Prevalence of *Mycobacterium bovis* infection in traditionally managed cattle at the wildlife-livestock interface in South Africa in the absence of control measures

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Abstract

Cattle are the domestic animal reservoir for *Mycobacterium bovis* (*M. bovis)* which also affects other domestic animals, many wild animal species and humans leading to tuberculosis. The study area is in a resource-poor community that is surrounded by several game parks, where *M. bovis* infection has been previously diagnosed in wildlife. A cross-sectional study was carried out to determine the prevalence of *M. bovis* infection in 659 cattle from a total of 192 traditionally managed herds using the BOVIGAM® interferon gamma assay (IFN-γ). Infection was confirmed by post mortem examination and *M. bovis* isolation from three test-positive cattle. Genotyping of the *M. bovis* isolates was done using spoligotyping and VNTR (variable number of tandem repeats typing). The apparent *M. bovis* prevalence rate in cattle at animal level was 12% with a true population prevalence of 6% (95 % Confidence interval (C.I) 3.8 to 8.1) and a herd prevalence of 28%. Spoligotyping analysis revealed that the *M. bovis* isolates belonged to spoligotype SB0130 and were shared with wildlife. Three VNTR profiles were identified among the SB0130 isolates from cattle, two of which had previously been detected in buffalo in a game reserve adjacent to the study area. The apparent widespread presence of *M. bovis* in the cattle population raises a serious public health concern and justifies further investigation into the risk factors for *M. bovis* transmission to cattle and humans. Moreover, there is an urgent need for effective bTB control measures to reduce infection in the communal cattle and prevent its spread to uninfected herds.

Keywords: bovine tuberculosis (bTB); cattle; *Mycobacterium bovis (M. bovis)*; wildlife-livestock interface

Introduction

Mycobacterium bovis causes chronic bacterial disease in cattle known as bovine tuberculosis (bTB); it also affects other domestic animals, wildlife animals, and humans leading to tuberculosis (Michel et al. 2010). Although cattle are known as the primary host, *M. bovis* has been detected in some wildlife

reservoirs such as the African buffalo (*Syncerus caffer*) in South Africa and the Zambian Kafue lenchwe antelope *(Kobus leche kafuensis)* in Zambia (Michel et al. 2006; Renwick et al. 2007; Munyeme et al. 2010). In South Africa *M. bovis* has been diagnosed in 21 wildlife animals that include leopards, cheetahs, lions, wild dogs, bush pigs and chacma baboons (Michel et al. 2006; Renwick et al. 2007).The disease indirectly results in high economic losses for buffalo ranchers, affects wildlife conservation (e.g. spill-over to lions (*Panthera leo*)), poses a threat to human health especially in HIV/AIDS patients and is a threat to sustainable livestock farming (Ayele et al. 2004; de Garine-Wichatitsky et al. 2013; Caron et al. 2014).

In most African countries including South Africa control programs are absent or inadequate especially in communal cattle due to a lack of resources and commitment from the relevant stakeholders (de Garine-Wichatitsky et al. 2013). The disease is of particular significance in Africa where a herd prevalence in cattle of up to 50% has been recorded in Zambia, Uganda and in Ethiopia (Oloya et al. 2007; Munyeme et al. 2009; Dejene et al. 2016). The presence of bovine TB wildlife reservoirs serves as a potential risk factor to cattle and communities living at the wildlife-livestock-human interface (Thoen et al. 2006; Michel et al. 2010; Miller 2015). The spill-back and spill-over of bovine tuberculosis at the wildlife-livestock interface has been documented in South Africa in the Kruger National Park and Hluhluwe-iMfolozi Game Park (HiP) respectively, and this may be due to shared resources such as contaminated pasture and direct contact with infected herds (Hlokwe et al. 2014; Musoke et al. 2015).

The human population is exposed to *M. bovis* infection through consumption of undercooked meat, unpasteurised milk (fermented milk), direct contact with infected animals and the situation is exacerbated by poverty, the HIV epidemic and reduced access to health care (WHO 2006; Gumi et al. 2012a; Welburn et al. 2015). South Africa has been categorized by the World Health Organisation (WHO) amongst countries with a high TB incidence. The country has a high HIV prevalence, estimated at 14% in the general population and HIV positive persons are 27 to 37 times more likely to contract tuberculosis than those who are HIV negative (WHO 2013; Human Sciences Research Council (HSRC) 2018).

