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ORIGINAL PAPER

Prevalence of bovine tuberculosis and animal level risk factors for indigenous cattle under different grazing strategies in the livestock/wildlife interface areas of Zambia

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Abstract A cross-sectional study was conducted to investigate the prevalence and animal level risk factors for bovine tuberculosis (BTB) in indigenous cattle of the livestock/wildlife interface areas in Zambia. A total of 944 cattle from 111 herds were investigated. The comparative intradermal tuberculin test (CIDT) was used to identify reactor animals for BTB. Animal level data on sex, age, parity and body condition score were registered. The overall animal prevalence of BTB as determined by the CIDT was 6.8% (95% CI: 4.2, 9.5%). In Lochinvar and Blue Lagoon areas, animal level prevalence were observed at 5.2% (95% CI: 2.2, 8.2%) and 9.6% (95% CI: 6.1, 13.2%), respectively. Kazungula, an area outside the livestock/wildlife interface, had a prevalence of only 0.8% (95% CI: 0.0, 2.3%). The age of the animal, its body condition score and the type of management system, were predictive of its BTB status. The study revealed that BTB was relatively high in the livestock/wildlife interface areas of Lochinvar and Blue Lagoon compared to Kazungula. These findings should raise a serious public health concern considering the extent to which the communities of the study areas are in contact with their animals and the levels at which they use untreated milk.

Keywords Bovine tuberculosis · Grazing strategies · Livestock/wildlife interface · Prevalence · Zambia

Introduction

Bovine tuberculosis (BTB) is an important disease of both economic and public health importance (Sudre et al. 1992). In cattle, the disease is mainly caused by the bacteria, *Mycobacterium bovis* (*M. bovis*) (Acha

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and Szyfres 1987). However, occasional occurrence of tuberculosis due to *Mycobacterium tuberculosis* (*M. tuberculosis*) species with concurrent tuberculous lesions has been reported in pigs (Lesslie and Brin 1970). In cattle, *Mycobacterium tuberculosis* accounts for a small proportion of usually sub-clinical cases (Ameni and Erkihun 2007; Pritchard 1988).

Bovine tuberculosis used to be a serious zoonosis and represented a tremendous public health problem in developed countries, mainly through the consumption of unpasteurised milk (Collins and Stollerman 1993). However, after the disease was controlled in cattle, through designed test and slaughter schemes, together with milk pasteurization and public education, these countries drastically reduced and in some cases controlled the transmission of BTB to humans (Dankner et al. 1993).

In low-income countries BTB is still prevalent and is responsible for significant economical loss in animal production through reduced milk yields, abattoir carcass condemnations, low meat yield through emaciation and low reproductive performance. BTB has also been implicated in the increase of human health problems in these countries (Cosivi et al. 1998).

The global prevalence of human tuberculosis due to *Mycobacterium bovis* has been estimated at 3.1% of all human tuberculosis cases (Cosivi et al. 1998; Sudre et al. 1992). In countries where a majority of poor rural pastoral communities depend on cattle for their livelihoods, BTB is reported to be a constant threat due to lack of pasteurization of milk (Ameni et al. 2003; Cosivi et al. 1998). Unfortunately, lack of information on BTB in many African countries, Zambia inclusive, has reduced the attention given to this infectious agent in terms of prioritizing resources devoted to control of animal diseases (Cosivi et al. 1998).

BTB is known to be a major cause of human extra-pulmonary tuberculosis specifically the gastro-intestinal tuberculosis, and is of particular concern in developing countries where bovine milk is often not pasteurized (Sonnenberg et al. 2001). The reason for this is that the major mode of exposure to of *M. bovis* infection in humans is through drinking of raw milk, and *M. bovis* in humans mainly manifests as an extra-pulmonary form, particularly causing cervical lymphadenitis.

