# Preliminary assessment of bovine tuberculosis at the livestock-wildlife interface in two protected areas of Northern Botswana.

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**Place where the work was carried out:** Botswana

## Abstract

Protected areas from Northern Botswana such as the Okavango Delta (OD) or Chobe National Park (CNP) are well known hot spots for the conservation of African wildlife. However, their infection status regarding bovine tuberculosis (BTB) at the domestic-wildlife interface has never been investigated. In order to provide preliminary baseline data on the circulation of *Mycobacterium bovis* in those sites, we performed a cross sectional survey in 130 buffalo in both protected areas (60 individuals from CNP and 70 from OD) and 818 cattle at the wildlife/livestock interface surrounding both conservation areas (369 in CNP and 449 in the OD). Whole blood samples were tested using a commercial interferon gamma assay (IFN-ɣ ) with modifications. The apparent BTB prevalence in buffalo was nil in CNP and 0.7 % $_{95\% CI}$ [0.2-1.9] in the OD, while the apparent BTB prevalence in cattle was 0.7 % $_{95\% CI}$ [0.2-2.1] in OD and 2.4 % $_{95\% CI}$ [1.2-4.7] in CNP. True prevalence values calculated on the basis of the locally applicable IFN-ɣ test performance suggested that BTB prevalence was nil in both buffalo populations and in cattle from the OD interface but reached 2.3 % $_{95\% CI}$ [0.2-4.5] in the cattle populations around CNP. The results of a questionnaire survey conducted among a sample of farmers living in the communities adjacent to each conservation area (97 and 38 persons in OD and CNP, respectively) suggested a higher risk for the circulation of *M. bovis* at the wildlife/livestock interface of the CNP than that at the one of the OD.. However, further comprehensive studies are needed to confirm the circulation of *M. bovis* and to monitor the inter-species and transboundary transmission of BTB in Northern Botswana.

**Key words**: *Mycobacterium bovis*, risk, prevalence, wildlife/livestock interface, interferon gamma assay, *Syncerus caffer,* Botswana

**Introduction**

*Mycobacterium bovis*, the causative agent of bovine tuberculosis (BTB) in cattle has shown the ability to infect a large diversity of natural populations of non domesticated species worldwide. Several cases of reservoirs of *M. bovis* following transmission from cattle to wildlife have been described in developed countries in the UK (Vicente et al., 2007, Delahay et al., 2007), the Iberian peninsula (Vieira-Pinto et al., 2011, García-Bocanegra et al., 2012), North America (Wobeser, 2009, O'Brien et al., 2011) and New Zealand (Nugent, 2011), jeopardizing efforts for the eradication of BTB in cattle.

The African continent, with its high densities in large mammals, large cattle populations and veterinary services with limited resources represents an ideal scenario for *M. bovis* spreading from domestic cattle to wildlife species such as the African buffalo (*Syncerus caffer*). The spillover of BTB from cattle to wildlife has already been described on several occasions affecting a diversity of wildlife species in a wide range of protected areas of Sub-Saharan Africa: In East Africa, BTB has been reported in a wide range of ungulate species in the Serengeti and Tarangire ecosystems (Cleaveland et al., 2005). In South Africa, contacts between livestock infected with *M. bovis* and wildlife have resulted in infections and spread of BTB among buffalo populations from Hluhluwe –Imfolozi (Jolles et al., 2005) and Kruger National Park (Rodwell et al., 2001). In the latter case, the progressive spread of the disease from the south to the north of the park resulted in the introduction of *M. bovis* into buffalo populations from Gonarezhou National Park in neighboring Zimbabwe (De Garine-Wichatitsky et al., 2010). In Zambia, a large proportion of the free-ranging population of Kafue Lechwe (*Kobus lechwe kafuensis*), a resident aquatic antelope in the swamps of Central Zambia, is reported to be infected with BTB with prevalences higher than 25% (Munyeme et al., 2010).

The Okavango Delta (OD) and Chobe National Park (CNP), are two well known areas for wildlife conservation and upmarket wildlife tourism destinations in Southern Africa, hosting for instance the largest African elephant population in the world (Darkoh and Mbaiwa, 2009). In 2011, the OD and CNP were integrated as core areas of the Kavango Zambezi TFCA, one of the largest complexes of protected areas in Southern Africa. The development of these transfrontier conservation initiatives allows better connectivity between wildlife territories from different countries on the one hand, but on the other hand also open up excellent opportunities for pathogens to move through borders and meet new and diverse host populations and pose huge challenges for the control of diseases at the livestock-wildlife interface (Bengis, 2005). Wildlife from protected areas in Northern Botswana has never been investigated for the presence of BTB. Despite the lack of a systematic surveillance program for BTB in cattle farmed adjacent to those areas, animal health authorities have performed occasional annual TB testing of cattle in the study area using the skin test (ICTT), without reports of positive results (unpublished reports). The main national abattoirs in the centre of the country (Lobatse and Francistown) perform routine lung inspections in cattle and have not reported any positive cases and the disease is officially considered absent from the national cattle population of Botswana. However, cattle from Northern Botswana are only marketed to local butcheries where there are no official veterinary inspections.