Despite the importance of the disease in livestock and as a zoonotic threat in South Africa, bovine tuberculosis in cattle managed under a traditional communal grazing system also referred to as communal cattle, is largely unmonitored and uncontrolled. Hence, its prevalence and transmission between domestic and wild animal populations in the most vulnerable and marginalized communal areas are not well understood. It was, therefore, the aim of this study to estimate the prevalence of *M. bovis* infection in communal cattle, confirm the cause of infection, as well as to investigate its similarity with strains previously isolated at the wildlife-livestock interface. In addition, cattle that were identified according to the farmers' perception of respiratory signs were tested using the interferon gamma assay to determine the usefulness of farmer's syndromic diagnosis in detecting *M. bovis* infected cattle. The association between gender and *M. bovis* infection in cattle was also explored.

Materials and methods

Study area

A cross-sectional study was carried out at four dip tanks in the Big 5 Hlabisa Municipality (uMkhanyakude District) in the northern part of KwaZulu-Natal in September 2016 (Mpempe and Nkomo dip tanks) and March 2017 (Nibela and Masakeni) (Fig. 1). Cattle from several villages are registered at a dip tank where animals gather for diseases control programs in weekly or fortnightly intervals depending on the season. Each dip tank served as an epidemiological unit (a dip tank is considered a whole herd because animals in a dip tank interact during grazing, at watering points and dip tank inspections). The study area is surrounded by provincial and private game reserves such as the iSimangaliso Wetland Park (formerly St Lucia), Munyawana (formerly Phinda), Hluhluwe-iMfolozi Park and Mkhuze Game Reserve. This is defined as the wildlife-livestock interface with an overlap in the rangelands of wildlife and livestock.

