Detection of *Brucella abortus* in Chiredzi district in Zimbabwe

Brucellosis is an endemic disease in Zimbabwe caused by the genus *Brucella*. *Brucella* seroprevalence was recently reported to be high in the wildlife-livestock interface in the Chiredzi district and the neighbouring Gonarezhou National Park (GNP) in Zimbabwe, and higher amongst communal cattle with an abortion history and access to grazing in GNP than amongst communal cattle with no abortion history or access to grazing in GNP. The aim of this study was to investigate *Brucella* species in brucellosis seropositive cattle in the Chiredzi district with access to GNP using isolation and identification. Isolation of *Brucella* species from whole blood (n = 18) and milk samples (n = 10) from seropositive animals with an abortion history was based on the rose Bengal test (RBT) and enzyme-linked immunosassays (enzyme-linked immunosorbent assay [ELISA]; indirect ELISA and complement ELISA), using microbiology and polymerase chain reaction (PCR) methods. *Brucella abortus* was cultured and identified from blood and milk collected from seropositive cows in both communal areas. The *Brucella*-specific 16-23S intergenic spacer (ITS) PCR and multiplex AMOS-PCR assays verified the identification of the cultures. Our results confirmed that *B. abortus* is present in cattle on communal farms in the Chiredzi district in Zimbabwe and might cause cattle abortions. The need for implementing control measures and raising public awareness on zoonotic transmission of brucellosis are recommended.

Introduction

Bovine brucellosis is a bacterial disease caused by *Brucella abortus*. In addition to its zoonotic importance, it also affects animal health and production (Godfroid et al. 2005; Pappas et al. 2005). Consumption of contaminated foods or occupational exposure remains the major source of infection in humans. Brucellosis is primarily recognised as an occupational hazard for veterinarians, farmers, laboratory technicians, slaughterhouse workers, and others who work with animals and their products. The main source of infection for the public is through ingestion of unpasteurised dairy products. The bacteria can also be transmitted through raw or undercooked meat from infected animals. The *Brucella* species generally considered pathogenic for humans, in decreasing order of virulence, are *Brucella melitensis*, *Brucella suis* and *B. abortus* (Baldwin & Goenka 2006).

Identification of *Brucella* spp. is important in surveillance and eradication efforts. Currently, mainly serological screening of potential hosts and to a lesser extent isolation and identification of the pathogen from potential hosts are used for the diagnosis of brucellosis. Culturing (isolation and identification) of *Brucella* spp. is recognised as the ‘gold standard’, but is time consuming and complex, and positive animals sometimes yield negative culture results (Alton et al. 1988; OIE 2008; Whatmore 2009). Most *Brucella* cultures have been isolated from aborted foetuses, milk, hygroma fluid, or lymph nodes from infected animals. Madsen (1989) and Mohan et al. (1996) identified *B. abortus* biovar 1 cultures from aborted foetuses. The same biovar (bv) was also isolated from an aborted foetus of a waterbuck in Wankie (Hwange) National Park (Condy & Vickers 1969) and from eland hygroma fluid on a game ranch in Zimbabwe (Condy & Vickers 1972). Matope et al. (2009) isolated primarily *B. abortus* bv 1 and to a lesser extent *B. abortus* bv 2 from aborted foetuses and milk samples from infected herds in Zimbabwe. These authors also isolated *B. melitensis* bv 1 from an aborted foetus of a goat in Zimbabwe (Matope et al. 2009). It is difficult to obtain positive *Brucella* cultures from blood and positive cultures are only obtained from 10% – 70% of infected human infections since successful isolation depends on the duration, the localisation of the infection and the type of *Brucella* species (Al-Attas et al. 2000).

