BRUCELLOSIS IN SOUTH AFRICA – A NOTIFIABLE MEDICAL CONDITION

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Executive summary

Brucellosis is an important zoonotic condition in many regions of the world. In South Africa brucellosis is a controlled animal disease and is a notifiable medical condition in humans. The predominant pathogen is *Brucella abortus*, with far fewer reported *B. melitensis* infections in animals and humans. The predominant clinical manifestation in animals is abortion. Transmission from animals to humans is mainly through consumption of unpasteurised milk. Occupational exposure typically occurs in farm, abattoir, veterinary and laboratory situations. Bovine brucellosis (*B. abortus*) occurs across all nine provinces, but most infected cattle herds occur in the central and Highveld provinces. Clinically, human brucellosis is highly variable in presentation, with *B. melitensis* more likely to produce severe or complicated disease. Treatment generally requires use of antibiotic combinations for prolonged periods. In animals, one of the control measures for brucellosis involves the use of attenuated live vaccines, which sometimes cause accidental human infections. Dairy products are rendered safe for human consumption through pasteurisation. Bovine brucellosis control in cattle is currently being prioritised under the South African Veterinary Strategy (2016-2026).

Introduction

Brucellosis is a bacterial disease of animals, and is transmissible to humans. *Brucella abortus* causes the majority of bovine and human brucellosis cases in South Africa, whereas the usual reservoirs of *B. melitensis* are goats and sheep. Pregnant livestock may abort, with *Brucella* bacteria being shed in the birth fluids and potentially in their milk. Brucellosis has also been documented in certain wildlife in the country.

Brucellosis is a controlled animal disease in South Africa, and human brucellosis is a notifiable medical condition. Persons working in occupations where contact with livestock and livestock tissues frequently occur are at highest risk of brucellosis (farmers and farm workers, abattoir workers, veterinarians, animal health technicians, etc.). Humans acquire *Brucella* infection by three routes:

- Direct contact with infected animal tissues or secretions through skin cuts/abrasions or conjunctival splashes;
- Inhalation of contaminated aerosols (uncommon);
- Consumption of unpasteurised dairy products (incl. milk, yoghurt, cheese).

It is important to note that pasteurised or adequately boiled milk or milk products, and cooked meat from infected animals, are safe to consume and do not transmit infection. Accidental self-injection with *Brucella* vaccine is a risk for farmers, vets and animal health technicians, and laboratory staff may also be accidentally exposed when dealing with *Brucella* cultures (discussed later).

Bovine brucellosis – a cattle herd disease

Brucella abortus is primarily a cattle disease but may affect most other mammals, including humans.[1] The disease in cattle typically presents with abortion, reduced fertility, orchitis, joint problems, a drop in milk production and decreased general production.[2-4] Currently, most cattle farmers in South Africa are at risk of acquiring brucellosis-positive cattle in their herds, as there is generally poor compliance with brucellosis vaccination and testing.[5] In South Africa, cattle are serologically screened using the Rose Bengal test (serum) or milk ring test (milk) followed by the complement fixation test (serum) on screen-positive samples.[6] Culture is considered the gold standard diagnostic.[6] Test results are interpreted at herd level and an entire epidemiological herd is placed under quarantine if positive cattle are identified, owing to the long incubation period of the disease. Testing individual animals before movement is considered a high-risk practice. Whole epidemiological herds should preferably be tested regularly to establish their brucellosis status.

Bovine brucellosis occurs in all nine provinces of South Africa's provinces, but is especially concentrated in the central and Highveld regions. Figure 1 shows the *B. abortus* outbreaks from January 2015 to May 2018 as reported to the Directorate Animal Health of the Department of Agriculture, Forestry and Fisheries (DAFF).