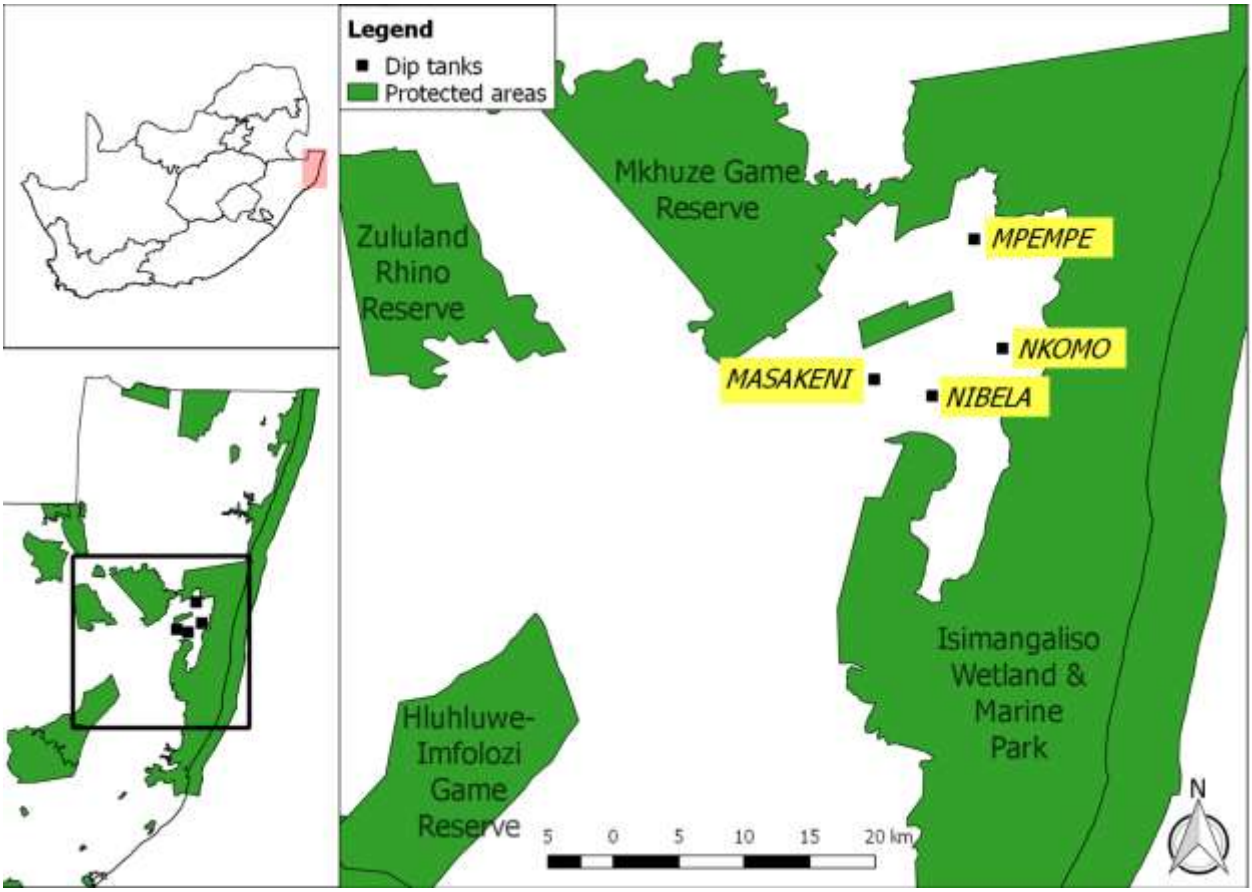

Figure 1: Map of the study area that is surrounded by conservation areas as shown on the map. The four dip tanks (Mpempe, Masakeni, Nkomo, Nibela) in Big 5 False Bay Municipality, uMkhanyakude District, northern KwaZulu Natal

Animals

Animals that were used in this study belonged to communal farmers practicing traditional (communal) farming. The animals were kept under the extensive management system where they freely mix and graze in communal pastures and share watering points. The animals are kept in enclosures known as “kraals” at night, but during periods of drought or the dry season the animals may be left out for months or moved

outside the communal area in search of grazing. During these periods there is no supplementary feeding and the animals receive little or no veterinary inspections.

Sample size

The cattle population at the four dip tanks was 7 836 from a total of 456 owners as calculated based on stock cards (W. McCall personal Communication, 21 June 2016). There were 2 345, 2 233, 2 025 and 1 235 from Mpempe, Nibela, Masakeni and Nkomo dip tank respectively. The sample size for the cross-sectional prevalence study was calculated for each dip tank using the simple random sampling formula according to (Thrusfield 2007).

A 95% confidence limit (CL) at 5% level of desired absolute precision and expected prevalence of 15% estimated from a pilot study carried out using cattle from one of the dip tanks in the study area (Nibela) (A.L. Michel, unpublished data,2015) were used for sample size calculation. The desired sample size was calculated after adjusting the sample size using the formula for finite population according to (Dohoo et al. 2009). The final number of animals that included a surplus of 9 for each dip tank were as follows; Masakeni (205), Mpempe (205), Nibela (205) and Nkomo (185), adding to a total of 800 animals.

Sampling procedure

Systematic random sampling was used to identify animals for the individual animal and herd prevalence at the dip tanks. For random sampling, at each dip tank, all cattle presented per herd were moved into the cattle crush and every 5^{th} animal was selected for the study excluding all the calves $\leq$ 6 months. In herds with fewer than 10 cattle, every 3^{rd} animal was selected.

Additional samples were collected from cattle identified by the farmers based on his/her perception of clinical signs consistent with respiratory disease. The animals were selected by the farmer if they had at

least one of the following symptoms, chronic coughing, labored breathing and/or lagging behind the herd during physical exercise. This was termed syndromic sampling.

Interferon gamma assay (IFN-γ) BOVIGAM®

Whole blood samples were drawn from identified animals into heparinized sterile tubes, gently mixed, maintained at below 25^{o} C until stimulated within 8hrs of collection. The assay was performed according to the modified interferon gamma assay as previously described by (Michel et al. 2011). Detection of IFN-γ in the plasma was achieved using the sandwich enzyme-linked immunoassay plates supplied by the manufacturer (Thermo Fisher Scientific, South Africa). Optical density (OD) values for the plasma were measured according to the stimulating agent i.e. purified protein derivatives (PPD); avian (PPD) (OD_{av}), bovine PPD (OD_{bov}), fortuitum PPD (OD_{fort}), poke weed mitogen (PWM) (OD_{pwm}) and Roswell Park Memorial Institute Medium 1640 (RPMI) (OD_{nil}). The interpretation of the results for reactor classification was according to the criteria described by (Michel et al. 2011). Animals with $OD_{bov} > 0.385$ and $OD_{bov} - OD_{av} > 0.20$ were classified as bovine reactors (bTB positive). Reactors with $OD_{av} > OD_{bov}$, or $OD_{bov} - OD_{av} < 0.1$ were considered negative.

