

## An overview of the eradication of *Brucella melitensis* from KwaZulu-Natal

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### ABSTRACT

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*Brucella melitensis* is a Gram-negative bacterium whose primary hosts are goats and sheep. Like the other *Brucella* spp., with the exception of *Brucella ovis*, it is not particularly host specific as it is pathogenic for a variety of other mammal species including humans. In humans the disease caused by it is rated as one of the most important zoonoses. Three outbreaks have been recorded in goats and sheep in South Africa; the first outbreak occurred in sheep in 1965 in the Mpumalanga and Northern Provinces (then both part of the Transvaal Province), the second occurred in sheep in 1989 near Pretoria, Gauteng Province, and the third and current outbreak was diagnosed in a flock of goats in northern KwaZulu-Natal in September 1994. Following the initial diagnosis of *B. melitensis* in north-eastern KwaZulu-Natal, a serological survey was conducted in order to identify foci of infection in the goat and sheep populations. Six positive foci were identified. In March 1996 a test-and-slaughter eradication campaign was initiated in these areas. Initial test results revealed a prevalence of between 1.23 % and 4.02 %. All positive animals were identified and slaughtered. Eradication programmes were repeated between March 1996 and June 2000, in the populations at risk, and the disease prevalence was reduced in all the affected populations.

**Keywords:** *Brucella melitensis*, eradication programme, goats, KwaZulu-Natal, outbreak

### INTRODUCTION

One of the most serious zoonoses in the world is caused by *Brucella melitensis* which is highly virulent for humans and is readily transmitted from reservoirs of infection in goats and sheep which are its primary hosts. Like the other *Brucella* spp., with the exception of *Brucella ovis*, it is not particularly host specific and is able to infect several other mammal species through inhalation or ingestion of infective organisms, via mucous membranes such as the conjunctiva, or via abrasions. In humans *B.*

*melitensis* is the cause of Malta fever or Mediterranean fever, which is one of the forms of undulant fever, the latter also being caused by *Brucella abortus* and *Brucella suis*.

*Brucella melitensis* is a facultative intracellular organism and, following its entry into the body, is transported actively within phagocytic macrophages or passively in the lymph to the regional lymph node where it multiplies. A bacteraemia follows and results in localization of the organism in various organs especially the uterus, udder, and lymph nodes. Invasion of the pregnant uterus often results in abortion with large numbers of the bacteria being shed in vaginal discharges and foetal tissues. In addition, *B. melitensis* organisms are shed in the milk for prolonged periods post-partum, which presents the greatest risk for human infection (Alton 1990b).

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Only three outbreaks have been documented in South Africa; the first occurred in sheep during 1965 in the Mpumalanga and Northern Provinces (then both part of the Transvaal Province) (Van Drimmelen 1965), the second in a herd of Boer goats near Pretoria, Gauteng Province in 1989 (Ribiero, Herr, Chaparro & Van Der Vyver 1990), while the third and current outbreak described below was diagnosed in north-eastern KwaZulu-Natal (KZN) in 1994 (Reichel, Nel, Emslie & Bishop 1996).

In September 1994 an investigation was launched to investigate the disease status of a herd of goats on a small holding adjacent to the Makhathini Research Station after the owner of the goats was diagnosed as having 'Malta fever' by a medical specialist in Empangeni. Twelve of the fourteen goats tested positive with the Rose Bengal test (RBT) and complement fixation test (CFT). The entire herd was slaughtered and *B. melitensis* biovar 1 was cultured from milk and tissue samples, including uterus, udder, and supramammary and iliac lymph nodes, from several of the animals (Reichel *et al.* 1996).

A province-wide serological survey was then initiated in order to identify further foci of infection in KZN, and was followed by an eradication campaign. These form the basis of this report.

## MATERIALS AND METHODS

### Serological survey

The province-wide survey took place between October 1994 and April 1995 when a total of 6 266 goats and sheep was tested. A stratified sample was taken as only mature nanny goats and ewes were serologically tested on each farm, or from the animals presented at prearranged rendezvous in each dip tank area. It was, however, a biased sample as only approximately 10% of the animals were sampled based on their being able to be caught and bled. A single 10 ml 'Vacutainer' sterile sample of blood was collected from the jugular vein of each goat or sheep obtained for testing. The samples were transported on ice to the Makhathini Research Station where they were centrifuged and the sera decanted into cryotubes and frozen. The frozen sera were screened by each of four laboratories using the RBT, CFT and serum agglutination test for the presence of antibodies against *B. melitensis*, namely Allerton Provincial Veterinary Laboratory (Allerton P.V.L.), Vryheid Veterinary Laboratory, Ermelo

Laboratory, and the Onderstepoort Veterinary Institute.

