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Wildlife-cattle interactions emerge as drivers of bovine tuberculosis in traditionally farmed cattle

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1.1 Abstract

Bovine tuberculosis (bTB) in cattle is recognized to be associated with several risk factors that include herd size, cattle movement, ownership of other domestic animals, confinement of cattle in enclosures at night, water sources, communal grazing area and proximity to wildlife, especially bTB maintenance hosts. A questionnaire survey was used to investigate the risk factors associated with *Mycobacterium bovis* (*M. bovis*) infection and transmission in traditionally farmed cattle at the wildlife/livestock interface in uMkhanyakude district, northern Kwa-Zulu Natal (KZN), South Africa. The questionnaire comprised of semi-structured questions that were used to gather data on livestock management practices and knowledge about bTB from 71 respondents from households that owned either bTB infected cattle herds or uninfected herds. Multiple correspondence analysis (MCA) was used to explore the association between the risk factors for *M. bovis* transmission and the bTB herd status. Bovine TB positive herds were associated with a herd size of $n > 15$, movement of cattle to areas adjacent to the game parks for grazing, cattle grazing inside the game parks as well as cattle sharing water and pasture with wildlife. The multivariable logistic regression model identified movement of animals to areas adjacent to the game parks and cattle sharing water with wildlife as highly significant risk factors for bTB infection in cattle. The findings of this study emphasized the need for the implementation of

bTB control strategies in both cattle and wildlife populations for the successful control of the disease.

Keywords: bovine tuberculosis (bTB); cattle; *Mycobacterium bovis* (*M. bovis*); risk factors; multiple correspondence analysis (MCA); wildlife/livestock interface

1.2 Introduction

Mycobacterium bovis (*M. bovis*) causes bovine tuberculosis (bTB) in cattle and tuberculosis in a wide range of other domestic, wild mammal species as well as humans (Ayele et al., 2004; Michel et al., 2006; Humblet et al., 2009; Michel et al., 2010; de Garine-Wichatitsky et al., 2010;). *M. bovis* infection is prevalent in many countries in the world and remains a potential threat to humans and animals in low-income countries, due to inadequate control programs (Etter et al., 2006; de Garine -Wichatitsky et al., 2013). The pathogen is mainly transmitted between animals through aerosols, although it can also be transmitted through direct contact with an infected animal or through the consumption of contaminated food and water (Gumi et al., 2011; Dejene et al., 2016).

In Africa, tuberculosis due to *M. bovis* infection has been reported to be prevalent in cattle populations of many countries and in several wildlife species that include the African buffalo, lion, chacma baboon, kudu, warthogs and several others (de Garine -Wichatitsky et al., 2013; World Organisation for Animal Health (OIE), 2019). In South Africa, sporadic outbreaks of bovine TB occur in cattle

while *M. bovis* infection in wildlife has been reported in 21 species and bTB is endemic in some of the wildlife populations (Michel et al., 2006; Renwick et al., 2007; Michel, 2015). While most wild animal species act as dead-end hosts, others, such as the African buffalo, are maintenance hosts that are able to transmit and sustain the disease (Renwick et al., 2007; Humblet et al., 2009; de Garine-Wichatitsky et al., 2013). Inter species transmission at the wildlife/livestock interface has been documented in Zambia and South Africa where *M. bovis* strains isolated from wildlife and cattle shared common genotypes (Hang'ombe et al., 2012; Musoke et al., 2015).

Research has shown that bTB in cattle is associated with several risk factors including a large herd size, entry of new animals, production areas, type of water sources, livestock management practices, ownership of other domestic animal species and communal livestock farming (Cleaveland et al., 2007; Munyeme et al., 2008; Oloya et al., 2007; Tschopp et al., 2009). Studies carried out in several countries have revealed that association of cattle with wildlife particularly at the wildlife/livestock interface is a significant risk factor for bTB transmission to cattle (Dejene et al., 2016; Griffin et al., 1996; Kaneene et al., 2002; Katale et al., 2013; Munyeme et al., 2009)

Traditional livestock farming in African countries is based on the sharing of communal watering points and livestock from different herds are allowed to graze freely on communally owned pastures (Shirima et al., 2003; Oloya et al., 2007;

Tschopp et al., 2009). Animals are kraaled (confined in enclosures) at night during the wet season and there is unrestricted movement of animals during the dry season due to limited availability of grazing and water (Oloya et al., 2007; Munyeme et al., 2008). Animals do not receive feed supplements, and access to veterinary public health services is usually limited in these areas (Michel et al., 2006; Oloya et al., 2007). Livestock in sub-Saharan countries implies traditional wealth (social status), is used in cultural practices, as a source of draught power and food, and has an economic role in supporting livelihoods in rural areas (Michel et al., 2006; Caron et al., 2014).