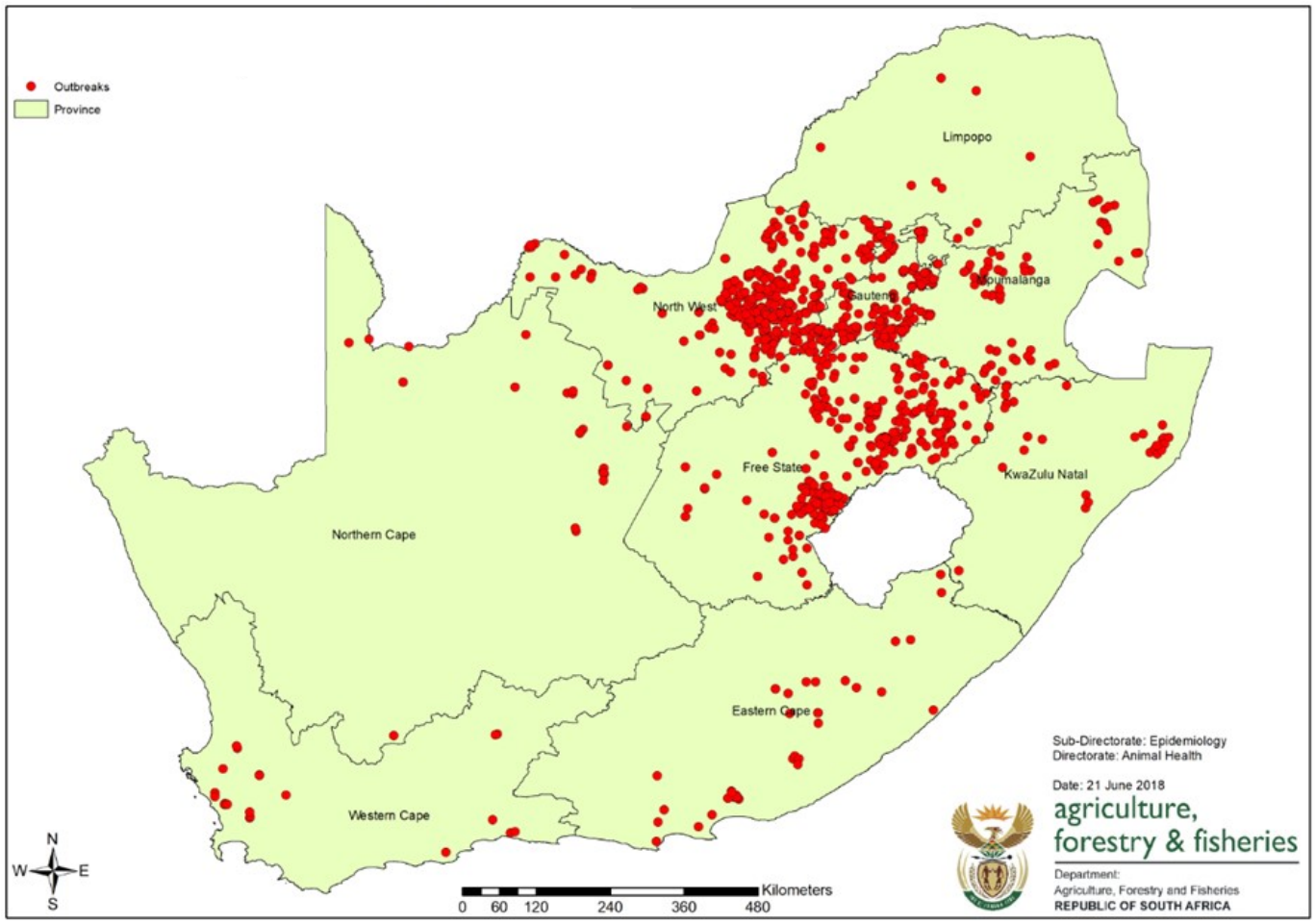

Figure 1. Reported *Brucella abortus* outbreaks in animals from January 2015 to May 2018 across all nine provinces of South Africa. Image courtesy of the Sub-Directorate: Epidemiology of the Directorate Animal Health, Department of Agriculture, Forestry and Fisheries (DAFF).

DAFF brucellosis policy development

As part of the South African Veterinary Strategy (2016-2026), bovine brucellosis was selected to be used as a 'model disease' by which to approach future livestock disease control efforts.[7] Review of the bovine brucellosis control policy in cattle is underway to facilitate implementable, cost-effective and sustainable control of the disease.

Several shortcomings in the Bovine Brucellosis Scheme R.2483 of 9 Dec 1988 have been identified, including the voluntary nature of the scheme, testing programmes that are not all herd based, movement of test-negative animals from quarantined farms, and capacity concerns to handle infected herds.[5] The first step in the Brucellosis Policy review was the drafting of the 'Discussion Paper on the Review of Bovine Brucellosis Control in South Africa' that was published for public comment in the Government Gazette on 5 May 2017.[5] Several challenges and key points were discussed and highlighted in the document. Several inputs and ideas where received from various stakeholders that will be considered going forward.