Post mortem examination

Three *M. bovis* positive reactor animals were selected and purchased from the owners for slaughter from Nibela, Mpempe, and Masakeni dip tanks, respectively. *M. bovis* infection was confirmed through post mortem examination that involved the careful examination of the lungs, liver, spleen, kidney and mammary glands for visible lesions, followed by excision and thin slicing of the mandibular, retropharyngeal, mediastinal, hepatic, mesenteric and supra mammary lymph nodes. The size of the bTB like lesions, consistency and degree of infection were noted. Relevant samples of at least 10g were collected from suspected tissues and placed into sterile plastic containers that were packed into sterile zip

lock bags. These were frozen and transported to the University of Pretoria-Department of Veterinary Tropical Disease BSL2+ laboratory ensuring that a cold chain was maintained for microbiological analysis.

Mycobacterium bovis culture and identification

The tissue samples were processed and decontaminated with 4% NaOH and 2% HCl as described by (Alexander et al. 2002) . The samples were inoculated on three slants of Löwenstein-Jensen (LJ) media supplemented with pyruvate and incubated at 37° C for up to 10 weeks (Alexander et al. 2002). Preliminary identification of *Mycobacterium tuberculosis* complex positive cultures were defined by slow growth on LJ pyruvate media, colony morphology and Ziehl-Neelsen staining to confirm the presence of acid-fast positive bacilli (AFB). Genomic DNA extraction from the isolates was done by boiling in 100µl of double distilled sterile water using a heating block at 95°C for 25 minutes (Hlokwe et al. 2013). Deletion analysis (Region of difference-RD) was used for *M. bovis* identification using the primers RD1, RD4 and RD9 with DNA amplification products of 146bp, 268 bp and 108 bp respectively, as previously performed by (Warren et al. 2006).

Sample collection and processing from African buffalo

Tissue samples were collected from bTB positive buffalo detected in the annual bTB monitoring program from 2012- 2017 in HiP by the Ezemvelo KwaZulu Natal Wildlife. Relevant samples were collected at post mortem, frozen and transported to the Department of Veterinary Tropical Diseases-University of Pretoria using a cold chain for microbiological analysis. *M. bovis* isolation and identification was carried out using the same procedures as described before for cattle tissues (Alexander et al. 2002) and isolates were characterised using spoligotyping (Kamerbeek et al. 1997) and Variable number of tandem repeats (VNTR) typing (Hlokwe et al. 2013).

Molecular characterisation

Spoligotyping

Spoligotyping assay was performed using a commercially available kit (Mapmygenome India Ltd.) to genotype the mycobacterial DNA as previously described by (Kamerbeek et al. 1997). Spoligopatterns obtained were referenced according to the international spoligotyping database (www.Mbovis.org). The spoligotype numbers were assigned according to the information described in the database.

Variable number of tandem repeats-Mycobacterial Interspersed Repetitive Units (VNTR-MIRU)

VNTR typing of the *M. bovis* isolates from cattle and wildlife was performed as described by (Hlokwe et al. 2013). A set of 13 tandem repeats loci were used, previously identified as stable and suitable for South African isolates namely ETR A, B, C, and E; Qub 11a, b, 18 and 26, MIRU 16, 23 and 26 and Mtub 12, M tub 21 (Inqaba Biotec, South Africa).

Statistical Analysis

Laboratory results were coded and stored in an Excel database before being exported to R for prevalence analysis using the © R Foundation for Statistical Computing, 2014. The apparent prevalence of *M. bovis* reactors was defined as the number of positive reactors divided by the total number of cattle that were tested at each dip tank. True prevalence was calculated using the formula, $TP = \frac{(AP+SP-1)}{(SE+SP-1)}$ (1)

whereby TP is true prevalence, AP the apparent prevalence, SE is sensitivity, and SP is specificity as stated by (Dohoo et al. 2009) . Interferon-gamma assay (IFN-γ) assay test performances that were used i.e. SE and SP were estimated using the values calculated in the pilot study mentioned before (A.L. Michel, unpublished results, 2015). The values of the SE and SP and their 95% confidence interval were as follows: SP: 0.912% [0.876% – 0.948%] and SE: 0.620% [0.496% – 0.746%]. The value of SE and SP were used to calculate the true prevalence and using all possible combination of the different limits of

their confidence intervals we defined an "error interval" for the true prevalence that considered the uncertainty around the real value of SE and SP.