Despite the importance of cattle in rural communities and risk factors identified in other countries, there is a limited understanding of the current risk factors for bTB infection and disease transmission to cattle at the highly complex wildlife/livestock interface in South Africa. The uMkhanyakude district is surrounded by game reserves where bTB is known to be endemic in wildlife and the disease was previously detected in traditionally farmed cattle at an overall animal prevalence of 12% (Michel et al., 2009; Sichewo et al., 2017). For improved disease control it is imperative to determine the risk factors associated with *M. bovis* infection and transmission in traditionally farmed cattle in this area. As anthropological factors are also of significance in the spread of bTB, the study included an assessment of the farming community's knowledge and awareness of bTB in cattle and wildlife.

2. Methodology

2.1 Study Area

The study was conducted in Big 5 False Bay Municipality in uMkhanyakude district in Northern Kwa-Zulu Natal, South Africa. The uMkhanyakude district (28°01'25"89 S, 32°17'30"30 E) is situated in the north eastern parts of Kwa-Zulu-Natal Province surrounded by numerous private and public conservation areas that include: St Lucia (iSimangaliso), Hluhluwe/Imfolozi, Mnyawana, Thanda, Thula thula, Mkuze and others as shown in figure 1. This region has low rainfall patterns that result in frequent water shortages and regular droughts.

The community in this study area is under traditional authority governance that influences land use in terms of agriculture (mixed crop and livestock farming) and tourism. The main cattle breeds are the local Nguni and the Brahman that are kept traditionally in a free-range grazing system using communal water sources and grazing grounds within village perimeters. Farmers from several villages gather regularly (weekly/fortnightly) for disease control activities at government constructed dipping tanks (dip tanks). Veterinary assistance at the dip tanks is mostly restricted to vaccination and control of external parasites. The animals are taken care of by members of the household that include young boys, or employees as cattle keepers.

2.2 Questionnaire survey

During August and September 2017, a questionnaire survey was conducted among the 192 households of cattle owners that had their herds tested for bTB in September 2016 and March 2017 during a prevalence study and were registered at four dip tanks (Masakeni, Mpempe, Nibela, Nkomo) as shown in figure 1. A case-control study was designed to determine the risk factors that were associated with bTB infected herds. The households and their herds were classified as either cases (owned a bTB positive herd) or controls (owned a bTB negative herd) according to the results from the prevalence study (Sichewo et al., 2019). Therefore, we classified all the positive herds (53 herds), 28% of total herds in the previous prevalence study (Sichewo et al., 2019) as potential cases and aimed at selecting the same number of controls by random selection amongst the 139 bTB negative herds in the same study.

The structured questionnaire was administered either to the cattle owner, to household members that owned cattle or to cattle keepers. The questionnaire was carried out at the homesteads through “face-to-face” interviews by one of the research team members who is a native speaker of the local language of isiZulu. The relevant data was simultaneously collected with data for a survey on risk factors to zoonotic TB transmission to humans (Sichewo et al., 2019). See attached questionnaire in English (supplementary files).

The purpose of the study was discussed with all the household members present and verbal consent obtained from the head of the family (cattle owner). The questionnaire gathered information on knowledge of bTB, general livestock management practices, herd size, cattle kraaling (housing) at night, introduction of new cattle into herd, ownership of other livestock species, watering and grazing resources available for the animals. The wildlife interaction section of the questionnaire included questions on livestock movement to grazing areas adjacent to game parks, grazing animals inside game parks and contact with wild animals. Contact with wild animals was defined as wildlife (any species) being observed by cattle keepers in the grazing or watering areas, while cattle were present. The cattle keepers observed an approximate distance that ranged from 25-100m between cattle and wildlife during co-grazing in the bush veld as well as during drinking at the water sources.

2.3 Ethical statements

Permission to carry out the study was granted by the Department of Agriculture, Forestry and Fisheries under their Section 20 (12/11/1/1/6/1) and the University of Pretoria, Faculty of Veterinary Science Animal Ethics approval number (V078-16). The permission for questionnaire administration to the farmers was obtained from the University of Pretoria-Faculty of Humanities Research Ethics Committee (GWO170814HS). Verbal consent from the tribal authorities was obtained prior to the start of any activity in the area. At the time of the interviews, written consent

was obtained from the participants after explaining that the study was voluntary, confidential and that they had the choice of ending their participation at any time without effect on the services received from the state veterinary officers.

2.4 Statistical analysis

The questionnaire data was cleaned by checking for missing information or incorrect entries and exported to R for analysis. Different approaches were applied to investigate the (variables) risk factors of bTB transmission to traditionally farmed cattle at the wildlife/livestock interface using the questionnaire data.