Various polymerase chain reaction (PCR) assays are available for differentiating *Brucella* at the genus, species and/or biovar level. Genus-specific PCR assays like 16-23S rRNA intergenic spacer (ITS) region (Keid et al. 2007) detect only *Brucella*, whereas multiplex PCR assays differentiate *Brucella* at the species level (Bricker & Halling 1994, 1995; Garcia-Yoldi et al. 2006; Halling, Tatum & Bricker 1993). The automated multiplex oligonucleotide synthesizer (AMOS) multiplex PCR...
assay distinguishes *B. abortus* (bv 1, 2 and 4), vaccine strains *B. abortus* RB51 and S19, *B. melitensis* (bv 1, 2 and 3), vaccine *B. melitensis* rev1, *Brucella ovis*, and *B. suis* (bv 1) (Bricker & Halling 1994, 1995; Halling et al. 1993).

Brucellosis is endemic in sub-Saharan African countries. In Zimbabwe it was first diagnosed from aborted cattle in 1913 (Bevan 1931). Various studies in the country showed a higher *Brucella* infection in commercial than communal areas (Madsen 1989; Matope et al. 2010; Swanepoel, Blackburn & Lander 1976). A recent study in the wildlife-livestock interface (Malipati and Pesvi) and non-interface (Chomupani and Pfumare) communal areas in the south-east lowveld of Chiredzi district (Figure 1) showed a significantly higher *Brucella* seroprevalence in cows with an abortion history and in cattle grazing in parks (Gonarezhou National Park [GNP] and Kruger National Park [KNP]) (Gomo et al. 2012). Although bovine brucellosis was demonstrated through serology (Gomo et al. 2012), no isolation or characterisation of the bacteria was done. Due to the potential health risk to community members, the objective of the present study was to further characterise the brucellosis species from infected herds in the Malipati and Pesvi communal areas in Chiredzi district, which were found seropositive using the rose Bengal test (RBT) and competitive enzyme-linked immunoabsorbent assay (cELISA) in the study by Gomo et al. (2012) and RBT and indirect enzyme-linked immunoabsorbent assay (iELISA) in this study. *Brucella*-specific PCR and AMOS-PCR assays were used to confirm the identity of the *Brucella* isolates.

**Materials and methods**

**Study area and sample collection**

The study was conducted in the Chiredzi district in the south-east lowveld of Zimbabwe as described earlier by Gomo et al. (2012). The Malipati and Pesvi communal areas in the Chiredzi district share boundaries with the GNP in Zimbabwe and the unfenced region of the northern KNP (separated by the Limpopo River), respectively (Figure 1). The two communal areas were selected based on high *Brucella* seroprevalence, reports of abortion and no history of vaccination (Chiredzi Veterinary Services, pers. comm., 2009; Gomo et al. 2012). Samples were collected from cattle at the Malipati and Pesvi dip tanks during 2008 and 2009. The Malipati dip tank is located about 1 km from the unfenced GNP and Pesvi dip tank lies adjacent to the unfenced KNP across the Limpopo river (dip tank 3 km from northern boundary of KNP). Whole blood (*n* = 18) as well as milk (*n* = 10) samples (Table 1) were collected from herds with an abortion history and that tested seropositive using RBT and cELISA by Gomo et al. (2012). The iELISA was done on samples from Malipati and Pesvi communal cattle (700 serum samples of 1038 tested cattle) that were part of the study of Gomo et al. (2012) to confirm their seropositive status.

**Cultures**

Only milk (*n* = 10) and blood samples (*n* = 18) collected from animals which had a history of abortion and had tested

![FIGURE 1: The location of the two communal areas (Malipati and Pesvi) that were surveyed in the Chiredzi district in Zimbabwe that borders the Gonarezhou National Park and the Kruger National Park.](image)

**TABLE 1:** Information of rose Bengal test seropositive bovines from which whole blood and milk samples were collected in the Pesvi and Malipati communal regions in Zimbabwe.