Therefore, the goal of this study was to generate baseline data on the epidemiological situation of BTB at the livestock-wildlife interface of the OD and CNP at the beginning of their integration within the larger KAZA TFCA. For that purpose, a cross sectional survey of BTB was implemented in buffalo and livestock populations from both areas. In addition, a semi-structured questionnaire to assess and compare the cattle management characteristics contributing to the presence of BTB were conducted among cattle farmers in both study areas.

**Figure 1:** Location of the study areas and sampling sites for buffalo and cattle populations.
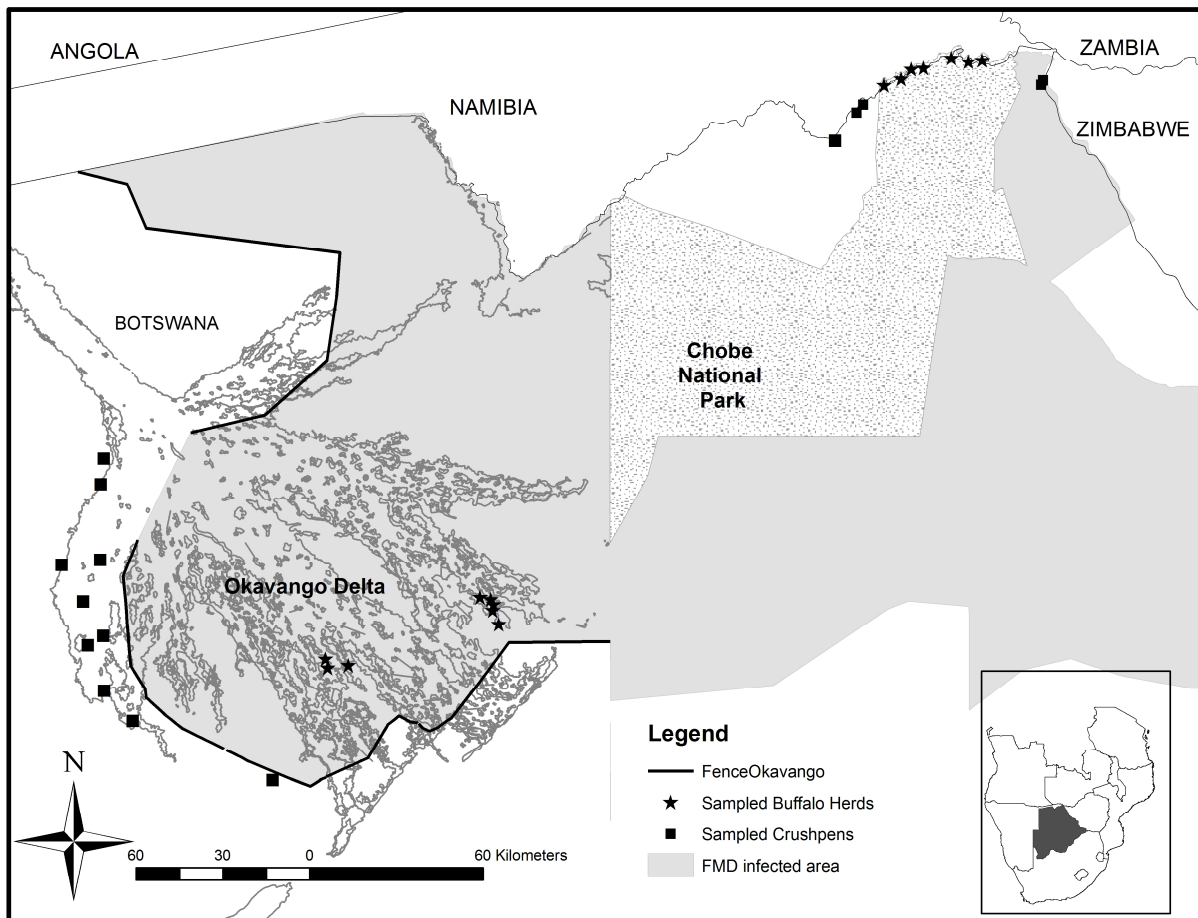