The 95% confidence interval for true prevalence for extrapolation at the population level was calculated using the following formula,

$$TP_{pop} = TP_{sample} \pm 1.96 * \sqrt{\frac{TP_{sample}*(1-TP_{sample})}{n'}} \quad (2)$$

Where TP_{pop} is the true population prevalence, TP_{sample} is the true sample prevalence and n' is the size of the sample calculated taking into consideration that we have a clustered sample as stated by (Dohoo et al. 2009). To calculate n' the following formula

$$n' = \frac{n}{1+(m-1)*\rho} \quad (3)$$

where using a conservative approach, we chose the biggest value for the cluster size (m) =5 and an intra-cluster coefficient (ICC) ρ of 0.1 working on the conservative assumption that bovine TB transmission occurs at the same level as brucellosis within a cattle herd (43, 44). Considering the uncertainty of SE and SP we also applied the equation (2) to the minimum and maximum of the error interval calculated for the true sample prevalence. We obtained a confidence interval for both values from which we kept only the lower limit of the minimum and the upper limit of the maximum to obtain an "error interval" at population level reflecting the uncertainty on SE and SP. Herd prevalence with a 95% confidence interval , was calculated as the number of farmers with at least one test positive animal divided by the total number of farmers. The Chi-square test (x^2) was used to compare the prevalence of the four dip tanks and the Fischer exact test was used to compare the prevalence of the random sample and the syndromic sample and to assess the association between sex and *M. bovis* infection with p values < 0.05 were considered statistically significant. Logistic regression was used to investigate the effect of diptank (location) and gender on *M. bovis* prevalence in cattle. The herd was considered as the random effect

whereas sex and dip tank were considered as the fixed effects. The stepwise backward approach based on AIC (Akaike Information Criterion) was used to select the final model.

Results

Cattle population

A total of 722 cattle were tested at four dip tanks using the interferon gamma assay. These were 139, 248, 142 and 130 cattle at Nkomo, Mpempe, Nibela and Masakeni dip tanks, respectively, randomly selected for bTB testing, leading to a total of 659 cattle that consisted of 488 females and 171 males from 192 herds. Syndromic sample collection was carried out from a total of 63 cattle made up of 17, 14, 15 and 17 cattle from Nkomo, Mpempe, Nibela and Masakeni dip tanks, respectively. The number of animals brought to the dip tanks by the owners was less than the calculated sample sizes due to the drought prevailing in the area which prompted farmers to move cattle to more suitable grazing areas outside the study area.

Interferon gamma assay (IFN-γ)

The overall apparent animal prevalence from the random samples was 12 % whereas the true sample prevalence was 6%. The apparent and the true prevalence at animal level and at population level estimates at the four dip tanks are shown in Table 1. There was a significant difference in the apparent prevalence of *M. bovis* reactors at the four dip tanks as shown by the (x^2) test of significance (p = 0.012).

Table 1. Apparent and true prevalence values of *M. bovis* infection in cattle at the four dip tanks at animal level and population level estimates of true prevalence

Dip tank name	AP_{sample} (%)	TP_{sample} (%)	TP_{sample} (error interval)	$TP_{population}$ (95% confidence interval)	$TP_{population}$ (error interval)
Mpempe	17.3	16	[7.9-27.3]	[10.6-21.4]	[3.9-33.8]
Masakeni	8.5	0	[0-7.4]	-	[0-12.8]
Nibela	16.9	15.2	[7.2-26.4]	[8.2-22.2]	[2.2-36.9]
Nkomo	7.9	0	[0-6.1]	-	[0-11.1]
Total	12	6	[0-15.3]	[3.8-8.1]	[0-18.5]

Where AP_{sample} -Apparent sample prevalence, TP_{sample}-True sample prevalence, $TP_{population}$-True population prevalence

Out of 63 animals identified by the farmers during syndromic sampling 10 animals were *M. bovis* positive reactors using the interferon gamma assay. The use of clinical signs had a low sensitivity of 16% $[6.8\% - 24.9\%]_{95\%}$ as compared to the 62% for interferon gamma test.