Numerous underlying brucellosis-related matters are constantly being addressed with the aim of building a more solid foundation for policy implementation. This includes, but is not limited to, public and farmer awareness, training of veterinarians and animal health technicians, promoting vaccination of heifer calves, SANAS accreditation and DAFF approval of laboratories, investigating potential incentive strategies, addressing abattoir-related matters and investigating authorizations to increase manpower. The second step of policy drafting is being addressed by the Bovine Brucellosis Working Group (which reports to the Ministerial Technical Committee of Veterinary Services).

Provincial Veterinary Services' initiatives

Several provinces are increasing their efforts at heifer calf vaccination. The Free State Province rolled out vaccination in 2017, focusing on high-risk herds, positive herds and farms neighbouring positive herds (mostly commercial sector). The vaccination efforts in the North West Province commenced in November 2017. To date, over 45 000 doses of vaccine have been acquired and over 20 000 heifers have been given initial and booster vaccine doses (mostly in the communal sector).

Human brucellosis in South Africa

Globally, brucellosis is considered one of the most common zoonoses with an estimated 500 000 new cases diagnosed annually. In Africa, human brucellosis incidence is largely unknown with the estimated burden of the disease varying widely from <0.09 to >8.43 per 100 000 population.[8-10] In South Africa, brucellosis is considered a priority zoonotic disease and despite being a notifiable medical condition, the true incidence of the disease is unknown. The last formally published incidence rate of >0.2 per 100 000 population was based on a survey conducted between 1956 to 1959.[11] A Department of Health analysis of national notifications between 1977 and 1984 showed annual incidence rates between <0.1 and 0.3 per 100 000 population,[12] and there has not been a subsequent update on the national incidence rate. There is currently no national human brucellosis surveillance program and over the recent past only a limited number of studies have been conducted in acute febrile illness patients and at-risk populations (e.g. farming community, abattoir workers and veterinary professionals; not published). Some sporadic human infections and related laboratory exposures have been reported to the National Institute for Communicable Diseases (NICD).[13-16] The case definitions for brucellosis notification in South Africa are provided as an addendum to this article.

Clinical disease

Owing to its ability to cause a wide spectrum of clinical manifestations with a tendency towards chronicity and persistence, brucellosis is one of the three 'great imitators', along with TB and syphilis. It evolves into a granulomatous disease capable of

affecting any organ system. The clinical features depend on the stage of disease as well as the organ/s involved. Fever is the most common feature, followed by osteoarticular involvement, sweating and constitutional symptoms. Hepatosplenomegaly is evident in a third of patients, and lymphadenopathy in 10%. Osteoarticular manifestations (sacroiliitis, spondylitis, peripheral arthritis and osteomyelitis) account for over half of the focal complications, while pulmonary disease may be evident in up to 16% of complicated cases, and genitourinary complications can be found in 10% of patients. Neurological involvement may be evident in about 6% of cases, with protean nervous system manifestations.

Human brucellosis diagnosis

Laboratory diagnosis of human *Brucella* infection is complicated and none of the currently available diagnostic tools can be used in solo to reliably detect the causative agent. Definitive laboratory diagnosis of human brucellosis is based on the isolation of bacteria from clinical samples (blood, bone marrow or other tissues). However, cultures give a low yield as *Brucella* is fastidious and the number of bacteria in clinical samples may vary widely. The isolation of *Brucella* is highly dependent on the stage of disease (acute versus chronic), antibiotic treatment, availability of appropriate clinical sample and the culturing method used.[17] Advances in automated blood culture systems (e.g. BACTEC™ and BacT/Alert™) have decreased the culture time and increased the recovery rate from sterile body fluids.[18] In recent years, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been shown to be an effective tool to identify *Brucella* spp. directly, from both culture plates and blood culture bottles.[19-21] However, this method is not routinely used as it requires access to a specialised protein profile database.

Serological testing is preferred in routine clinical practice as it is non-hazardous, faster and more sensitive than bacterial culture. Many serological assays are available for the diagnosis of human brucellosis. The most commonly used assays include the serum agglutination test (SAT), the Coombs anti-*Brucella* test, the Rose Bengal test, complement fixation and more recently enzyme-linked immunosorbent assay (ELISA). However, interpretation of these assays is often difficult and several factors should be considered when interpreting serological results: i) many patients are seronegative in the acute phase of the disease, which necessitates the serological testing of paired sera or performing more than one serological test, ii) a high proportion of the population in endemic regions may have persistent antibody titres due to ongoing exposure to *Brucella*, and appropriate cut-off values should be determined, iii) antibodies can remain detectable despite successful therapy, and iv) cross-reaction with other lipopolysaccharide (LPS) containing Gram-negative bacteria (e.g. *Salmonella*, *Yersinia*) may occur.[19] Serology results should be interpreted in combination with clinical signs and symptoms. Serological assays available at public and private pathology laboratories in South Africa include SAT and ELISA.