## Material and Methods

*Study areas*

The OD and CNP are located in two different districts of Northern Botswana (Ngamiland and Chobe Districts, respectively) and represent the largest wildlife areas in this part of the country. They are both integrated in the Foot and Mouth Disease Infected area, a large part of the northern region of Botswana devoted to wildlife conservation which separates buffalo populations from the primary cattle export and buffer zones (Figure 1) by the use of veterinary cordon fences. The Chobe, Zambezi and Okavango rivers are the largest in the region, providing abundant water throughout the year. Rainfall is strongly seasonal, occurring mostly from December to April (wet season).Vegetation consists mainly on deciduous dry

woodland and scattered grasslands. Wildlife abundance is fundamentally depending on rainfall and water availability and varies cyclically throughout the years (Alexander et al., 2012).

The OD is the largest inland delta in the world encompassing 12000 km$^2$ and hosting large populations of wildlife including an estimated total of 31 500 buffaloes; An additional population of 7 500 individuals has been estimated for the CNP, according to the latest census data (Chase, 2011). The OD is delineated from livestock areas by a double veterinary cordon fence to prevent contacts between large mammals (particularly buffalo) and cattle (Darkoh and Mbaiwa, 2009). The CNP encompasses 10 700 km2 of savannah grassland. The boundaries of CNP are natural - The Chobe river in the north constituting the natural border between Botswana and Namibia- and there is no physical separation between cattle and wildlife.

*Buffalo study design*

In buffalo herds, the sampling process was opportunistic, depending on the ability of finding herds from the air. Buffalo herds were located from a helicopter, separated in smaller groups (5-10 individuals) and then darted in quick succession using a combination of 8 mg etorphine hydrochloride (M99, Novartis, South Africa), 80 mg azaperone (Stresnil, Janssen Pharmaceutical Ltd., South Africa) and 1500 IU hyaluronidase (Hyalase, Kyron Laboratories, South Africa) in adults and 6mg etorphine Hydrochloride and 60mg azaperone with 1500 IU hyaluronidase in sub-adults. All animals were positioned in sternal recumbence as soon as the ground teams moved into the capture to collect the samples. Blindfolds were placed on all buffaloes and dart wounds were treated with 200 mg cephalexine (Rilexine 200 LC Virbac Animal Health, S.A.). Once all sampling procedures were completed the buffaloes were simultaneously reversed with a combination of 50 mg naltrexone (Naltrexone, Kyron Laboratories, South Africa) and 12 to 16 mg of diprenorphine (M50:50, Novartis, South

Africa) depending on the age. In this manner, a total of 130 buffaloes were captured and sampled in the OD (n=70) and in the northern area of CNP (n= 60) in October 2010. The animals in the OD were captured in two different sites: Moremi Game Reserve (a total of 36 animals, belonging to 4 different herds) and in Khurunxagha (34 animals, 4 herds). Sampled individuals were sprayed with paint to avoid re-sampling of the same individuals or groups.

*Cattle study design*

The cattle population is estimated to be around 400 000 heads in every district, consisting of indigenous cattle breeds reared under extensive communal management practices. As in many other countries in Southern Africa (Jori et al., 2009), livestock herds sharing the same grazing lands in Northern Botswana congregate regularly (approximately twice a month) at crush pens. The average number of cattle herds in a crush pen ranges between 200 and 1 000 animals. Crush pens are also used by the Botswana animal health authorities to undertake preventive and control measures against notifiable animal diseases such as foot and mouth disease (FMD) or contagious bovine pleuropneumonia (CBPP). Cattle herds brought to the crush pens cohabitate and share the same grazing spaces and are managed under the same grazing strategy, which has been identified as a significant risk factor of BTB in East and Southern Africa before (Oloya et al., 2007, Munyeme et al., 2008, Munyeme et al., 2009). Crush pens were hence considered as distinct epidemiological units.

In Chobe district, most of the surface is devoted to the CNP and the total surface for cattle grazing is limited. A total of 10 254 cattle heads (approximately 350 herds) are regularly mustered for dipping at 19 crush pens. Eight of those crush pens are located within a range of 15 km from the boundaries of the CNP. The Ngamiland district represents a much larger territory and encompasses a total 51 000 cattle heads (approximately 1000 herds) distributed in 391 crush pens (Ministry of Agriculture, 2010). Thirty six of those are within less than 15 km from the veterinary cordon fence surrounding the OD.