Some of the animals tested belonged to speculators but the majority were the property of rural subsistence farmers whose animals are maintained in communal grazing systems. Ectoparasite control in animals belonging to these rural subsistence farmers is facilitated by State-run dip tanks (constructed by the Government in the early 1900s to eradicate bovine East Coast fever and Corridor disease) which serve the community within a radius of approximately 7 km from each tank. It was decided to consider all goats and sheep from the same dip tank area as constituting a single herd in order to simplify the control of the *B. melitensis* outbreak. No identification of animals was undertaken during the survey but positive animals could be traced back to the dip tank area and owner by means of consecutive sample numbering and recording of owner information on data record sheets. The dates and times at which the animals were sampled were prearranged through meetings with farmers and tribal authorities in dip tank areas. The tribal authorities played a key role in soliciting the support of the local communities.

A high degree of agreement in the test outcomes was reached between the laboratories, and the three screening tests used. This together with the fact that no *B. melitensis* Rev 1 vaccination had previously been carried out in the area resulted in the RBT (Alton 1990a) being used as the sole screening test in the subsequent eradication campaign.

### Eradication campaign

This campaign was initiated in a bid to eradicate *B. melitensis* from KZN. Test-and-slaughter exercises were conducted between March 1996 and June 2000 in the goat and sheep populations of the three dip tank areas Machobeni (two exercises: total of 373 animals), Nondabula (three exercises: 1 817 animals), and Ntenga/Mamfene (six exercises: 2 684 animals) that had been found to contain positive animals during the serological survey.

The test-and-slaughter initiative was preceded by lengthy extension and information transfer opportunities with stockowners and representatives of the various dip tank communities. The tribal authorities were also approached for their authorization and support of the disease eradication programme. Following the education of the affected communities with respect to the nature of the zoonosis and the need to eradicate it in order to promote and pro-

tect human health, test opportunities were carried out as follows:

- On day 1 of the test-and-slaughter exercise each goat and sheep presented at the pre-arranged rendezvous was caught, had a sterile blood sample drawn from a jugular vein and was identified by means of a unique number sprayed in orange spray paint on its side. Each owner's details were recorded on a data sheet and correlated with the test numbers allocated to his goats or sheep. The blood samples were then taken to the Directorate of Veterinary Services mobile laboratory based at the Makhathini Research Station, where they were centrifuged and the sera decanted and screened using the RBT.
- On day 2 all animals which tested positive for *B. melitensis* on day 1, were collected and transported to a quarantine facility at the Makhathini Research Station, pending their transportation to Cato Ridge abattoir in KwaZulu-Natal. The owners of positive animals either received a similar disease-free animal or a market-related cash sum in compensation for their positive animal that had been confiscated. This modus operandi was repeated until all the animals in the dip tank area had been screened, which usually lasted between 4 and 6 weeks.

At the conclusion of each exercise the positive animals were transported directly from the quarantine facility at Makhathini Research Station to Cato Ridge abattoir where the animals were slaughtered under appropriate quarantine conditions. Tissue samples from several lymph nodes (especially supramammary and iliac), udder and foetus (if pregnant) of some of the slaughtered animals were collected and cultured at Allerton PVL in an attempt to isolate *B. melitensis* biotype 1.

## RESULTS

### Serological survey

From October 1994 to April 1995, 6 266 goats and sheep were tested of which 19 were diagnosed positive for *B. melitensis* (3.03 % prevalence). Positive animals were identified as having come from six foci of infection: a speculator's goat herd in Pongola, a speculator's goat herd adjacent to the Makhathini Research Station (Ubombo) (this being the herd in which the infection was first diagnosed), Ntenga/Mamfene dip tank areas (Ubombo), Machobeni dip tank area (Ingwavuma), and Nondabula

dip tank area (Ingwavuma). The herds of both speculators were slaughtered out in their entirety but the animals in the four dip tank areas were left for inclusion in the subsequent test-and-slaughter campaign.