Various polymerase chain reaction (PCR) assays targeting different gene loci have been developed for the diagnosis of human brucellosis from pure cultures and clinical specimens (i.e. serum, whole blood, urine samples, various tissues, etc.).[19] Independent of the disease stage, PCR is more sensitive than blood cultures and more specific than serological tests.

Treatment

Treatment of human brucellosis is often complicated by treatment failures and relapses. Meta-analysis has shown that dual or triple regimens including an aminoglycoside (doxycycline-streptomycin/gentamicin or doxycyline-rifampicin-streptomycin/ gentamicin) significantly reduce treatment failure and relapse rates, and are currently recommended as first-line treatment regimens. Duration of treatment is at least six weeks for doxycycline and rifampicin, and up to two weeks for aminoglycoside

therapy (daily intramuscular injections) - see Tables 1 and 2 for details. Patients require prolonged follow-up to monitor for further complications or relapse.

Table 1. Treatment regimens for brucellosis.[22]

Form of brucellosis infection		Recommended antibiotic regimen	Duration
Uncomplicated - adults		Doxycycline plus streptomycin or gentamicin (preferred regimen)	6 weeks 1 - 2 weeks
		OR doxycycline plus rifampicin	6 weeks
- children	<8y	Cotrimoxazole plus rifampicin	4 - 6 weeks
	≥8y	Doxycycline plus rifampicin	
Focal - adults			
Spondylitis		Doxycycline plus streptomycin or gentamicin	12 weeks 2 weeks
		OR doxycycline plus rifampicin	12 weeks
		OR doxycycline plus ciprofloxacin	12 weeks
Neurobrucellosis		Doxycycline plus rifampicin plus (ceftriaxone OR cotrimoxazole)	Prolonged, until CSF normalises
Endocarditis		Doxycycline plus rifampicin plus streptomycin or gentamicin Surgery if indicated	6 weeks to 6 months, depending on clinical response
Focal – children	<8y	Cotrimoxazole plus streptomycin or gentamicin	6 weeks at least 2 weeks
	≥8y	Doxycycline plus streptomycin or gentamicin	6 weeks at least 2 weeks
		Rifampicin can be added to either regimen	6 weeks at least
Brucellosis in pregnancy		Rifampicin with/without cotrimoxazole (avoid in last week before delivery: risk of kernicterus)	6 weeks
Complex focal, relapsed or refractory infection, or antibiotic toxicity/resistance		Consider adding quinolone or cotrimoxazole as second-line to doxycycline or rifampicin; triple therapy has better cure rates	

Table 2. Antibiotic dosages for brucellosis treatment.

Cotrimoxazole	Trimethoprim 10 mg/kg/d (max. 480 mg/d), sulfamethoxazole 50 mg/kg/d (max. 2 g/d)	In 2 doses/day
Doxycycline	2-4 mg/kg/d (max. 200 mg/d)	In 2 doses/day
Rifampicin	15-20 mg/kg/d (max. 2 g/d)	In 1 or 2 doses/day
Gentamicin	5 mg/kg/d	In 1 to 3 doses/day
Streptomycin	20-40 mg/kg/d (max. 1 /d)	In 2 doses/day
Ciprofloxacin	1 g/d	In 2 doses/day
Ofloxacin	400 mg/d	In 2 doses/day

Brucella vaccine exposure in humans

Two vaccines are used for immunising animals against *B. abortus* in South Africa: S19 (widely available through farmers' co-ops) and RB51 (veterinarian-prescribed). *Brucella melitensis* Rev 1 vaccine is used for sheep and goats. These are live attenuated vaccines and are potentially able to cause infection in humans; S19 being more likely to do so than RB51.[23] Accidental occupational exposure to *Brucella* vaccine is well described, and is usually via injection. Accidental spray of vaccine into the conjunctiva and open wounds has also occurred, and should be managed as for needlestick inoculation. Accidental exposures to *Brucella* vaccines should be managed as follows:

- For S19 exposure, take a blood sample for baseline serological testing and storage of serum. There is no serological test available for RB51.
- For S19, recommended post-exposure antibiotic prophylaxis (PEP) is doxycycline (or doxycycline plus rifampicin) for at least 3 weeks; RB51 is resistant to rifampicin, so doxycycline (or doxycycline plus cotrimoxazole or a fluoroquinolone) is used, for at least 3 weeks.[26]
- Those with contraindications to doxycycline (e.g. pregnancy, or attempting to become pregnant,) should use cotrimoxazole for at least 3 weeks.
- Symptoms and daily temperature should be actively monitored for at least 4 weeks, and passive reporting of symptoms should continue for 6 months.
- Any raised temperature and/or symptoms of infection (sweating, fever, chills, arthralgia, myalgia, anorexia, etc) should trigger clinical examination and laboratory investigation (repeat serological testing to check for seroconversion, blood culture, full blood count, inflammatory markers, etc). If infection is confirmed, appropriate antibiotic combination therapy as for any other invasive brucellosis disease must be initiated.

Laboratory exposure to *Brucella* species

Laboratory-acquired *Brucella* infections and outbreaks are well known.[24,25] Live cultures are easily aerosolised, and the infective dose is very low. Additionally, it is an uncommon isolate in most clinical microbiology laboratories, and unidentified *Brucella* samples and cultures may arrive without warning. High-risk exposures for laboratory staff are if they work (or are within 1.5 m of others who work) with *Brucella* cultures on an open bench, or if they open or sniff culture plates, or if they come into direct skin or mucous membrane contact with cultures, suspensions or aerosols of the organism. Working with *Brucella* in a class II biosafety cabinet without additional BSL-3 protection (gown, gloves, mask, goggles) is also high-risk exposure. Certain procedures involving *Brucella* are high risk for all persons in a laboratory, because they potentially produce aerosols: centrifugation without using sealed containers, vortexing, sonicating, shaking, diluting, and accidental spilling or splashing of infected material. Low-risk laboratory exposure is working more than 1.5 m away from manipulations of cultures on an open bench or a class II biosafety cabinet, but without the high-risk aerosol-generating procedures described above. Laboratory exposures should be managed as follows:

- All staff exposures in the laboratory at the time should be risk-assessed, as defined above.
- High-risk exposures: PEP is doxycycline for 3 weeks, alternatively cotrimoxazole, as described above for live vaccine exposure. The US CDC recommends doxycycline plus rifampicin[24] but the combination does not appear to provide increased protection and there is more chance of adverse effects and reduced compliance.
- Low-risk exposures: employees should be offered the option of PEP. All immunocompromised and pregnant workers should be considered for PEP.

- High- and low-risk exposures: blood samples for baseline serological testing should be taken and the serum stored, and employees should be under active medical surveillance. Symptoms and daily temperatures should be monitored for 6 weeks, and passive reporting of symptoms continued for 6 months.
- Any raised temperature and/or symptoms of infection (sweating, fever, chills, arthralgia, myalgia, anorexia, etc) should trigger clinical examination and laboratory investigation (repeat serological testing to check for seroconversion, blood culture, full blood count, inflammatory markers, etc). If infection is confirmed, appropriate antibiotic combination therapy as for any other invasive brucellosis disease must be initiated.
- A full incident investigation should be done and extended to other potential sites if necessary (e.g. referring laboratories).

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Addendum. Case definitions for notification of human brucellosis

Brucellosis (*Brucella* spp.)

Clinical Description

An illness characterized by acute or insidious onset of fever and one or more of the following: night sweats, arthralgia, headache, fatigue, anorexia, myalgia, weight loss, arthritis/spondylitis, meningitis, or focal organ involvement (endocarditis, orchitis/epididymitis, hepatomegaly, splenomegaly).

Laboratory Criteria for Diagnosis

Definitive:

- Culture and identification of *Brucella* spp. from clinical specimens
- Evidence of a fourfold or greater rise in *Brucella* antibody titer between acute- and convalescent-phase serum specimens obtained greater than or equal to 2 weeks apart

Presumptive:

- *Brucella* total antibody titer of greater than or equal to 160 by standard tube agglutination test (SAT) or *Brucella* microagglutination test (BMAT) in one or more serum specimens obtained after onset of symptoms
- Detection of *Brucella* DNA in a clinical specimen by PCR assay

Case Classification

Probable:

A clinically compatible illness with at least one of the following:

Epidemiologically linked to a confirmed human or animal brucellosis case

Presumptive laboratory evidence, but without definitive laboratory evidence, of *Brucella* infection

Confirmed:

A clinically compatible illness with definitive laboratory evidence of *Brucella* infection