The multiple relationship between risk factors and bTB herd status (positive or negative) was explored using a non-linear multivariate approach referred to as multiple correspondence analysis (MCA). MCA is an exploratory descriptive data analysis technique that is used to provide a detailed description and comprehensive analysis of the relationship among a set of categorical variables (predictor) with the outcome variables (Elmoslemany et al., 2009).

By presenting the value of the outcome variable on the same graph as the categorical variables, the clusters of predictor values around the outcome variable can be also be visualised. In this case, it was used to detect and explore the spatial association between the mentioned risk factors for bTB transmission to cattle categorical (predictor) variables and the bTB herd status as displayed by

the clustering of the categorical variables around the outcome variable that is either a positive or negative herd. When reading the graph, the closeness of the points that represent the categorical variables to the outcome variable shows the strength of the association i. e points that are in the same quadrant or approximate vicinity indicate an association (Rencher, 2002; Zuur et al., 2010) given that these variables are well represented by the two dimensions used to produce the graph.

All variables of interest and biological relevance were included in the MCA as a preliminary analysis to explore the data set. The variables included were; herd size (less than or greater than 15), ownership of other domestic animals (pigs, goats, sheep), source of water for the animals (communal, borehole or own well), cattle -wildlife interactions (cattle sharing water with wildlife, cattle co-grazing with wildlife, movement of animals to areas adjacent to the game parks, cattle grazing inside game parks), kraaling of animals at night, occurrence of TB like lesions in animals at slaughter, knowledge and awareness of bTB in wildlife and cattle.

The independent effects of risk factors (categorical variables) such as contact of cattle with wildlife, herd size, the introduction of animals into the herd and other potential risk factors on the bTB herd status (dependent variable) were examined using the two-tailed Fisher's exact test. The number of bTB positive as well as the numbers of bTB negative herds were specified in a binary categorical response variable, comprising the bTB positive and bTB negative herds.

Predictor variables with p-value lower than 0.3 according to the Fisher-test were considered potential risk factors and used for the subsequent construction of a multiple logistic regression model (Generalized Linear model (GLM-family = binomial). A forward stepwise approach was used to build the final multivariable model based on the selection of model with the lowest AIC (Akaike Information Criterion), and this is ideal for relatively small data sets containing great numbers of variables (Dohoo et al., 2009; O'Hagan et al., 2016). The collinearity was tested in the final model calculating the variance inflated factor (VIF) for each remaining factor (Zuur et al., 2010).

Descriptive analysis, multiple correspondence analysis (MCA), independent effects of risk factors (categorical variables) on bTB herd status (univariable analysis) and multivariable data analysis (logistic regression) were accomplished using the statistical R software version © 3.4.4, 2018, (The R Foundation for Statistical Computing, Vienna, Austria). The packages used included the 'base' package of R including "stats" for univariate analysis and "FactoMineR" for the multivariate analysis (MCA). We tested for collinearity on the GLM using the variance inflation factor using the "car" package and Hosmer-Lemeshow goodness of fit test using the "Resource Selection" package.

3. Results

3.1 Descriptive analysis

A total of 71 respondents from households that owned cattle registered at one of the four dip tanks in the study area were recruited into the study based on their results from the previous study (Petronillah Rudo Sichewo et al., 2019) and their availability and willingness to participate in the interviews. Amongst the 53 households that owned bTB infected herds and 53 randomly selected households that owned bTB negative herds, respectively 42 (59 %) and 29 (41 %) households were available and willing to participate in the study. Due to unavailability of some owners and reluctance to participate in others it was not possible to match the number of bTB infected herds with new randomly selected herds from the remaining bTB negative herds. According to gender, 65% of the respondents were male and 35% were females.

The results of the questionnaire survey (Table 1) showed that several species of domestic animals other than cattle were kept by the cattle owners in a mixed herding system (94% kept goats, 80 % pigs and 7% sheep). All the cattle were grazed on communal pastures (100%), 69% used a communal water source and all herds had close contact during dip tank visits, communal grazing and/ or drinking water. More than half of the respondents (69%) kraaled their cattle at night, especially during the rainy season. The situation was somewhat different during the dry season when most of the cattle were moved over long distances in

search of water and pasture, therefore the animals slept out in the pastures as was explained by the participants during the interviews.

Sharing of water and grazing by livestock and wildlife (sighting of wildlife and cattle drinking water or grazing at the same place, at the same time) during the past 12 months was reported by 58% and 47% of respondents., respectively, In addition, 66% reported the movement of their animals to areas adjacent to the game reserves during the dry months of the year. As shown in Table 1, the awareness of bovine TB in cattle among respondents was very high, 86% of them had heard about the disease in cattle. Generally, most respondents were knowledgeable about bTB in cattle since 61% of them managed to mention at least two clinical signs of the disease in cattle. On the other hand, most of the respondents were not aware of tuberculosis in wildlife, 89% of them revealed that they had never heard about TB in wildlife.