In Zambia, reports on the Kafue Basin indicate that *M. bovis* infections could be a problem not only in cattle, but in humans too (Cook et al. 1996; Cosivi et al. 1998). These reports have also indicated the presence of *M. bovis* both in wildlife (Cook et al. 1996; Cosivi et al. 1998; Munag'andu et al. 2006; Pandey 1998; Sitima 1997). Whilst reports on the existence of tuberculosis in wildlife of the Kafue basin are persistent, no studies have been conducted in cattle sharing grazing land with the Kafue lechwe (*Kobus leche Kafuensis*). The Kafue lechwe antelopes in Zambia are suspected to be biological reservoir hosts for BTB (Gallagher et al. 1972; Stafford 1991). There is a need therefore, to systematically investigate the levels of BTB in cattle reared in the purported high risk area of the Kafue Basin.

The overall aim of this study was to determine the prevalence of BTB in cattle sharing grazing land with Kafue lechwe antelopes in the livestock/wildlife interface areas of the Kafue Basin of Zambia, and identify animal level risk factors of its occurrence.

Materials and methods

Study areas

The Kafue Basin is a floodplain along the Kafue River of about 6,000 km^2 located in the South-Central region of Zambia, (Fig. 1)- (Sheppe 1985). It lies between the lines of latitude 26°-28°E and 15°20′-15° 55′ S. It is comprised of Lochinvar National Park (410 Km^2; 15°43′-16°01′S, 27°11′-27°19′ E), Blue Lagoon National Park (420 Km^2; 15°21′-43′S, 27° 15′-27°32′ E) and the Kafue flats Game Management Areas (5 175 Km^2) (Mwima 1995). The comparative area of Kazungula District lies in the most Southern part of the country, located by coordinates 17°05′S to 17°45′S and 25°38′E to 25°59′E along the Zambezi Basin. Cattle reared in the Kazungula District have no apparent contact with wild animals as they are outside the livestock/wildlife interface areas.

Study design

A cross-sectional study was conducted between August 2003 and February 2004. Due to lack of comprehensive information on the cattle herds in the study areas, a base line study was conducted to estimate the population of cattle herds (N) in the areas. Based on the baseline study, we estimated that

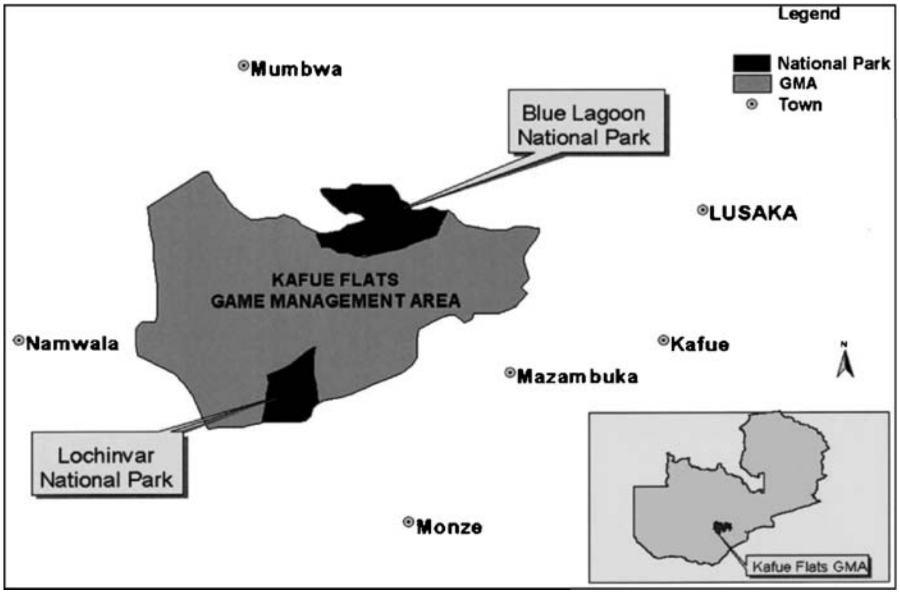

Fig. 1 Map of the Kafue Basin region (Zambia) showing the interface areas where cattle graze in close proximity with wildlife (insert, is the full map of Zambia)

there were approximately 110 cattle herds in the Blue Lagoon area, 100 in Lochnivar and 50 in Kazungula. This population of herds constituted the study population from which actual testing was conducted (sample population).