### Eradication campaign

In the Ntenga/Mamfene area in which six test-and-slaughter exercises were carried out the prevalence decreased from 1.23 % to 0.15 % at the end of the campaign. In the Machobeni area (two exercises) the prevalence was reduced from 4.02 % to 0 %, while in the Nondabula area (three exercises) it was reduced from 2.42 % to 0 %.

Bacteriological examination of the tissues of several of the serologically positive goats confirmed the presence of *B. melitensis* biotype 1.

## DISCUSSION

### Serological survey

Financial and time constraints, as well as personnel inexperience, resulted in a rushed and biased sampling of the population at risk. However, six positive foci were identified which allowed for the rapid implementation of an effective control programme. In order to verify the accuracy of the initial survey results it will be necessary to resample all the goats and sheep in the dip tank areas which were previously classified as being disease-free, and confirm the absence of disease there.

### Eradication Campaign

With repeated test-and-slaughter exercises, the number of animals presented for testing on successive samplings decreased. This can be attributed to stockowner disillusionment with the compensation measures and the effort required to drive a herd of goats up to 7 km in order to have the animals tested. This resulted in animals belonging to the responsible owners being presented and bled repeatedly during successive test-and-slaughter exercises, while those belonging to owners less concerned about the disease were presented and bled once or twice and were then absent.

No evidence could be found that *B. melitensis* Rev 1 vaccine had been used in either the goat or sheep populations in the affected area. This, together with the high proportion of agreement between the RBT, CFT and SAT tests used to screen sera during the survey, supported the decision to use the RBT as

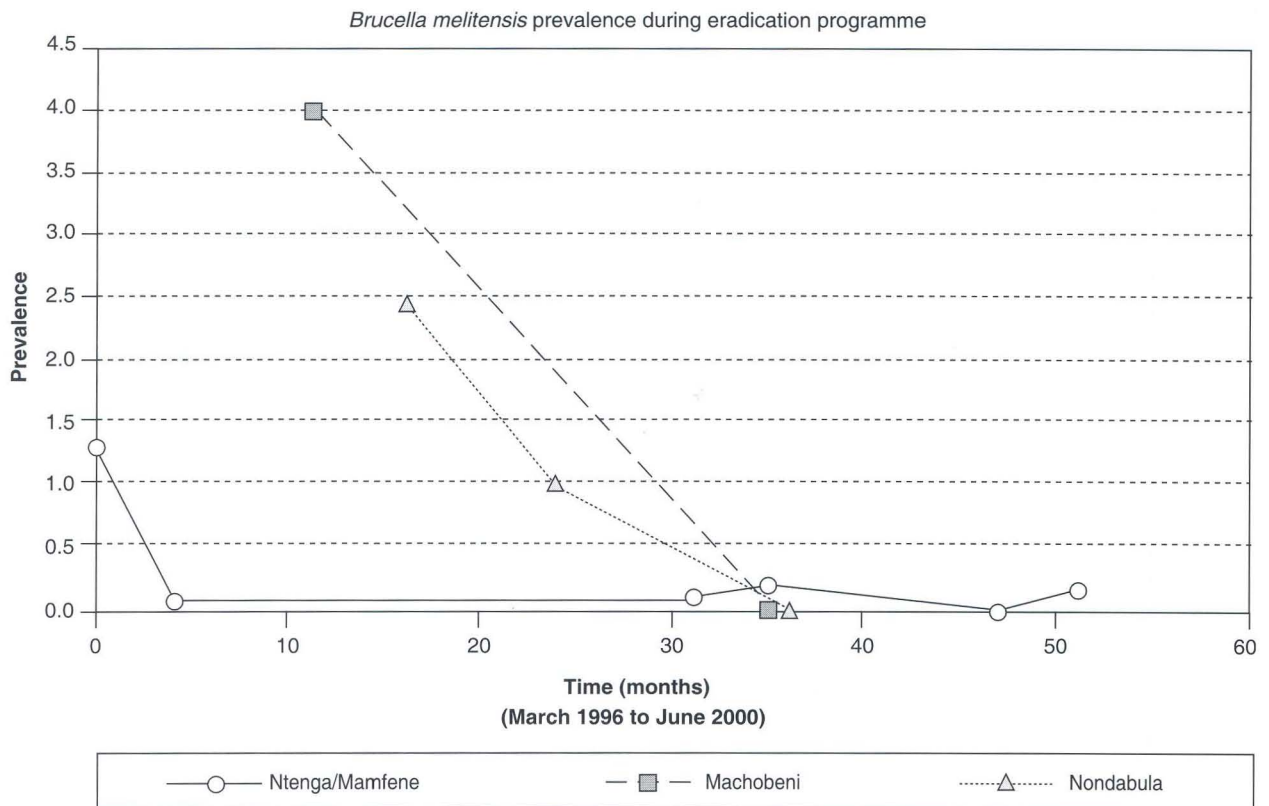


FIG. 1 Changes in the prevalence of *Brucella melitensis* after test-and-slaughter procedures were initiated