3.2 Risk factors for bovine tuberculosis transmission to cattle

3.2.1 Multiple Correspondence Analysis (MCA)

The selection of the two dimensions to be used in the plotting of the MCA map was based on the quality of the representation of the bTB status on these dimensions as well as on the highest amount of the total variance explained by these dimensions. The quality of representation of bTB status on the dimensions was determined using squared cosine (\cos^2). The \cos^2 of bTB status on the 1st

and 2nd dimension were, respectively 0.8 and 0.05, this was higher than on the 3rd and 4th dimensions. The 1st and 2nd dimensions accounted for 22.56% and 10.27%, respectively of the variance of the whole dataset and therefore were considered to adequately represent the bTB status variable and most of the other variables. The variables kept on the figure 2 and figure 3 were the ones the most clustered around bTB status.

The first results of the MCA analysis (Figure 2) showed that the factors that are associated with the bTB status of a herd include movement of cattle to areas adjacent to the game reserves, cattle grazing inside game reserves and the cattle herd size greater than 15. The knowledge around bTB (knowledge of the disease and its transmission) is uncorrelated with the bTB status of the herd.

Further analysis was done to determine the specific categories of variables that are associated with a bTB positive herd or bTB negative herd as shown in Figure 3. The farmers responded yes to the practices that they carried out and no if there were not carrying out the practice. Categories associated with bTB positive herds were cattle sharing water with wildlife_ yes, cattle co-grazing with wildlife_ yes, cattle grazing inside game reserve_ yes, movement of cattle to areas adjacent to the game reserve_ yes and herd size greater than 15_ yes. Bovine TB negative status was associated with practices that did not involve wildlife-cattle interaction such as cattle not sharing water or grazing with wildlife, cattle not

grazing inside game reserve or areas adjacent to the game reserve as shown in figure 3.

3.2.2 Univariate analysis

Based on the univariable analysis 12 out of 15 of the variables were associated with bTB positive herd and these included herd size (a herd size between 1-15, between 15-40 and greater than 40), kraaling of animals at night, use of boreholes as a source of water for animals, movement of animals to areas adjacent to the game reserve, cattle co-grazing with wildlife, cattle sharing water with wildlife, cattle grazing inside the game reserve, introduction of new cattle into the herd, ownership of other animals such as goats, sheep and pigs (Table 3. 2). Using own well as a source of water for animals, communal water source and communal pasture (grazing area) were not associated with a bTB positive status.

3.2.3 Regression analysis

The variables included in the final multiple logistic regression, selected using AIC values, were movement of cattle to areas adjacent to the game reserve, cattle sharing water with wildlife, cattle co-grazing with wildlife. Using the multivariable logistic regression analysis movement of animals to areas adjacent to the game reserve and cattle sharing water with wildlife were identified as significant risk

factors for bTB infection in cattle (Table 3). There was high collinearity between many risk factors due to the uniformity in the management systems nevertheless the variables included in the final model showed very low collinearity with VIF inferior to 3 for all of them (Zuur et al., 2010). The result of the Hosmer-Lemeshow goodness of fit test comparing the observed value and the one fitted by the logistic regression showed that there was no statistical difference (p-value=1) and that the retained model fit very well with the observations.

4 Discussion

This study identified the movement of cattle to areas adjacent to game parks for grazing as well as shared water sources between cattle and wildlife as highly significant risk factors for *M. bovis* infection in traditionally farmed cattle in the area under study. The significant risk factors suggest intra- and inter- species transmission of bTB facilitated by the livestock management practices. *M. bovis* infection has previously been confirmed in African buffalo populations in the private and public game reserves that surround the study area with cattle being implied as the likely original source of the infection (Michel et al., 2009; Hlokwe et al., 2011). Genotyping of *M. bovis* isolates from cattle and buffalo at this wildlife-livestock interface revealed a shared spoligotype pattern (SB0130) as well as VNTR-MIRU (Variable number of tandem repeats-Mycobacterial

Interspersed Repetitive Units) profiles, indicating an exchange of *M. bovis* between species (Sichewo et al., 2019). African buffaloes are a wildlife maintenance host responsible for the persistence of *M. bovis* in a multi-species ecosystem. They can also act as a source of infection to neighboring communal cattle, either through buffalo-cattle interactions or indirectly through infection of wild spillover hosts such as suids and antelopes which interact with domestic cattle (Kalema-Zikusoka et al., 2005; Musoke et al., 2015; Roos et al., 2018). The sharing of water and pasture at the interface of livestock and wildlife areas is believed to facilitate the spread of bTB to communal cattle which has been supported in this study by the finding that 58% of the respondents reported observations of cattle and wildlife drinking water from the same source during the annual dry season, exacerbated during extensive droughts. However, the predominant direction of transmission at the wildlife/livestock interface remains unclear and is probably bi-directional and determined by several factors such as population density, prevalence of infection, distribution and movement of infected and susceptible species (Bengis et al., 2002; Renwick et al., 2007; Musoke et al., 2015).