Assuming low heterogeneity between herds, we used a detection power (1-β) of 90%, the level of significance (α) at 95% and the desired absolute precision at 5 %. We further assumed the sensitivity and specificity of the comparative intradermal tuberculin test (CIDT) to be 80% and 100%, respectively (Monaghan et al. 1994; Quirin et al. 2001). The BTB prevalence previously reported for cattle in Zambia varies from 10% to 20% at animal level. The average herd size was assumed to be at 100 animals. We thus planned to sample individual cattle from herds at a 10% testing fraction. Based on these assumptions, we used *Herdacc*™ Version 3 (Jordan 1995) to estimate the herd specificity (HSp) and herd sensitivity (HSe). Our predicted HSp and HSe were 100% and 73.9%, respectively, at 10% testing fraction when a herd was classified positive if at least one animal tested positive on CIDT. Thus applying the estimates in the sample size calculation formula for simple random testing, and correcting for a finite population we planned to sample a total of 125 herds, represented by 53, 48 and 24 herds for Blue Lagoon, Lochinvar and Kazungula, respectively. To select this number of herds and to avoid selection bias, a simple random mechanism of choosing farms was designed using a lottery system. In each study area, the farms were given numbers on a piece of paper. These numbers were then put in a suitable receptacle from which random selection of farms were made. Testing of farms from the receptacle was done without replacement. In areas where herd owners were un-cooperative, other herds sharing similar exposure factors to those excluded, such as herd size, grazing strategy etc were chosen for the study. At animal level, the situation was slightly different. For those animals that were tested from crush pens, we used a systematic random testing, were as true random testing was difficult to attain in animals that were restrained by casting in the kraals. In these study areas, three grazing strategies were practised. Those animals that were grazed within village perimeters without migration in search of pasture were known as village resident herds (VRH), while those that went in search of pasture during drier times coming back to the villages in the rainy season were known as transhumant herds (TH). However, some herds of cattle remain permanently resident in the plains and these are known as interface herds (IFH).

Epidemiological data

Information about each animal such as age, sex, pregnancy status, lactating status, parity, body condition score etc, were collected And entered into a data sheet.

Intradermal skin test

For the determination of prevalence of BTB in cattle, the comparative intradermal cervical tuberculin test (CIDT) was applied. The procedure was conducted as described in the manual of Office International Des Epizooties (OIE 2004). Briefly, two areas of about 2 cm diameter of the cervical skin were clipped (or short cropped), washed with soap and disinfected with 70% ethanol. The initial skin thickness was measured followed by intradermal injection of 0.1 ml of bovine and avian purified protein derivatives (PPD), respectively. The results of hyper-sensitization were read after 72 hours by measuring again the skin thickness at the inoculation sites. A strict standard level of interpretation was used to classify reactors according to the OIE manual (OIE 2004). Negative reactors were indicated by a differential increase in skin thickness of less than 2 mm after subtracting the increase of skin thickness at the avian PPD injection site from the one at the bovine PPD injection site (OIE 2004). Inconclusive reactors were indicated by skin thickness increases of between 2 mm and 4 mm. A positive reaction was indicated by an increase in skin thickness of more than 4 mm.

Data analyses

The database was maintained in Excel® before transferring to Stata 9 for Windows (Stata Corp. College Station, TX, USA). This included bio-data about each animal such as sex, age, parity and body condition score. Animal level prevalence estimate for BTB with confidence intervals were computed using the survey command estimates in Stata with adjustments for strata (study area) as described by Dohoo et. al. (2003). At animal level, the effect of age, sex, and other factors with a plausible (causal-effect) relationship to BTB status were investigated. Independent effect of categorical variables on BTB status were assessed using the two sided Fisher exact test while those of continuos variables were assessed using the Kruskal_Walli's tests. Only variables that had p-values (two-sided) ≤0.25 and those without many missing values (>15) were retained for multivariable analysis in a logistic regression model. The model was manually constructed using forward selection according to details of logistic model building as described by Dohoo and others (Dohoo et al. 2003). The model's goodness of fit was assessed using the Hosmer - Lameshow goodness-of-fit test, while its significance was assessed by using the likelihood Ratio Test with sensitivity and specificity being assessed using the ROC analysis.

