Documenting the absence of brucellosis in cattle, goats and dogs in a "One Health" interface in the Mnisi community, Limpopo, South Africa

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ABSTRACT

This study shows the absence of the world's most common bacterial zoonoses caused by *Brucella abortus* and *Brucella melitensis* in cattle, goats and dogs in an agro-pastoral community in South Africa, where heifer vaccination against brucellosis with the live Strain 19 vaccine is compulsory. The study site is bordering wildlife reserves with multiple wildlife species infected with brucellosis. The results showed a low seroprevalence (1.4%) in cattle. Seroprevalence in cattle decreased with age after four years in females, males were less positive than females and a tissue culture from a Brucellin skin test positive male was negative. The results indicate that *Brucella* seropositivity in cattle is due to S19 vaccination and not natural infections. This conclusion is reinforced by the absence of *Brucella*

seropositivity in goats (1/593 positive result) and dogs (0/315), which can be seen as potential spill over hosts. Therefore, the close proximity of brucellosis-infected wildlife is not a threat to domestic animals in this controlled setting with vaccination, fencing and movement control.

Keywords: South Africa; Transfrontier Conservation Area ; Brucellosis; Serology; Cattle; S19 vaccine; Goat; Dog

INTRODUCTION

In transfrontier conservation areas the humans, domesticated animals and wildlife live in close proximity with the transfer of disease between them of growing concern. Brucellosis has been serologically identified in several herbivore wildlife species including African buffalo (*Syncerus caffer*) (Bengis et al., 2002). South Africa's largest national park, adjacent to the research site, has over 37 000 buffalo with a brucellosis seroprevalence between 8.7- 47.6% (Gorsich et al., 2015).

Brucella abortus is the most abundant zoonotic *Brucella* species in South Africa with cattle as the main domestic reservoir (Mcdermott and Arimi, 2002). Brucellosis in small ruminants is generally caused by *B. melitensis* but spill over of *B. abortus* from cattle is a possibility (Godfroid et al., 2011) as it is with dogs (Wareth et al., 2017).

In South Africa, heifers between 4 and 8 months are vaccinated with *Brucella abortus* strain 19 vaccine containing 5 x 10^{10} colony forming units (CFU) (R. Macdonald, Onderstepoort Biological Products, personal communication, 2014).

The objectives of this study are to determine the presence of brucellosis in the domestic animal species and whether there is a brucellosis public health risk in a rural community established at the border of a wildlife conservation area.

MATERIALS and METHODS

Research site

The Mnisi community research site, surrounding the Hluvukani Animal Clinic (HAC), borders the Kruger National Park (KNP) in South Africa (Figure 1), with 40000 people (Berrian et al., 2016) of whom 1497 are cattle owners with 16418 cattle, 3350 goats and an estimated 4000 dogs.

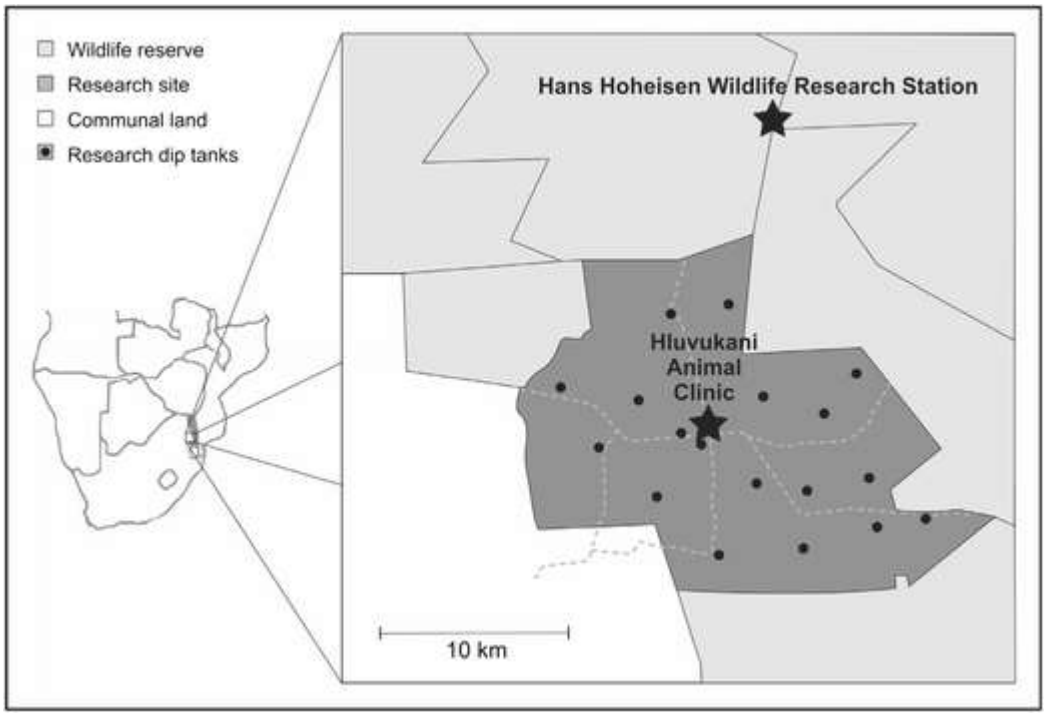

Figure 1. Research site in the Mnisi Community in South Africa. Black dots are research diptanks (see legend).