The MCA further strengthened the view that all the activities which involved sharing of resources by wildlife and livestock and therefore potential direct or indirect contacts i.e. when cattle graze in areas adjacent to game parks or inside game parks or share watering points were associated with bTB infected herds. Although wildlife and cattle may share pastures and water, close physical contact

between them is rarely observed (Meunier et al., 2017). This means that opportunities for direct aerosol transmission between wild and domestic animal species may be few, compared to intra-species transmission. Shedding of *M. bovis* from nasal secretions of cattle, on the other hand, is common and can, apart from aerosol transmission lead to contamination of food and water, especially where those resources are shared (Menzies and Neill, 2000). Animals exposed to a contaminated environment can either contract *M. bovis* via the respiratory or the alimentary tract (Cleaveland et al., 2007; Phillips et al., 2003; Meunier et al., 2017). A study carried out in New Zealand demonstrated a potential respiratory route of transmission after observing cattle behavior that predisposed them to tuberculosis (Sauter and Morris, 1995).

Mathematical modelling of the transmission dynamics between buffalo and cattle populations has confirmed independently that the involvement of all transmission routes helps to sustain cross-species transmission at the wildlife/livestock interface. The analysis of the same model established that bTB had a negative impact on cattle populations more than buffalo populations when all modes of transmission are considered (Phepa et al., 2016). The rate of transmission is critically affected by the survival of *M. bovis* in the environment owing to factors such as exposure to sunlight, the temperature, soil pH and moisture levels (Cleaveland et al., 2007). The surroundings of natural water bodies in the habitat where the study area is located provide moist and often shady conditions favorable for *M. bovis* survival (Kaneene et al., 2002). Fine and co-workers

demonstrated that *M. bovis* can persist in contaminated water for up to 8 weeks (Fine et al., 2011). It has been previously suggested that buffalo rarely shed high quantities of *M. bovis* in nasal or oral secretions making effective transmission via water less likely, (Michel et al., 2007) while *M. bovis* can survive for 6 weeks in infected carcasses and for 4 weeks in faecal matter of free ranging buffalo depending on the weather conditions (Tanner and Michel, 1999). Moreover, during the rainy season *M. bovis* bacilli from wildlife carcasses or excreta could contaminate grazing areas and water bodies through surface runoff water (El-Sayed et al., 2016).

Reflecting on the findings from this study it can be concluded that the sustained indirect contact between cattle and wildlife in the study area provides favorable conditions for the transmission of *M. bovis* to cattle, especially during the dry season. The intermittent inter-species transmission intervals are relevant to the endemic bTB status of the communal cattle herds due to indirect contact with infected grazing areas or water bodies and their surroundings as cattle, once infected, can maintain the infection. Consequently, the control of bTB in this communal cattle population faces multiple major challenges. Several studies in different developed countries have established that the presence of a wildlife reservoir is a serious hindrance to bTB control and may render eradication from cattle impossible (Olea-Popelka et al., 2005; Graham Nugent, 2011; Fitzgerald and Kaneene, 2013). The lack of regular bTB testing and removal of infected

cattle in communal farming systems diminishes the chances for timely disease detection while the free movement of cattle promotes the spread of the disease.

The study also established that the farmers' awareness (89%) and knowledge (61%) of bovine TB as a disease of cattle was generally high and can probably be attributed to a recent educational campaign conducted during and after the bTB testing of cattle in the study area. However, there was no association of the level of awareness to the bTB status of the herd, which can probably be explained by the recent acquisition of this knowledge. In comparison, a lower level of awareness was recorded in Zimbabwe (48%) from communal cattle owners living in a similar environment (small scale farmers living at a wildlife/livestock interface and facing drought problems) and this was attributed to the absence of a relevant educational program (Gadaga et al., 2015).

Despite the farmers' basic knowledge of bTB as a disease of cattle and ability to list symptoms, they had little information on how the disease is spread between infected herds or from wildlife. Moreover, most of the respondents had never heard of bTB in wildlife and its link to cattle bTB, thus, there was no conscientious application of protective livestock management practices to prevent the spread of the disease. These findings indicate a great need for educational programs on bTB transmission dynamics to promote risk-averting practices at the wildlife/livestock interface since it has been observed that cattle owners play a central role in bTB control in cattle and inadequate knowledge of

the disease epidemiology hinders the execution of prevention and control measures (Shitaye et al., 2007).