Results

A total of 944 cattle were tested from 111 herds under investigation. Median herd size was 39 (95% CI: 36, 41) animals across all the three study areas (Table 1). In Blue Lagoon and Kazungula District, the targeted numbers of herds that were to be tested were attained, while in Lochinvar only 34 out of a targeted number of 48 herds were tested. Out of 944 animals tested, 52

Table 1 BTB in Zambian traditional cattle (*n*=944) according to study areas incorporating targeted herds and actual numbers tested between August 2003 to February 2004

Study area	Targeted No. of Herds	No. of herds (no. of cattle)	Median herd size (Centile range)	Individual BTB prevalence (95% CI)	Herds with BTB prevalence (95% CI)
Blue Lagoon	53	54 (490)	51 (25–127)	9.6 % (6.1–13.2)	47.9 % (33.5–62.3)
Lochinvar	48	34 (280)	25 (13–37)	5.2 % (2.2–8.2)	42.9 % (26.0–59.5)
Kazungula	25	23 (174)	66 (48–127)	0.8 % (0.0–2.3)	4.4 % (0–13.0)
Overall	126	111 (944)	39 (25–89)	6.8 % (4.2–9.5)	36.8 % (28.0–45.6).

tested positive on tuberculinisation, giving a BTB prevalence of 6.8% (95% CI: 4.2, 9.5) after adjusting for sampling weights (Table 1). Table 1 also shows the distribution of reactor animals both at individual and herd level according to area of study which formed the primary study strata.

Table 2 shows the animal BTB prevalence in cattle by sex, age and grazing strategy. The results showed that animals from the livestock/wildlife interface areas (IFH) had by far the highest BTB prevalence (11.4%, 95% CI: 7.4, 15.4), followed by cattle that practiced the transhumance grazing strategy (TH) (5.3%, 95% CI: 1.9, 8.7). Village resident cattle had a markedly lower BTB prevalence of (1.6%, 95% CI: 0.3, 2.9). The prevalence of BTB was shown to increase proportionately with age as indicated in Table 2.

A number of factors were found to be associated ($p<0.05$) with increased risk of testing positive for a tuberculin test (Table 3). Significant differences were observed between animals in the study area (the livestock/wildlife interface) and those in the control area of Kazungula in relation to body condition score, age, sex, grazing strategy and size of the herd. Survey logistic regression analysis results showed that there was a strong association between being BTB positive and having a poor body condition score (OR=15.6) (Table 3). The model also showed significant association between being BTB positive and age as well as type of grazing strategy under which animals where managed (Table 3). The results also showed that animals that were reared in the interface areas were almost seven times more likely to test positive for BTB than village resident herds (OR=6.7) (Table 3). The model also showed an increasing prevalence with increasing age, although the association was statistically significant only for animals older than seven years ($P<0.001$).

Table 2 Prevalence of bovine tuberculosis (BTB) in Zambian traditional cattle (n=944) by sex, age and grazing strategy in three study areas (August 2003 to February 2004)

Variable	Level	% proportion	95% CI
Sex	Females	6.0	2.9–9.1
	Males	8.4	2.3–14.6
Age	1–3 years	4.9	2.4–7.4
	3.5–5 years	5.7	2.4–9.0
	5.5–7 years	3.6	0.0–8.9
	>7 years	15.1	5.2–24.9
Grazing type	VRH	1.6	0.3–2.9
	TH	5.3	1.9–8.7
	IFH	11.4	7.4–15.4
Overall Prevalence	All Areas	6.8	4.2–9.5

Table 3 Results from a predictive logistic regression model for bovine tuberculosis (BTB) in Zambian traditional cattle (n=944), animals tested between August 2003 to February 2004