An overall herd prevalence of 28% [95% CI, 21.3-34%] (53 out of 192) was found. The dip tank herd prevalences were: Nibela 42% [95% CI, 25.6-57.8%); Mpempe 39% [95% CI, 25.3-52%], Masakeni 18 % [95% CI, 7.8-27.9%] and Nkomo 17 % [95% CI, 6-27.3%]. There was a significant difference in the herd prevalences at the four dip tanks (x^2) test of significance ($p = 0.007$).

Based on sex the apparent prevalence for females and males was 14% (68 out of 488) and 12% (21 out of 171) respectively, with no significant association between sex and *M. bovis* infection (Fisher's exact test: $p = 0.107$). Nevertheless, at Mpempe dip tank there was a significant difference in infection according to gender with more females being infected than males (Fisher's exact test: $p = 0.013$).

The final model chosen as having the lowest AIC (Akaike Information Criterion) only included the fixed factor dip tank and Nkomo dip tank was used as the reference level. According to the logistic regression there was a statistically slightly significant difference between Mpempe and Nkomo dip tank ($p<0.1$) and a significant difference between Nibela and Nkomo dip tank ($p<0.05$) as shown in Table 2. There was no significant difference between Nkomo and Masakeni dip tanks. The logistic regression also confirmed that sex was not a risk factor.

Table 2: Relationship between dip tank (location) and *M. bovis* prevalence using logistic regression (Nkomo dip tank used as a reference level, °p value≤0.1, *p-value≤0.05)

Dip tank name	O.R(Odds ratio)	p-value
Mpempe	2.34	0.09°
Masakeni	1.20	0.75
Nibela	2.87	0.05*

Post mortem findings

Macroscopic lesions typical of bTB of different sizes and numbers were observed in all the affected organs that had been collected from all the slaughtered animals with one of the animals presenting with generalized tuberculosis. The lesions in the organs appeared yellowish with granulomas that had central caseous necrosis and mineralization. In other cases, encapsulated nodules containing yellowish white exudates were also observed.

***Mycobacterium bovis* culture and identification**

A total of 12 isolates were obtained from the three slaughtered animals from pooled samples from the lungs, kidneys, tonsils, udder, mammary glands and associated lymph nodes including retropharyngeal,

mesenteric, mediastinal, popliteal, prescapular and inguinal lymph nodes. The acid-fast bacilli were classified as *M. bovis* using deletion analysis for *Mycobacterium* species differentiation.

Spoligotyping

Spoligotyping confirmed the species identification of the 12 isolates from cattle and 22 from wildlife as *M. bovis*. All the isolates showed identical profiles belonging to the SB 0130 spoligotype, which is characterised by the absence of spacers 3, 9, 11, 16, 39-43. This is one of the 5 spoligotypes that have been described to occur widely in KwaZulu Natal in both cattle and buffalo from the HiP (Hlokwe et al., 2014).

Variable number of tandem repeats (VNTR)

In Figure 2 the results of the VNTR analysis of 12 *M. bovis* isolates from the slaughtered cattle were compared with a subset of *M. bovis* isolates cultured from 8 buffaloes and one baboon from game reserves surrounding the study area. A total of 7 VNTR profiles labelled 1-7 (shown on Figure 2) were revealed based on the 13 loci MIRU-VNTR panel with two profiles being shared by buffalo and cattle (VNTR 1 and 4); three profiles were observed exclusively in buffalo (VNTR 3,6 and 7), one in the baboon (VNTR 2) and one in cattle (VNTR 5). The most common VNTR profile was found in 13 of the isolates shared between buffaloes and cattle. It is interesting to note that all cattle (BSL 358, 359, 360) shared the same VNTR profiles (VNTR 4) although they belonged to three different dip tanks. Animal BSL 358 from Mpempe dip tank yielded 3 different VNTR profiles with 2 (VNTR1,4) being identical to profiles detected in buffaloes.