the sole screening test during the eradication campaign. The use of this test facilitated rapid in-field screening of samples, which allowed for swift recovery of positive animals. Cultures from tissue samples from a number of the slaughtered goats confirmed the presence of *B. melitensis* biotype 1, but it is probable that a number of serologically false positive animals were slaughtered. The serious nature of this zoonosis and the policy of the Directorate of Veterinary Services to eradicate the disease support the use of a test with a high sensitivity. Alton (1990a) and Herr (1994) consider the RBT to be one of the best screening tests provided that no Rev 1 vaccination has occurred in the test population.

Compensation of stockowners proved to play a key role in the success of the eradication campaign. Initially owners were compensated financially at above market-related prices with up to R600 being paid for a castrated goat. However, stockowners became suspicious of the motives behind the campaign and became reluctant to accept financial compensation for positive animals. This situation was solved by the purchase of disease-free animals by the Directorate, which were then exchanged for

positive goats during the eradication campaign. This proved to be an extremely effective means of compensation, which was acceptable to the stockowner community.

Although sheep are susceptible and grazed together with infected goats, all sheep tested during the campaign were *B. melitensis*-free. Alton (1990b) reported similar situations in the Mediterranean region and Middle East. The conclusion reached is that the local Nguni sheep breed is resistant to the infection.

The apparent slow rate of spread experienced in KwaZulu-Natal is in contrast to all other documentation of the disease from the Mediterranean region, Near and Middle East, the Americas and Africa. This cannot be explained since the majority of the susceptible goat population is grazed communally by day and confined in traditional kraal facilities by night. Contamination of dust must occur in these kraals which should facilitate aerosol infection of uninfected animals.

Brucellosis in humans might be confused with other fever-causing diseases, especially malaria, some of

which have a high prevalence in the area. Unpasteurized milk and dairy products, from goats and to a lesser extent sheep and cattle, comprise the primary source of human infection (Alton 1990a). However, the communities in the affected areas apparently do not use goat milk for human consumption (Reichel *et al.* 1996). The cause of the disease was established by Bruce (1887) who isolated the organism, which was subsequently named *Brucella melitensis*, from the spleens of soldiers who had died of 'Malta or Mediterranean Fever' on the island of Malta. The soldiers had contracted the disease through the ingestion of infected goats' milk.

The histories of positive goats were obtained from their owners in an attempt to try to trace the source of infection. A number of the positive goats had been purchased in Swaziland and illegally imported into South Africa. The stockowners concerned alleged that goats were cheaper in Swaziland than in South Africa and that they purchased and imported these goats in order to speculate, celebrate important cultural events, and pay 'Lobola' (a payment made to one's future wife's family). The route of this illegal entry of animals into South Africa is in close proximity to the Machobeni dip tank which lies less than 10 km from the south-eastern Swaziland border.

Following the diagnosis of *B. melitensis* in South Africa, Swaziland veterinary officials conducted a survey in their goat populations. Samples were forwarded to Allerton P.V.L. and the presence of the disease was confirmed in several dip tank areas in Swaziland, with prevalences of up to 12 % being reported in the south-eastern region (Dr P. Danso, personal communication 1996). A control programme was initiated in Swaziland in 1998 and in order for the South African eradication programme to be successful, it will probably be necessary to

coordinate the Swaziland and South African eradication initiatives in a regional control programme.

At face value the results indicate that the eradication programme has been extremely successful in reducing the disease prevalence. The remaining low prevalences might be the result of false positive RBT reactors, and the true *B. melitensis* prevalence is probably even lower than levels reported here. Future testing efforts will involve initial screening using the RBT, and all positive sera will be tested with the CFT, which has a higher specificity than the RBT. Two consecutive negative CFT tests 6 months apart would confirm the absence of *B. melitensis* from the population being tested (Herr, Bishop, Bolton & Van Der Merwe 1979).

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