Study design

This was a cross-sectional study in cattle, goats, and dogs. The strategy in cattle was to estimate the seroprevalence using Rose Bengal plate agglutination test (RBT) and indirect enzyme linked immunosorbent assay (iELISA), confirm brucellosis using a brucellin skin test (ST) and isolate *Brucella spp*. through organ culture of a skin test positive animal.

The cattle study was conducted in two stages. The first stage, from May to June 2010, blood was collected from 1470 cattle. All RBT positive cattle were also tested with the iELISA for confirmation. In addition, every three out of five seronegative cattle (875 samples) were also tested with iELISA. In

the second stage, from April to September 2012, the entire herd of any seropositive animal by RBT or iELISA from the first stage were tested by RBT and iELISA. Animals that tested seropositive to both RBT or iELISA and were older than two years (to decrease the likelihood of animals being false positive due to vaccination (Saegerman et al., 1999)) were tested by the ST.

The strategy for goats and dogs was to establish the presence of *B. abortus* or *B. melitensis* infection using RBT and confirm with iELISA and also in goats the ST. During April to June 2012, blood was sampled from 593 goats at randomly selected herds and 315 dogs at the diptanks and Hluvukani Animal Clinic.

Disease diagnostics

Serology

The RBT was performed as described (Alton et al., 1988) using standardised *B. abortus* antigen from Onderstepoort Veterinary Institute, South Africa. The indirect enzyme linked immunosorbent assay (iELISA) test used was provided by Pourquier® (IDDEX, Montpellier, France).

Brucellin skin test

The brucellin skin test was performed as described (Saegerman et al., 1999) using standardized antigen, prepared from *B. melitensis* B115 rough strain (BRUCELLERGENE OCB®, Synbiotics Europe, France). A skin thickness increase of more than 1.1 mm three days later was considered positive. In goats, the test was done below the eyelid and the skin thickness measured again 48 hours later.

Bacterial culture

Tissues for *Brucella* culture were obtained by slaughtering the animal. Pre-scapular and superficial inguinal lymph nodes, testes, epididymes and the spleen were sent to Onderstepoort Veterinary Institute Laboratory on ice for culture to identify *Brucella* spp.

Data analysis

Serological data was analysed in logistic regressions (Stata, StataCorp). Sex was first used as only explanatory variable. Then, age was used as continuous explanatory variable in females only for lack of positive data in males. Phase 1 ELISA data was weighted to compensate the fact that sampling

fractions were different among RBT positive and RBT negative samples. In the absence of positives in a category, a Fisher exact test was applied and 95% confidence interval was calculated using the exact method. Apparent prevalence estimates were transformed to true prevalence using 87% sensitivity and 97.8% specificity for RBT and for iELISA 97.2% and 97.1% respectively (true prevalence 1) and a higher specificity (true prevalence 2) of 99.8% was also used for the iELISA (Godfroid et al., 2010).

Ethics approval

Ethics approval for the study was obtained from the University of Pretoria Animal Use and Care Committee (V026-12).

RESULTS

Cattle

In phase 1, all estimated true prevalences had negative values, with the exception of "true prevalence 2", which ranged between 1 and 2.5% (table 1). The difference between males and females was not significant ($p>0.1$) while, in females, seroprevalence did not significantly increase with age. The 95% CI upper limit of odds ratios were 1.04 and 0.98 for RBT and iELISA respectively. In phase 2, prevalence in females was significantly different from 0, unlike males, with a significant sex effect in iELISA data only ($p=0.002$). In females, seroprevalence did not increase with age either. The 95% CI upper limits of the odds ratio were 1.03 and 1.17 for RBT and iELISA, respectively.

Table 1. Estimated apparent and true prevalences in phase 1 and phase 2 using RBT and iELISA as diagnostic tools (with 95% CI)

	Sex	*N*. obs.	*N*. pos.	Apparent prevalence (%)	True prevalence 1 (%)	True prevalence 2 (%)[a]
Phase 1						
RBT	Females	1102	19	1.7 (1.1–2.7)	− 0.6 (− 1.3–0.6)	
	Males	368	2	0.5 (0.1–2.1)	− 2.0 (− 2.4–0)	
iELISA	Females	673	22	2.7 (1.7–4.0)	− 0.3 (− 1.2–0.8)	2.5 (1.6–4.0)
	Males	223	3	1.2 (0.4–3.7)	− 1.8 (− 2.7–0.8)	1.0 (0.2–3.6)
Phase 2						
RBT	Females	345	16	4.6 (2.9–7.4)	2.9 (0.8–6.2)	
	Males	71	1	1.4 (0.2–9.3)	− 0.9 (− 2.4–8.4)	
iELISA	Females	345	35	10.1 (7.4–13.8)	7.7 (4.7–11.6)	10.3 (7.4–14.0)
	Males	71	0	0 (0–4.1)	− 3.1 (− 3.1–1.3)	− 0.2 (− 0.2–4.1)

* Using a 99.8% specificity instead of 97.1% for true prevalence 1

Nine cattle were tested with the ST with 3 being positive at 2.5, 1.89 and 1.78 mm skin thickness change. The animal deemed the most likely to be infected, was the male that should not have been vaccinated, was slaughtered and organs cultured for *Brucella* spp. with a negative result.

