in antibody titre.^[6] There are numerous commercial kits and serological methods commonly used and recommended, including the Rose Bengal test, serum tube agglutination and enzyme-linked immunosorbent assay (ELISA).^[2] Serological testing using an ELISA, MASTAZYME BRUCELLA (MAST Diagnostics, UK) was performed for all exposed staff members at baseline and then at 6 and 24 weeks.^[6]

Five staff members had borderline or low-level positive IgG or IgM levels detected on serological testing at baseline. Repeat testing of the same samples as well as repeat samples sent 3 - 4 weeks later revealed considerable variability, particularly in IgM results.

In the absence of local studies to determine appropriate cut-off values in SA, and in view of the known intrinsic variability of IgM assays as well as known cross-reactivity with Enterobacteriaceae, borderline results can be difficult to interpret. Failure to demonstrate a (four-fold or greater) rise in the titre for specific antibody in paired sera usually excludes recent infection.^[6] None of the staff members tested had significant increases in titre (four-fold or greater rise in immunoglobulins) over the 24-week period. All exposed staff members remained asymptomatic throughout the 6-month follow-up period.

Informed consent was obtained from the patient, and the study was approved by

the Human Research Ethics Committee, University of Cape Town, SA (HREC REF: 220/2015).

Veterinary investigation

The state veterinarian's investigation revealed that two other people who had lambed goats on a nearby farm in 2014 had become ill and were subsequently diagnosed with brucellosis, based on serological testing. The state veterinarian placed the farm under quarantine and initiated sampling of the cattle, sheep and goats. Animals on surrounding farms were also sampled. Serological testing using either the Rose Bengal test or the complement fixation test was performed. These methods detect nonspecific antibodies to both *B. melitensis* and *B. abortus*.

Of the 100 goats sampled on the farm in question, 44 tested positive for *Brucella* on serological testing. All animals on the farm were quarantined, but none had been sacrificed at the time of writing and no samples were available for culture and confirmation of the *Brucella* species. As goats do not contract *B. abortus*, it was concluded that the most likely strain causing the outbreak was *B. melitensis*. No animals on other surrounding farms had tested positive for *Brucella* at the time of writing.

Discussion

The Department of Agriculture, Forestry and Fisheries relies on notifications of diseases and important epidemiological incidents from veterinarians. The first documented cases of veterinary *B. melitensis* in SA were in sheep in 1965 in the Transvaal Province.^[7]

Vaccination is the foundation of a functional *Brucella* control programme in livestock. This involves the *B. abortus* strain 19 (S19) and *B. abortus* RB51 vaccines for cattle and *B. melitensis* Rev. 1 vaccine for sheep and goats. These are both live attenuated vaccines with the potential of being pathogenic to humans if not administered appropriately.^[1,8] Vaccination regulations in SA as stipulated by the Animal Diseases Act 35 of 1984 have specific guidelines on vaccination of animals against *Brucella*. There are currently no safe, efficacious vaccines recommended for routine use in prevention of brucellosis in at-risk humans. Prevention strategies therefore require robust animal programmes and adherence to prevention/eradication and control protocols.^[1] From a public health perspective, the key targets would be the prevention of infection due to direct/indirect contact with infected livestock or live vaccines and prevention of food-borne illness, as well as strengthening the diagnosis and management of human cases with increased awareness and safe laboratory practices when *Brucella* species are suspected. From a veterinary perspective, occupational hygiene involves safe injection practices for animals, monitoring and surveillance of disease in livestock, and having adequate protocols in place to manage an outbreak effectively. The prevention of food-borne illness includes community education campaigns around the consumption of unpasteurised dairy products or undercooked meat.

Patients with brucellosis often present with nonspecific signs and symptoms. Symptoms may persist for weeks to months. In the context of a country with a high

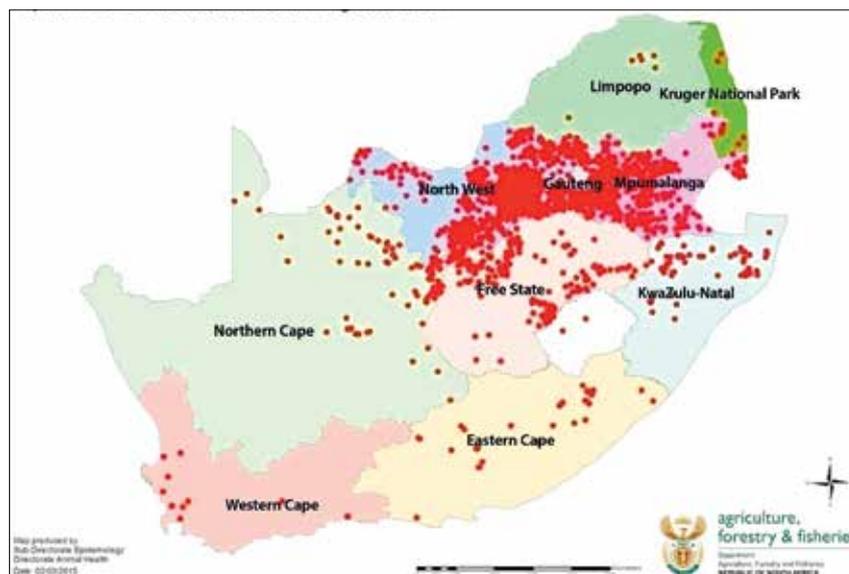


Fig. 1. Map of SA indicating outbreaks of *Brucella abortus* in animals, 2010 - 2014 (courtesy of the Department of Agriculture, Forestry and Fisheries, SA).

prevalence of tuberculosis and HIV infection, patients presenting with features of chronic brucellosis may be misdiagnosed and mistreated. As outbreaks of *B. melitensis* in animals in SA are generally infrequent^[9] and human cases are even less common, there is a lack of awareness among clinicians and laboratory staff, who have limited experience in diagnosing and handling this organism, with subsequent delay in diagnosis. A high index of suspicion and a proper social and exposure history are therefore essential in making a timely diagnosis. Effective communication between the clinician and the laboratory regarding suspected infections is imperative to ensure that all biological samples are handled appropriately. The testing laboratory should have protocols in place to ensure staff safety and maintain good safety practices. When working with a World Health Organization biosafety risk level 3 organism such as *Brucella*, all work should also be performed in an appropriate biosafety cabinet, in a biosafety level 3 facility, by individuals trained for this work.^[10]

Conclusion

A multidisciplinary *Brucella* control programme can be effective in preventing human infections with an approach that integrates three key elements: veterinary service, public health, and the medical healthcare system.

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