In both districts, crush pens to be sampled were chosen according to their proximity to the park boundaries (less than 15 km) and the availability of the National Veterinary Services teams to operate in the area during their routine FMD vaccination campaign. In this manner, 10 crush pens were selected in OD interface and 6 in the one from CNP.

The sample size was calculated with a 95% significance level ($\alpha$=0.05) a 5% margin of error for the calculation of individual animal prevalence and a 15% margin of error for the calculation of herd prevalence. Since there was no previous information available on BTB prevalence in Botswana, we used the overall prevalence values observed in neighboring Zambia in recent years: 6.8% of individual animal prevalence and 36.8% herd level prevalence (Munyeme et al., 2009, Munyeme et al., 2008). The required sample size to estimate the prevalence at the interface of every region (CNP and OD) was equivalent to 89 individuals and 40 herds. The sampling strategy was designed in order to screen a maximum number of cattle herds per cush pen, on the premise that several animals in the herd would test positive to the interferon gamma assay (IFN-ɣ ) if the herd was infected . Therefore, in order to maximize the chances of detecting *M. bovis* circulating at low levels among the cattle herds, 10 herds were chosen randomly in each crush pen and then 5 to 10 animals sampled per herd. In this manner, a total of 449 samples from 100 herds belonging to 10 crush pens were collected in the area adjacent to OD in April 2010 and 369 samples from 45 herds belonging to 6 different crush pens were collected CNP interface at the end of April 2011.


*Interferon gamma assay* (IFN-ɣ)

Whole blood samples were drawn from buffalo and cattle by venipuncture of the jugular vein using heparinized sterile vacutainers. They were transported to the nearest local hospital laboratories –Maun in Ngamiland District and Kasane in Chobe District within 8 hours after collection, where whole blood cultures were stimulated with mycobacterial antigens and

incubated at 37 °C for 20-24 hrs. For reasons of increased specificity as described previously, the stimulation was carried out in a triple comparative format and included, apart from commercially available avian PPD and bovine PPD, the in-house produced PPD from *M. fortuitum* (Michel et al., 2011). The following day plasma was harvested and stored at -20°C before being sent to the ARC-OVI laboratory for detection of interferon-gamma using the Bovigam assay (Michel et al., 2011) .

According to the modified protocol of the IFN-ɣ assay, the bovine PPD stimulated plasma samples of all animals were first tested to classify them into either reactor or non-reactor animals with regard to their responsiveness to bovine PPD. Plasma samples measuring an optical density ($OpD_{bovine}$ OpD) of less or equal to 0.38 were classified as negative for BTB without further analysis (Michel et al., 2011). For samples yielding a $OpD_{bovine}$ greater than 0.38 all antigen stimulated plasma samples for the respective donor animal were subsequently tested to discriminate between BTB positive, avian, multiple (Fortuitum) reactor animals and analyzed according to the formulae in Table 1. Briefly, a multiple reactor constituted an animal whose immune response profile resembled that of a bovine reactor with an additional strong immune response to Fortuitum PPD.

**Table 1:** Interpretation criteria for reactor classification in the modified Bovigam test according to Michel *et a*l. 2011.

| Bovine reactor | Avian reactor | Multiple reactor | Equal reactor |
|---|---|---|---|
| OpDbov - ODav > 0.2 and OpDfort - OpDnil ≤ 0.15 | OpDav > (OpDbov+ 0.1 x OpDbov) | OpDbov - OpDav > 0.2 and OpDfort - OpDnil > 0.15 | (OpDbov+ 0.1 x OpDbov) > OpDav > (OpDbov - 0.1 x OpDbov) |

Testing of Fortuitum PPD stimulated plasma in the Bovigam assay has, in particular, proven valuable in situations where buffaloes and cattle experience sensitization of the immune system by environmental, non-tuberculous mycobacteria, which may result in a cross-reactive interference with the specific immune response to *M. bovis* (Grobler et al., 2002, Michel, 2008, Michel et al., 2011). IFN-ɣ test performances ranging between 78.9 % and 81.8 % of sensitivity and 94 and 99% of specificity have been reported for domestic cattle (Michel, A. unpublished results).