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Other strain number</th>
<th>Location</th>
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<th>Sex</th>
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<th>cELISA‡</th>
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</table>

Bold indicates *Brucella* cultures isolated from milk and blood. M, Male; F, female; iELISA, indirect enzyme-linked immunoabsorbent assay; P, iELISA results from rose Bengal test seropositive samples positive for the iELISA results; N, iELISA results from rose Bengal test seropositive samples negative for the iELISA results. *†* Indicates animals with previous abortion history. *‡* Indicates animals with previous abortion history. *§* The bovine brucellosis iELISA kit (Institut Pourquier) was used according to the manufacturer’s instructions at the Department of Veterinary Tropical Diseases, University of Pretoria, South Africa, on serum samples.
positive for *Brucella* antibodies using serological tests were cultured for *B. abortus* isolation at the Central Veterinary Laboratory (CVL) in Harare, Zimbabwe (Table 1). Milk samples were centrifuged at 6000 g – 7000 g for 15 min; skim milk was discarded and the cream and sediment were mixed and spread on *Brucella* selective medium and blood agar (BA) (Quinn et al. 1994). The inoculated *Brucella* selective media and BA plates were placed in a jar with gas (6% [oxygen] O₂, 10% [carbon dioxide] CO₂, and 84% [nitrogen] N₂) at 37 °C and examined for 10 days. Plates that did not show any growth after 10 days were discarded as negative. Suspected *Brucella* colonies were transferred to BA, on which *Brucella* appeared small (1 mm diameter), round, grey and non-haemolytic. Suspected colonies were Gram and modified Ziehl Neelsen (Stamp’s) stained and the reactions to oxidase and catalase were observed (Quinn et al. 1994). Speciation of *Brucella* colonies was done using microbiology tests (excluding the phage tests) as indicated by Alton et al. (1988) and OIE (2008).

For blood culturing, each 5 mL blood sample was added to biphasic medium (tryptcase soy solid and liquid phase; Ruiz 1961) and incubated at 37 °C with 5% CO₂ atmosphere for 10 days (Ruiz et al. 1997). The solid phase was prepared with 12 mL of tryptcase soy agar and the liquid phase consisted of 30 mL tryptcase soy broth. Inoculated solid and liquid phase bottles were checked every 24 hours to evaluate haemolysis and turbidity. Once the bacterial growth was detected by turbidity and haemolysis, the colony was sub-cultured and Gram stain was performed to confirm the presence of Gram-negative rods in the broth and on the agar slant. Colonies were stained with Gram and modified Ziehl Neelsen (Stamp’s) stains and the reactions to oxidase and catalase were observed (Quinn et al. 1994). *Brucella* speciation was done as described for the milk cultures.

**Polymerase chain reaction**

DNA was extracted from isolates obtained from blood and milk cultures using the Qiagen DNA mini kit (Qiagen) according to the manufacturer’s instructions. Each 16-23S ITS PCR (Keid et al. 2007) amplification reaction was prepared in a total volume of 25 µL containing 50 mM potassium chloride (KCl), 10 mM Tris(hydroxymethyl)amino-methane hydrochloride (Tris-HCl) (pH 9.0), 2.0 mM magnesium chloride (MgCl₂), 200 µM of each deoxynucleotide triphosphates (dNTP), 0.4 µM of each primer, 2.5 µL DNA template and 1.5 U GoTaq® Hot Start Polymerase (Promega). Polymerase chain reaction conditions included an initial denaturation at 95 °C for 5 min followed by 35 cycles consisting of 30 s of denaturation at 95 °C, 30 s of annealing at 56 °C, and 30 s of elongation at 72 °C, with a final elongation at 72 °C for 5 min.

The AMOS-PCR condition was used as previously described by Bricker and Halling (1994, 1995). The PCR reaction consisted of 1.5 mM MgCl₂, 1X PCR buffer (Promega), 250 µM dNTPs, 5' primer cocktail consisting of *B. abortus, B. melitensis, B. ovis* and *B. suis* specific primers each (0.2 µM) and 1 µM IS711-specific primer, 1 U GoTaq® Hot Start Polymerase (Promega) and 2.5 µL DNA per 25 µL reaction.

All the PCR samples were analysed by electrophoresis in a 2% agarose gel, stained with ethidium bromide (0.5 mg/mL), and the DNA bands were visualised under ultraviolet (UV) light (UVF transilluminator model TM-20). The DNA of *Brucella* reference strains obtained from the *Brucella* culture collection, France (BCCN), namely: *B. abortus* bv 1 (544 = BCCN R4), *B. abortus* bv 2 (86/8/59 = BCCN R5), *B. abortus* bv 4 (292 = BCCN R7), *B. melitensis* (16M = BCCN R1), *B. suis* bv 1 (1330 = BCCN R12), *B. ovis* (63/290 = BCCN R17) and *Brucella canis* (RM6/66 = BCCN R18) were included as positive controls.