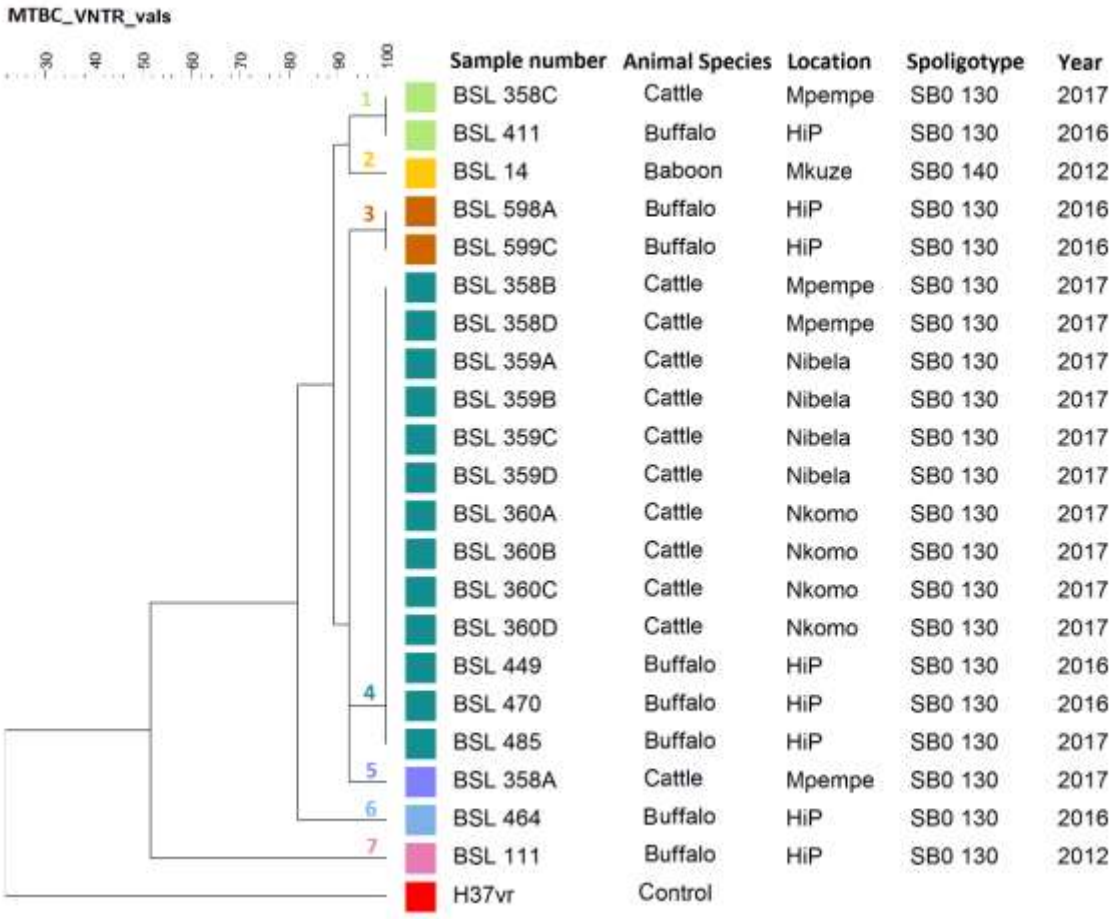

Figure 2: UPGMA tree depicting VNTR profiles of isolates from communal cattle at the 4 dip tanks in Big 5 False Bay Municipality and buffalo from Hluhluwe-iMfolozi Park (HiP). The animals are identified by a BSL number; letters refer to organ type. Colours differentiate the VNTR profiles and the corresponding numbers are labelled on the tree (1-7). Dip tanks: Mpempe, Nkomo, Nibela; VNTR-variable number tandem repeat

Discussion

The study investigated the prevalence of *M. bovis* infection in cattle in a traditional farming community at the wildlife-livestock interface where bTB control measures are absent. It also determined the usefulness of syndromic detection of bovine tuberculosis in cattle by farmers. The presence of *M. bovis* was confirmed through post mortem, isolation and spoligotyping of the pathogen from tissue samples.

An apparent bTB prevalence of 12 % was detected which is considerably higher than that described at the wildlife-livestock interface in Zambia (6.8%), Tanzania (2,4%) and in Ethiopia (3.4 %) (Munyeme et al. 2009; Tschopp et al. 2010; Katale et al. 2013). Differences in farming practices, cattle breeds, production systems and agro-ecological zones may account for the inter-study variations. The sensitivity of syndromic (respiratory signs) diagnosis was lower than when using the interferon gamma test (16 % versus 62 %). This suggests that even in the presence of advanced cases of bTB disease as observed at post mortem and the owners' evaluation of a change in an animal's health status, respiratory signs are an unreliable indicator of bTB. However, the low sensitivity in syndromic diagnosis can also be attributed to few animals showing respiratory signs during most stages of the disease except in advanced cases (Ayele et al. 2004; World Organisation for Animal Health (OIE) 2009).