**Table 2:** Summary of the questionnaire implemented among cattle farmers in the two study areas in Northern Botswana

**Section 1 : General cattle management**

1. Are you the main person who looks after the cows? (Y/N)
2. How many cattle (bulls, cows and calves) do you own?
3. How many of them are older than 10 years?
4. What breed?
5. Do your cattle graze in a fenced area or in a Communal land?
6. Does it change depending on the season?
7. Do they graze with other stock? cattle ? other species ?
8. How far do your cattle move for grazing (in football fields)?
9. Do you keep your animals in a kraal at night? (Y/N)

**Section 2 : Contacts with wildlife**

1. At what distance National Park boundaries do your cattle graze (in football fields)?
2. Have you seen wildlife grazing outside the park, on your communal land? (Y/N)
3. Which wild animals do you most frequent see grazing outside the park (Buffalo/Kudu/Impala/Wildbeest/warthog)
4. How often does it happen? (times per year? season ?)
5. How long do they stay together? (mns, hrs, days)
6. Do your cattle ever enter the park for grazing? (Y/N )
7. How often (frequency in days, weeks or months)?
8. How long do they stay? (frequency in days, weeks or months)?
9. Do they share water sources with wild animals? (Y/N)

**Section 3: Animal purchase**

1. Did you inherit your cattle or bought them outside? heritage / outside
2. Where do you buy your cattle from? Local market/Community /Outside
3. Have you introduced new cattle into your herd since the last year? (Y/N)
4. If yes, where did you buy the cattle from? Local market/Community /Outside
5. How often do you deworm your cattle? Every 3 month/6 month /year?

*Questionnaire*

Owners of sampled herds were asked to complete a simple questionnaire designed to collect information on herd size and composition, cattle management, contacts with wildlife species in grazing and drinking areas, veterinary care provided to livestock and the origin of purchased cattle. The contents of this questionnaire are summarized in Table 2. The questionnaire was implemented during 20-30 minutes by the veterinary technician undertaking the cattle sampling by "face to face" interviews. The questionnaire was delivered in local language (Tswana). Questions were asked referred to the preceding twelve months. These data were collected to characterize herd habitat use and assess potential risk factors for disease transmission such as contacts with wildlife or purchase of cattle from outside the community. As in other similar questionnaires, contact was defined as wildlife and cattle grazing together in an area of the size of a football field (Jori et al., 2011, Brahmbhatt et al., 2012).

*Data analysis*

Questionnaire data and laboratory results were first coded and stored into a Microsoft Access database. Descriptive epidemiological measures were analyzed using Epi-Info software (CDC, Atlanta, USA).

True prevalence was calculated on the basis of the formula $(R + Sp - 1)/(Se + Sp - 1)$ using the sensitivity (Se) and specificity (Sp) of the test and the proportion of subjects (R) screened positive by that test using EpiTools (http://epitools.ausvet.com.au/content.php?page=home). Confidence limits are based on variance estimates incorporating additional uncertainty associated with sample sizes used to calculate specificity and sensitivity values (Rogan and Gladen, 1978).

**Results**

*Buffalo*

Among the 130 buffalo sampled(60 from CNP and 70 from OD), 4% were younger than 1 year, 25% were between 1 and 2 years, 15% between 2 and 5 years and 56% were older than 5 years. Fifty eight per cent of the animals were females and 42% were males.

**Table 3:** Details of the 15 herds from which the130 buffalo individuals were captured and tested (location, sample size, estimated herd size and type of herd).

| Location | Herd ID | Sample size | Estimated herd size | Type of herd |
|---|---|---|---|---|
| Kabulebule | CH1 | 4 | 250 | Mixed |
| Kabulebule | CH2 | 2 | Megaherd* | Mixed |
| Ihaha | CH3 | 2 | 40 | Mixed |
| Serondela | CH4 | 20 | 300 | Mixed |
| Simwanza | CH5 | 8 | 30 | Mixed |
| Simwanza | CH6 | 4 | 25 | Bachelor |
| Ngoma | CH7 | 20 | Megaherd* | Mixed |
| Moremi | NH1 | 10 | 150 | Mixed |
| Moremi | NH2 | 1 | 5 | Bachelor |
| Moremi | NH3 | 15 | Megaherd* | Mixed |
| Moremi | NH4 | 9 | 50 | Mixed |
| Khurunxaragha | NH5 | 8 | 250 | Mixed |
| Khurunxaragha | NH6 | 12 | 350 | Mixed |
| Khurunxaragha | NH7 | 6 | 150 | Mixed |
| Khurunxaragha | NH8 | 9 | 150 | Mixed |

The estimated size of the herds captured varied between 5 and 2 000 animals, the median size being 250 individuals (IQR [150-1500]). Two of the herds sampled were bachelor herds and

the rest were mixed herds, (herd size 20 and 5 animals, respectively). Details on the different buffalo herds can be found in Table 3.

In CNP, all the animals tested negative. In OD two animals were found IFN-ɣ positive for BTB. They were adult buffalos older than 5 years and belonged to two separate herds (Moremi and Khurunxaga) captured 50 km apart. Therefore, the apparent prevalence among buffaloes in OD was 2.9%, $CI_{95\%}$ [0.8-9.8]. No strong IFN-ɣ responses to mycobacterial antigens other than bovine PPD were observed.