**Ethical considerations**

Research involving animals have been approved by the animal use and care committee of the University of Pretoria and were done according to the national code of welfare standards for each animal species.

**Results**

The seroprevalence was 8.3% of the 700 cattle samples from the Malipati and Pesvi in the Chiredzi district based on RBT and iELISA. The prevalence of the individual communal areas were 9% (n = 490) and 6.7% (n = 210) in Malipati and Pesvi, respectively. Table 1 indicates the RBT, cELISA and iELISA. The prevalence of the individual communal areas was 9% (n = 490) and 6.7% (n = 210) in Malipati and Pesvi, respectively. Table 1 indicates the RBT, cELISA and iELISA and were done according to the national code of welfare standards for each animal species.

**Discussion**

*Brucella abortus* was isolated and confirmed with AMOS-PCR assay as *B. abortus* bv 1, 2 or 4 strains (Bricker & Halling 1994, 1995) from seropositive cows with an abortion history in the Malipati and Pesvi interface regions in the Chiredzi district in Zimbabwe. These isolates were established from *Brucella* infected cattle samples from the Malipati and Pesvi regions with a seroprevalence of 10.3% (Gomo et al. 2012). The *Brucella* seroprevalence rate reported in this study (8.3% of n = 700) using RBT and iELISA was relatively similar to the seroprevalence of 10.3% (n = 1038) using RBT and cELISA reported by Gomo et al. (2012). Since none of the sampled...
cattle had been vaccinated against brucellosis, the detected antibodies were most likely due to a natural infection by Brucella species, which was confirmed by cELISA results (Gomo et al. 2012) and AMOS-PCR assay as wild-type B. abortus. The cELISA and AMOS-PCR differentiate between natural infections and vaccine strains (Bricker & Halling 1994, 1995; Nielsen et al. 1989).

Due to the unavailability of phage tests that identify biovars, the B. abortus isolates could only be identified to species level using microbiology tests (Alton et al. 1988). The AMOS-PCR confirmed the two isolates as B. abortus bv 1, 2 or 4 since the multiplex PCR cannot distinguish B. abortus bv 1, 2 and 4 from one another (Figure 2; Bricker & Halling 1994, 1995).

Previous studies have demonstrated the presence of B. abortus bv 1 and 2 in cattle in different parts of Zimbabwe (Madsen 1989; Matope et al. 2009; Mohan et al. 1996). The majority of the B. abortus isolates were found to be biovar 1 (84.6%, 11/13) with the remaining ones being biovar 2 (Matope et al. 2009). Brucella abortus bv 1 appears to be the predominant cause of brucellosis in cattle in Zimbabwe (Matope et al. 2009). Similarly, in neighbouring South Africa, biovar 1 has been shown to contribute about 90% whilst biovar 2 accounted for 10% of all the B. abortus isolates (Bishop, Bosman & Herr 1994).

Only two cultures were obtained from milk (n = 10) and blood (n = 18) samples from seropositive cows with an abortion history (7%). Sensitivity of culturing Brucella species from blood varies from 10% – 70% of suspected human infections (Al-Attas et al. 2000; Pappas et al. 2005; Ruiz et al. 1997) depending on the growth conditions (Ruiz et al. 1997), duration, localisation of the infection and type of Brucella species (Al-Attas et al. 2000; Ruiz et al. 1997). The biphasic method was used to isolate Brucella from whole blood samples as described by Ruiz (1961) and incubated for 10 days since Ruiz et al. (1997) obtained 100% cultures over a maximum time of 216 hours (9 days). We obtained a low percentage of cultures (7%) from known Brucella seropositive samples with unknown stage of brucellosis.