Goats and dogs

A total of 593 goats from 92 herds were tested with one sample RBT positive, but iELISA negative. That animal and the other two female herd members were tested with a ST and the results were deemed negative. Three hundred fifteen dogs were negative by RBT. No iELISA test were therefore done.

DISCUSSION

Our studies show a constant seroprevalence in female cattle with age, which is opposite to the expected increasing seroprevalence with age in animals exposed to natural challenge, e.g. unvaccinated buffalo in the Kruger National Park (Gorsich et al., 2015). We also found the apparent prevalences in males to be lower than in female, unlike in a wildtype infection (Gomo et al., 2012). We did not culture the S19 vaccine strain, which would have reinforced our conclusion that these antibody titres were due to vaccination rather than true wildtype infection.

The absence of evidence of the disease in goats and dogs is supportive of the postulated absence of brucellosis in cattle. An unpublished study testing 64 humans handling livestock study site diptanks that found no positive reactions with the human BrucellaCapt® agglutination test (Vanessa Quan, personal communication, July 19th 2017).

The absence of brucellosis in domestic animals in this area, which has frequent fence transgressions by wildlife, indicates the control measures in cattle (strain 19 vaccination of heifers), movement restrictions for cattle and goats because of foot and mouth disease and fencing between wildlife and domestic animals are adequate to keep brucellosis out of the domestic animals in this setting and therefore there is no risk at time of study of transmission to human beings from domestic animals.

Competing interests

The authors declare they have no competing interests.

Acknowledgements

The study was funded by a grant from the Belgium Institute of Tropical Medicine through collaboration with the University of Pretoria and research funds from the University of Pretoria. The authors wish to acknowledge the assistance of the Mpumalanga Veterinary Services and specifically the animal health technician Gypsey Mathumbo and veterinarians Oupa Rikhotso and Bjorn Reininghaus.

References

Alton, G.G., Jones, L.M., Angus, R.D. and Verger, J.M., 1988. Techniques for the Brucellosis Laboratory, (Institut National de la Recherche Agronomique (INRA); 75007 Paris; France)

Bengis, R.G., Kock, R.A. and Fischer, J., 2002. Infectious animal diseases: the wildlife/livestock interface, Rev. sci. tech. Off. int. Epiz, 21, 53–65

Berrian, A.M., van Rooyen, J., Martínez-López, B., Knobel, D., Simpson, G.J.G., Wilkes, M.S. and Conrad, P.A., 2016. One Health profile of a community at the wildlife-domestic animal interface, Mpumalanga, South Africa, Preventive Veterinary Medicine, 130, 119–128

Godfroid, J., Nielsen, K. and Saegerman, C., 2010. Diagnosis of brucellosis in livestock and wildlife, Croatian Medical Journal, 51, 296–305 (Medicinska Naklada)

Godfroid, J., Scholz, H.C., Barbier, T., Nicolas, C., Wattiau, P., Fretin, D., Whatmore, A.M., Cloeckaert, A., Blasco, J.M., Moriyon, I., Saegerman, C., Muma, J.B., Al Dahouk, S., Neubauer, H. and Letesson, J.-J., 2011. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century, Preventive Veterinary Medicine, 102, 118–131

Gomo, C., de Garine-Wichatitsky, M., Caron, A. and Pfukenyi, D.M., 2012. Survey of brucellosis at the wildlife–livestock interface on the Zimbabwean side of the Greater Limpopo Transfrontier Conservation Area, Tropical Animal Health and Production, 44, 77–85

Gorsich, E.E., Ezenwa, V.O., Cross, P.C., Bengis, R.G. and Jolles, A.E., 2015. Context-dependent survival, fecundity and predicted population-level consequences of brucellosis in African buffalo, Journal of Animal Ecology, 84, 999–1009

Mcdermott, J.J. and Arimi, S.M., 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact, Veterinary Microbiology, 90, 111–134

Saegerman, C., Vo, T.K., De Waele, L., Gilson, D., Bastin, A., Dubray, G., Flanagan, P., Limet, J.N., Letesson, J.-J. and Godfroid, J., 1999. Diagnosis of bovine brucellosis by skin test: conditions for the test and evaluation of its performance, Veterinary Record, 214–218

Wareth, G., Melzer, F., El-Diasty, M., Schmoock, G., Elbauomy, E., Abdel-Hamid, N., Sayour, A. and Neubauer, H., 2017. Isolation of *Brucella abortus* from a dog and a cat confirms their biological role in re-emergence and dissemination of bovine brucellosis on dairy farms, Transboundary and Emerging Diseases, 64, e27–e30