*Cattle*

Among the 449 tested cattle from OD interface, 3 BTB reactors were detected in 3 different crush pens, resulting in a BTB reactor rate of 0.7% (3/449) $CI_{95\%}$ [0.2-2.0] at animal level and of 3%, $CI_{95\%}$ [0.6-8.6] at herd level. In addition, prevailing IFN-ɣ responses to avian PPD were detected in two animals, classified as avian reactor animals. One animal showed strong

**Table 4:** Descriptive characteristics of cattle farming and management derived from farmers responses in the OD interface (n=97) and in the CNP interface (n=38). Percentages were compared with the MannWhitney Wilcoxon test. Medians were compared with the Kruskal Wallis test *.

| Characteristic | OD | CNP | p value |
|---|---|---|---|
| Cattle grazing in the protected area | 99% | 97.4% | NS |
| Deworming | 18.2% | 81.8% | < 0.005 |
| Kraal at night | 94.8% | 100% | NS |
| New cattle purchased last year | 9.8% | 4.8% | NS |
| Sharing water with wildlife | 77.3% | 94.7% | < 0.005 |
| Cattle grazing with sheep | 88.7% | 0% | < 0.005 |
| Cattle grazing with goats | 99.0% | 23.7% | < 0.005 |
| Cattle sourced outside community | 13.8% | 75.6% | < 0.005 |
| Median herd [IQR] | 38 [21;81] | 23 [12.5;46.5] | < 0.005* |
| Median number of animals > 10 years age | 15 [6 ; 20] | 5 [3 ; 11] | 0.005* |

IFNg reactivity to both bovine and Fortuitum PPD and was classified as a multiple reactor animal.

In the CNP interface, 9 BTB reactor animals were found in three different crush pens. Individual animal prevalence was 2.4% (9/369) $CI_{95\%}$ [1.3-4.6] and herd prevalence was 26.7% (8/30) $CI_{95\%}$ [12.3-45.9] (Table 4). Apparent individual animal prevalence and herd prevalence were both significantly higher in CNP than in the OD interface (p<0.05).

Herd prevalences in the test positive crush pens from CNP interface were 5.4% (3/56), $CI_{95\%}$ [1.1-14.8] in Lesoma, 2.8% (3/108) $CI_{95\%}$ [0.6-7.9] in Mawana and 2.9%(3/105) $CI_{95\%}$ [0.6-8.1] in Muchenje. Those inter-herd differences were not significant. Furthermore, 16 avian reactor animals and two multiple reactor animals were identified.

*True prevalence calculations*

True prevalence values were calculated for the different study areas and species on the basis of different test performances observed in known infected and uninfected South African cattle herds (Michel, unpublished data) (Table 5). For cattle, these calculations yielded a value of 2.3% [0.2-4.5] in the CNP interface and a value of 0.1% [0; 1.3] in the OD interface.

*Questionnaire results*

Differences between questionnaire results obtained among cattle farmers in the OD or CNP interfaces can be seen in Table 3. The median herd size was significantly different, being higher for CNP (38 vs. 23) than for OD. Farmers in the CNP interface purchased cattle from outside the community more often than in the OD interface (75.6% vs 13.8 %) and this difference was significant. Small livestock (sheep and goats) grazed frequently with cattle, particularly in the OD interface (more than 89% of the respondents). In both areas, the majority of farmers (at least, 95% or more) corralled their cattle at night and let their cattle graze within the boundaries of the protected area. The frequency of basic veterinary care to

**Table 5:** Apparent and true prevalence values calculated in buffalo and cattle in the study areas where some immune reactors were detected.

| Species and location | Apparent prevalence | True prevalence [1] |
|---|---|---|
| Buffalo OD interface | 2.9 [0.8; 9.8] | 0 |
| Cattle OD interface | 0.7 [0.2; 1.9] | 0.1 [0; 1.3] |
| Cattle CNP interface | 2.4 [1.3;4.6] | 2.3 [0.2;4.5] |

1. Calculated on the basis of the test performances assessed for buffalo (Michel et al, 2011) and cattle (Michel, A. unpublished results).

livestock, such as deworming was significantly higher outside of the CNP than outside the OD. Contacts with wildlife **were** also mentioned more frequently in the CNP than in the OD interface, both in grazing areas and at watering points (Figure 2).