The low sensitivity of culturing Brucella in this study clearly indicates that other culturing techniques should be investigated. The lysis centrifugation (LC) technique has been reported by Espinosa et al. (2009) to be the preferred technique for Brucella culturing at all stages of brucellosis, since it yields 25% more positive results and provided results 10 days earlier than the biphasic method. The LC technique is a yield-optimisation method that uses lysis of erythrocytes in a citrate solution followed by isolation of Brucella bacilli by centrifugation that concentrated the bacilli and assist growth (Espinosa et al. 2009). The use of the LC technique for Brucella culturing with a longer incubation period (40 days [Espinosa et al. 2009] compared to 10 days in our study) should rather be used for Brucella culturing from blood and milk in future.

Evidence of Brucella infections in cattle in the study area has been serologically demonstrated previously (Gomo et al. 2012). The isolation of B. abortus from Brucella seropositive animals confirms the presence of brucellosis and indicates that B. abortus might causes abortions in the studied areas since both the cows from which B. abortus were isolated had an abortion history. The purchase of unknown Brucella-status cattle from the commercial to the communal sector for the purposes of restocking herds and genetic improvements and an increased uncontrolled movement of cattle due to agrarian reforms in the country are reported as the likely source of spread of brucellosis into the communal sector (Matope 2008; Matope et al. 2010). In addition, sharing of grazing land and watering points between cattle and wildlife at the studied interface is also likely to be a source of transmission of the disease in both directions as B. abortus has been isolated from cattle (Madsen 1989; Matope et al. 2009; Mohan et al. 1996; this study) and wildlife (Condy & Vickers 1969, 1972). The identification of B. abortus known to occur in cattle and wildlife in Zimbabwe is significant since it is one of the species generally considered pathogenic for humans (Baldwin & Goenka 2006). The tradition of consuming unpasteurised milk in rural areas, low awareness of the zoonotic importance of brucellosis, close intimacy with livestock and provision of assistance during parturition may increase the risk of human exposure to B. abortus infections in the study area. Despite the prevalence of brucellosis in the study area, no published information is available with regard to human brucellosis. However, public awareness in the Chiredzi communities should be increased to reduce the risk of human exposure to B. abortus infection.

**Conclusion**

Brucella abortus was isolated from blood and milk collected from seropositive cows in the Chiredzi district and therefore the community members in the Chiredzi regions like Pesvi and Malipati should be informed of the risk of human exposure to Brucella infection. The isolation of B. abortus from seropositive cows confirms that this species could be associated with cattle abortions in the Chiredzi district in Zimbabwe. However, further studies are recommended to determine the distribution of B. abortus biovars and human brucellosis prevalence in the area. The need for implementing control measures and raising public awareness on zoonotic transmission of brucellosis is recommended. Serially,
serological testing for brucellosis before translocation, culling of seropositive animals, increased controlled livestock movement and calfhood vaccinations should be instituted for the control of the disease. In addition, simple, user-friendly extension material to make cattle owners aware of this disease and its control should be produced and disseminated to them and the extension staff.

Acknowledgements

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Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this paper.

Authors’ contributions

C.G. (University of Pretoria) and H.V.H. (University of Pretoria) designed the project and wrote the manuscript. C.G. (University of Pretoria) and S.M. (Central Veterinary Laboratory) were involved in morphology identification of the culture and serological testing of the sera. C.G. (University of Pretoria) conducted the molecular studies. M.D.G.-W. (CIRAD), A.C. (CIRAD) and D.M.P. (University of Zimbabwe) were involved with the serological surveys and made intellectual and editorial contributions to the manuscript.

References


Bricker, B.J. & Halling, S.M., 1994, ‘Differentiation of Brucella abortus by 1, 2 and 4, Brucella melitensis, Brucella ovis, and Brucella suis bv 1 by PCR’, Journal of Clinical Microbiology 32, 2660–2666. PMID:7852552
Godfroid, J., Cloekertaet, A., Liardat, J.P., Kohler, S., Fretin, D., Walravens, K. et al., 2005, ‘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’, Veterinary Research 36, 313–326. http://dx.doi.org/10.1051/vetres:2005003