Although the overall observed herd prevalence of 28% was lower than that reported in Zambia and other African countries, it is a significant indicator of widespread infection in the study area (Oloya et al. 2007; Munyeme et al. 2009; Tschopp et al. 2009; Katale et al. 2013; Dejene et al. 2016). Significantly different individual animal prevalences were detected at the four dip tanks, implying different levels of *M. bovis* exposure. The observed significant differences indicate variations in disease transmission opportunities within dip tanks influenced by recognized risk factors such as the local *M. bovis* burden of the pastures, herd size, overcrowding on the communal pastures, at watering points and the mixing of animals at the dip tank (Cosivi et al. 1998; Shirima et al. 2003). Equally applicable are risk factors such as different types of drinking water sources and uncontrolled cattle movement which has been shown to increase the likelihood of meeting infected cattle or wildlife resulting in exposure to infection (Phillips et al. 2003; Oloya et al. 2007).

There was no significant association between sex and *M. bovis* infection except at Mpempe dip tank, where more females were infected than males. This is in line with other studies carried out in Tanzania, Ethiopia and Nigeria whereby susceptibility to bTB is not gender related (Mfinanga et al. 2004; Cleaveland et al. 2007; Gumi et al. 2011, 2012b; Katale et al. 2013). Significant differences in *M. bovis*

prevalence have been reported as a result of farming practices i.e. the sharing of a bull by a community (Humblet et al. 2009). It has been observed in other studies that the long lifespan of cows and bulls, either in the same or different herds (following exchange), can actively contribute to prolonged *M. bovis* exposure and dissemination (Kazwala et al. 2001; Mfinanga et al. 2004).

Studies have highlighted that most cattle owning households in KwaZulu Natal province consume milk as fermented milk (amasi) on a daily basis and this represents a risk of exposure to *M. bovis* as it was demonstrated in another study in the same province that *M. bovis* may survive in sour milk (Michel et al. 2015). Moreover, the high HIV prevalence of over 15% that was reported by the Health Barometer for the province during 2015/2016 period means the communities are considered more vulnerable to opportunistic infections such as zoonotic TB (Bezerra 2010; Massyn et al. 2016). Further investigation into the role of *M. bovis* in human TB and associated risk factors is necessary.

Bovine TB in cattle also poses a significant risk to wildlife health with adverse effects on conservation and ecotourism as well as livelihoods of private game ranchers in general, but in particular in the vicinity of the study area which is a prime game ranching and tourist destination (de Garine-Wichatitsky et al. 2013). Previous studies have isolated the spoligotype SB0130 in cattle and buffalo and demonstrated its dominance in KwaZulu/Natal (Hlokwe et al. 2014). The detection of identical VNTR profiles (VNTR 1 and 4) in cattle and buffalo strongly indicates an exchange of *M. bovis* between wildlife and cattle. The chronic nature of the lesions in combination with the detection of three VNTR types in three cows and additional VNTR types in wildlife (Hlokwe et al. 2011), suggests that *M. bovis* has been circulating endemically in and possibly between cattle and buffalo populations. Therefore, the high bTB prevalence demonstrated in cattle warrants further investigation into alternative, practical and affordable control measures for *M. bovis* infection in cattle and wildlife.

Conclusion

The findings of the study have highlighted the consequences of uncontrolled *M. bovis* infection in communal cattle. Identical spoligotypes and strains of *M. bovis* isolates from cattle and wildlife indicates transmission between livestock and wildlife animals There is a need for further investigation of zoonotic TB transmission to humans and the assessment of the risk factors for bTB transmission to cattle and humans.

Declarations

Ethical consideration

This study was carried out with approval from the University of Pretoria –Animal Ethics Committee (**V078-16**) and the Department of Agriculture, Forestry and Fisheries under The Section 20 (**12/11/1/1/6/1**). Verbal and oral consent through signed forms was given by the farmers.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Author's contribution

Conceptualization of the study, methodology, interpretation of the data and the writing of the manuscript-Anita Michel, Eric Etter, Petronillah Sichewo

Investigation, sample collection and laboratory work-Anita Michel, Petronillah Sichewo

Data analysis: Eric Etter, Petronillah Sichewo

Funding acquisition: Anita Michel

Supervision: Anita Michel, Eric Etter

All authors read and approved the final manuscript

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