**Figure 2**: Comparative proportions of reported contacts between cattle and wildlife in CNP and OD interfaces. All differences were significant ($p < 0.05$).
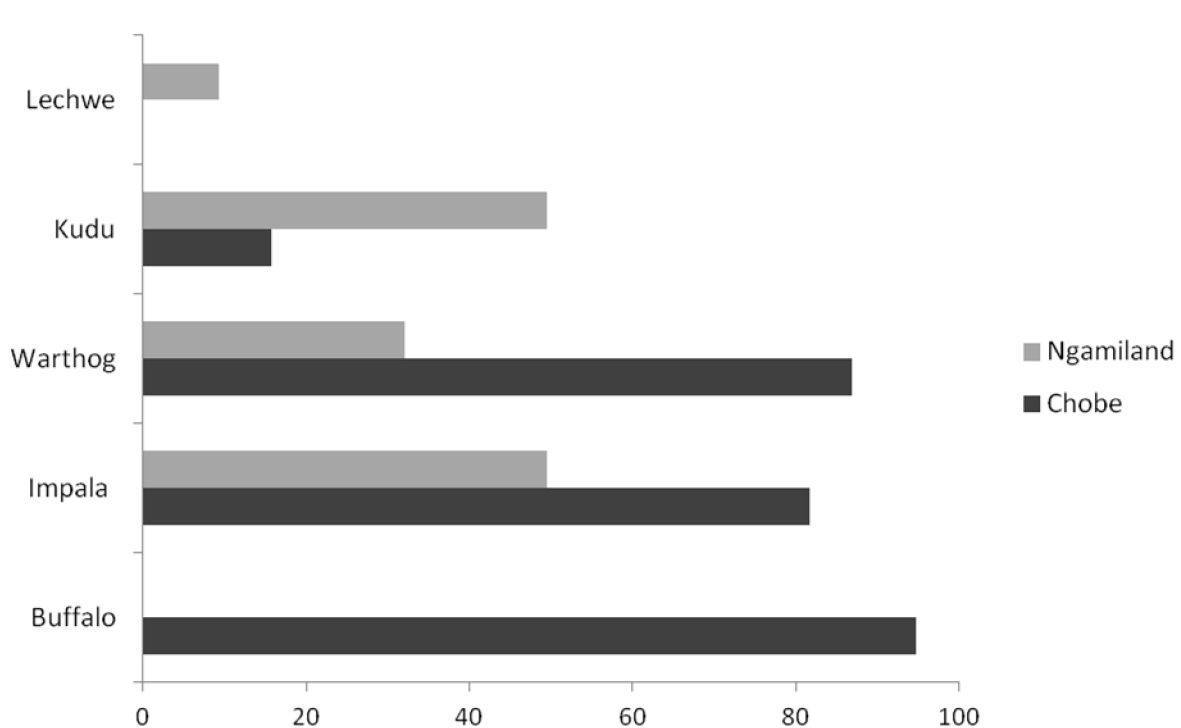


**Discussion**

Bovine tuberculosis (BTB) poses a serious threat to free-ranging wildlife and domestic animals in sub-Saharan Africa, and a significant zoonotic potential. Since the first reported case in buffalo from Kruger National Park (KNP) in 1990, BTB has become a major cause for concern in free-ranging buffalo populations which are the main wildlife reservoir for the disease in Southern Africa (Rodwell et al., 2001). It can easily spread to other hosts such as antelopes, primates, predators and scavengers and other vulnerable species (Michel et al., 2006, Renwick et al., 2007). Under favourable conditions, *M. bovis* can survive in the harsh

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African environment for up to 6 weeks in winter and up to 4 weeks during the rest of the year (Tanner and Michel, 1999). Therefore, even a relatively low prevalence may pose a substantial health risk to other wildlife species (particularly predators and scavengers) and domestic animals living at the interface with infected wildlife. Moreover, BTB is a zoonotic disease of potential concern among HIV positive populations in Southern Africa (Etter et al., 2006, Michel et al., 2010). Considering that legal and illegal hunting activities are common in Northern Botswana (Alexander et al., 2012), the processing of uninspected, raw animal products is another possible way of human exposure to *M. bovis*, in addition to the consumption of raw milk.

The findings of the first BTB prevalence study conducted in buffaloes in the OD and CNP conservation areas suggest that *M. bovis* is most likely absent from these populations. Although two buffaloes from the OD reacted positively to the IFN-ɣ assay, the calculation of the true BTB prevalence indicated absence of the disease. The diagnostic performance of the IFN-ɣ assay has been evaluated in African buffaloes and was found to be 86% sensitive and 92% specific (Michel et al. 2011). Based on this suboptimum performance, it would be possible to encounter two false positive test results in a BTB negative subpopulation of 60 buffaloes. However, the limited test sensitivity could also have accounted for a number of false negative and hence, could have missed some truly infected animals on the other hand.