The limitation of the present study was the lack of willingness of some farmers to participate in the study. This was mostly from the group of farmers that owned uninfected herds who possibly could not understand the relevance of the disease to their current herd status. This did not significantly impact on our result as not much additional information would have been generated as these communal farmers have uniform management practices and variations arise due to different geographical locations.

5. Conclusions

Livestock management practices that promote proximity of cattle to wildlife have been identified as important risk factors for *M. bovis* infection. Consequently, successful control of bTB is impossible in the presence of a wildlife maintenance host (buffalo). Collaborative efforts are required from both the state veterinary officers and wildlife managers to reduce the risk of bTB in cattle through reducing opportunities for wildlife/livestock contact but also through exploring more suitable, alternative control measures.

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Conflict of interest; The authors declare no conflict of interest

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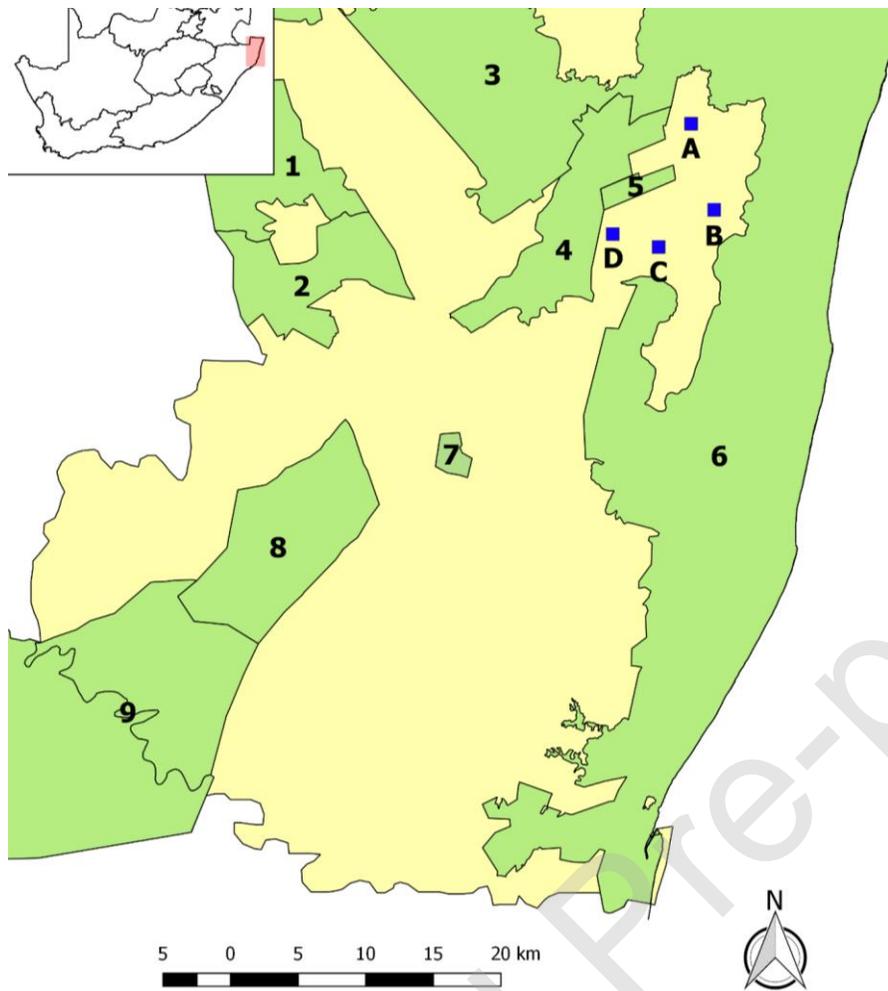
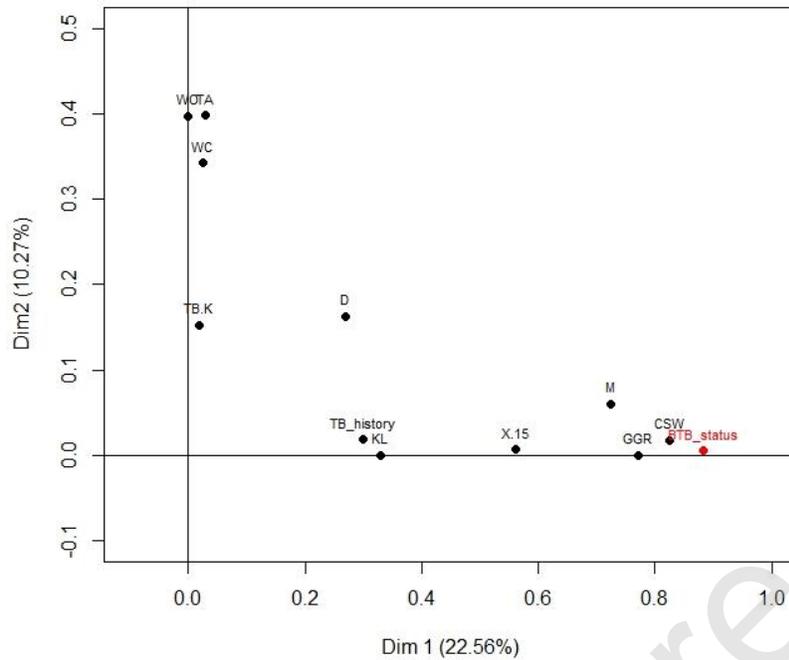


Figure 1: Map of the study area that is surrounded by game parks as shown on the map (1-Zululand Rhino Reserve, 2-Thanda game reserve, 3-Mkuze game reserve, 4-Phinda Game reserve, 5-Makasa Nature Reserve, 6-iSimangaliso Wetland Park, 7-Ubizane game reserve, 8& 9-Hluhluwe-iMfolozi Game Reserve. Locations of the four dip tanks Mpempe, Nkomo, Nibela, Masakeni are labelled A, B, C, D, respectively.



Key:

CSW: cattle sharing water with wildlife

KL: kraaling of animal at night

M: movement of animals close to game park

TB. K: Knowledge of bTB

WO: own well

X-15: herd size

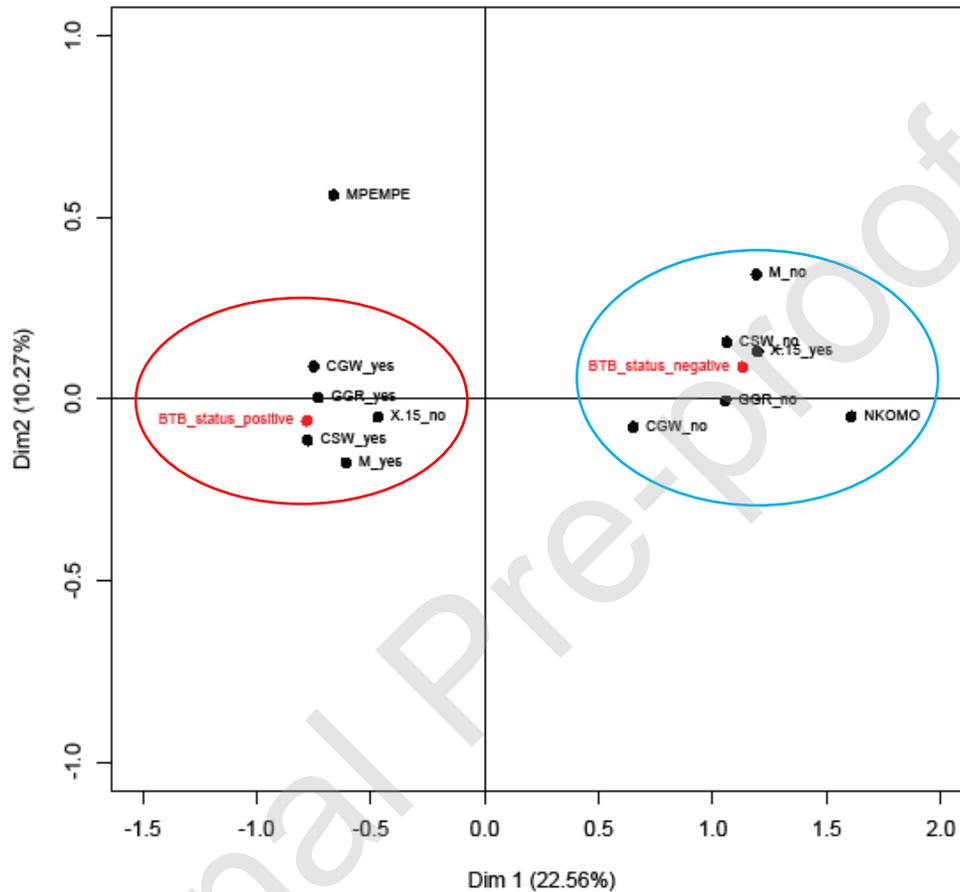
GGR: cattle grazing in game reserve

D: Dip tanks

WC: communal water source

TA: Awareness of bTB in cattle

Figure 2: Multiple correspondence analysis map of risk factors associated with bTB status of a herd