In a similar way, preliminary evaluation of the IFN-ɣ assay in several infected South African cattle herds, showed that the sensitivity of the test ranged from 71% to 86% while the specificity was found to be 99% or above throughout different uninfected herds (Michel, unpublished data). Based on these performance indicators, the true BTB prevalence calculated for 9 positive cattle herds at the interface with CNP was almost identical to the apparent prevalence (2.3% and 2.4% respectively), supporting the hypothesis of a low level of infection circulating in this cattle population. To the contrary and similarly to the results

obtained in the case of buffaloes, the corresponding data for 3 positive cattle from herds at the OD interface do not support the hypothesis of an *M. bovis* infection (Table 5).

Our findings therefore suggest that BTB infection is probably present at a low prevalence in cattle at the wildlife/livestock interface of the CNP. Data currently available for buffalo and cattle at the OD interface do not allow any conclusive diagnosis concerning their BTB infection status, but we speculate that BTB may be absent from these populations, as suggested by the true prevalence calculations (Table 5).

We are aware that it would have been imperative to confirm the BTB infection status of test positive reactor cattle and buffaloes by slaughter and subsequent culture of the causative agent, but this was not possible due to financial and logistical constraints. We therefore consider our findings instrumental in the motivation for a follow-up study in Northern Botswana in the near future.

The results of the questionnaire survey overall suggest that important risk factors that could facilitate the circulation of BTB are more frequent in the CNP than in the OD wildlife/livestock interface. In particular, the outcome of the questionnaire among farmers revealed that prevailing cattle management practices such as the occurrence of water points shared between wildlife and livestock and the percentage of farmers purchasing cattle outside the community were significantly more common among communal farmers from the CNP interface (Tables 3 and 4). Shared water sources are a recognized risk factor for the transmission of FMD between domestic and wild species (Bastos et al., 2000, Dion and Lambin, 2012) and is equally applicable to other pathogens such as BTB.

Both, cattle movement and the proximity of cattle to BTB infected areas are considered among the most consistently identified herd level risk factors for BTB worldwide (Skuce et al., 2012) and probably explain the significant difference in the apparent BTB prevalence observed between cattle from both study areas (p< 0.0006). Furthermore, it is noteworthy to

point out that the CNP is adjacent to the borders of 3 countries (Namibia, Zimbabwe and Zambia) and exposed to important formal and informal transboundary exchanges of livestock. In addition, BTB is endemic in wildlife and livestock in neighboring Central and Northern Zambia (Munyeme et al., 2009, Munyeme et al., 2010) and the reported infected Kafue Flats areas (Munyeme and Munang'andu, 2011) are located less than 300 km from CNP. Moreover, BTB has been detected in cattle the Zambian region of Kazungula, located less than 20 km from the northern border of CNP in Botswana (Munyeme et al., 2009, Munyeme et al., 2008).

We consider unlikely that the outcome of the survey was affected by recall bias because it was primarily aimed to collect qualitative rather than quantitative data from recent events. However, some of the answers could have been biased by the fact that the questionnaire survey was implemented by technicians from the Botswana Veterinary Services. In particular, several recent FMD outbreaks suspected to be caused by contacts between cattle and buffalo (http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=9856) have resulted in the enforcement of additional veterinary control measures such as restrictions in livestock movements, increased frequency in vaccination, etc.. These measures have led to considerable disruptions of the local cattle farming activities (Mendelshon and El Obeid, 2004) and could have been the reason why none of the farmers in the OD interface reported any contacts between cattle and buffalo (Figure 2). Those contacts are known to occur frequently despite the presence of a veterinary cordon fence and they facilitate the spread of FMD virus and potentially other pathogens including BTB, between buffalo and cattle.


**Conclusions**

This study highlights the difficulties in assessing BTB at the wildlife/livestock interface in areas where the prevalence is likely to be low. Our findings suggest that *M. bovis* infection

could be circulating at low prevalence levels in the cattle population at the wildlife/livestock interface of the CNP, but this result still requires confirmation. With the currently available test data from buffaloes in the OD and CNP, we consider that at the time this study, the presence of BTB in the sampled buffalo populations was unlikely. However, considering that important risk factors predisposing a potential introduction of *M. bovis* from neighboring countries to the CNP are not negligible, additional field studies to monitor the circulation of BTB in cattle through the implementation of a regular surveillance programme at the wildlife/livestock interface of CNP is strongly recommended.

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