Key

CSW: cattle sharing water with wildlife

CGW: cattle co-grazing with wildlife

GGR: cattle grazing inside reserves

X-15: herd size < or > than 15

M: movement of animals to areas

NKOMO/MPEMPE: Dip tanks

adjacent to reserves

Figure 3: Multiple correspondence analysis map of risk factors categories associated with bTB positive and bTB negative herds

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Table 1: Results of questionnaire survey on risk factors and awareness on bovine tuberculosis by the respondents (included cattle owners, cattle keepers and family members of households that owned cattle)

Variable	Level	Responses		M. bovis reactors herds	
		n	%	n	%
What is the type of grazing system used for your livestock?	1. Communal pasture 2. Own paddock 3. Own pasture	71 - -	100	42	100
What is the source of water for livestock?	1. Own well 2. Borehole 3. Communal water (surface)	17 24 5 7 49 69		10 5 27	24 12 64
Do you ever move your animals to areas adjacent to game parks for grazing?	1. Yes 2. No	47 66 24 34		41 1	98 2
Do you ever move your animals inside the game parks for grazing?	1. Yes 2. No	42 59 29 41		39 3	93 7
Have you seen your cattle grazing with wild animals during the past 12 months?	1. Yes 2. No	33 47 38 54		31 11	74 26
Have you seen your cattle with wild animals at watering points during the past 12 months?	1. Yes 2. No	41 58 30 42		40 2	95 5
Have you ever bought or received animals from another area?	1. Yes 2. No	6 8 65 92		6 36	14 86
Are the cattle kraaled at night?	1. Yes 2. No	49 69 22 31		37 5	88 12

What are the other domestic animals that you own?	1. Goats 2. Sheep 3. Pigs	67 94 5 7 10 14	40 1 6	95 2 14
What is your cattle herd size?	1. 1-15 2. 16-40 3. >40	24 34 28 39 19 40	0 23 19	0 55 45
Have you ever heard about bovine TB in cattle?	1. Yes 2. No	61 86 10 14	35 7	83 17
If yes, knowledge of clinical signs of bTB in cattle?	1. Yes 2. No	43 61 28 39	36 6	86 14
Have you ever heard about TB in wild animals?	1. Yes 2. No	8 11 63 89	7 35	17 83

Table 2: Univariable analysis of variables (risk factors) for bovine tuberculosis transmission to traditionally farmed cattle at the wildlife/livestock interface

Risk factor	Case (n=42) % (n)	Control(n=29) % (n)	p value	OR	95% CI
Herd size					
1-15	0(0)	34(24)	<0.0001*	0	0-0.06
16-40	55(23)	17 (5)	<0.001*	7.3	2.04- 34.19
>40	45(19)	0(0)	<0.0001*	Inf	5.03-inf
Water source					
Communal	64(27)	76(22)	0.43	0.58	0.17-1.84
Borehole	12(5)	0(0)	0.07*	Inf	0.66-inf
Own well	24(10)	24(7)	1	0.98	0.29-3.54
Cattle-wildlife interaction					
Movement of cattle to areas adjacent to the game reserve	98(41)	21(6)	<0.0001*	138.25	17.20 – 6301
Cattle co-grazing with wildlife	74(31)	7(2)	<0.0001*	35.6	7.14 - 357.15
Sharing water with wildlife	95(40)	3.5(1)	<0.0001*	410	40 – 16384
Cattle grazing inside the game reserve	93(39)	10(3)	<0.0001*	96.65	17.6- 865
Other livestock management practices					
Kraaling	88(37)	41(12)	<0.0001*	10	2.83- 42.85

Introducing animals into the herd	14(6)	0(0)	0.075*	Inf	0.86-inf
Bovine TB clinical signs in cattle	86(36)	24(7)	0.013*	4.13	1.29 – 15.04
Communal pasture (grazing)	100(42)	100(29)	-	-	-
Ownership of other animals					
Goat	95(40)	93(27)	0.01*	1.4	0.10-21.48
Sheep	2(1)	14(4)	0.15*	0.15	0.003-1.7
Pigs	14(6)	14(4)	0.22*	1.04	0.22-5.55

p-values of Fisher's exact test, odds ratios (OR) and 95% confidence intervals

(CI), Inf-refers to infinite number

Note: *These values had Fisher's exact p-

value ≤ 0.3 and were identified as risk factors for inclusion in the multivariable

analysis.

Table 3: Significant risk factors for bovine tuberculosis transmission to cattle as determined in a multi-variable model

Risk factor	Odds Ratio	CI _{95%}	p-value
Movement of animals close to game park	24.19	[11.00 - 531.67]	0.04332*
Cattle sharing water with wildlife	175.36	[131.05 - 690.42]	9.45e 05***

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