Variable	Level	*B*	OR	P-Value	95% CI
Body C score	Excellent	–	1	–	–
	Good	0.2	0.8	0.20	0.6–1.1
	Average	0.8	2.2	0.03	1.1–4.4
	Poor	2.7	15.6	0.00	9.2–26.4
Age category	1–3 yrs	–	1	–	–
	3.5–5 yrs	0.2	1.3	0.30	
	5.5–7 yrs	–0.1	0.9	0.78	0.6–1.4
	>7 yrs	0.4	1.5	0.00	1.2–1.9
Herd type	Village	–	1	–	–
	Transhumant	0.8	2.2	0.04	1.0–4.8
	Interface	1.9	6.7	0.00	2.6–17.2

Discussion

This study focused on tuberculosis in indigenous cattle breeds in the Zambian livestock/wildlife interface areas across different cattle grazing strategies. It also explored the relationship between cattle sharing grazing land with wildlife (mainly Kafue lechwe antelopes). In this type of set up, BTB infection was found to be prevalent in the livestock/wildlife interface areas. The animal prevalence of BTB varied among the 3 grazing strategies identified in the study areas. Significant differences were recorded between those in the interface areas and those outside. Animal BTB prevalence in Lochinvar was recorded at 5.2% and Blue Lagoon 9.6%, both found in the wildlife/livestock interface areas whilst Kazungula which is outside the interface area had a prevalence of 0.8%. These findings hint on the probable existence of a likely focus of infection around this geographical zone of the country (Sitima 1997). However, the reasons for such spatial distribution of infection are not well known, although it is suspected that the lechwe antelope is the biological reservoir host of

BTB in the Kafue Basin area (Cook et al. 1996; Pandey 1998; Sitima 1997). Cattle raised in the livestock/wildlife interface areas of the Kafue Flats were more likely to have a positive tuberculin reaction on CIDT than those found in Kazungula District although relatively larger herd sizes were recorded in Kazungula (Table 1) and the mixing of animals and other characteristics were similar to those found in the Kafue Flats, with the exception of lack of wildlife contact. Animals that were reared in the interface areas (IFH), were almost seven times more likely to have a positive test reaction than those reared in the villages (VRH) (OR=6.7). These results strengthened the earlier suggestion of an existing concentric focus of infection towards the wildlife sanctuary in the Kafue Basin as postulated by Pandey (Pandey 1998). The Kafue Basin has been identified as an area with favourable ecological conditions for the spread of infectious diseases between livestock and wild animals due to the interaction that exists between cattle and wildlife, coupled by the favourable marshy environmental conditions (Munag'andu et al. 2006). The gregarious nature of lechwe antelopes with higher herd densities obtaining in drier seasons is thought to have had facilitated the survival of *M. bovis* in these wetlands (Gallagher et al. 1972; Siamudaala et al. 2003; Stafford 1991). Yearly seasonal flooding may also help in the propagation of micro-organisms in the environment, while over-crowding of animals during the dry season at watering points may enhance the direct animal to animal transmission of the disease (Corner 2006). Thus, from the biological stand point of spread and maintenance of *M. bovis*, both environmental, host and agent attributes may be regarded as being optimal in the Kafue Basin region (Corner 2006; Zieger et al. 1998).

Data from this study indicated that sex had no effect on BTB status. This is in accordance with findings from other studies (Oloya et al. 2006; Omer et al. 2001). According to the biology of the disease, tuberculosis positive cattle go through a period of desensitization before and after calving and as many as 30% may give false negative reactions returning to a positive status 4 to 6 weeks later (Radostist et al. 1994). In our study less than 0.5 % of the animals had given birth within a month or two prior to being tested, which imply that the number of false negatives due to this aspect could not have an effect on the validity of the results.

In our study, age was found to be related to the distribution of BTB reactors across all areas and grazing strategies with low prevalence values being recorded in younger age groups and high values in older ones. These results agree with the established observation by other researchers (Ameni et al. 2003; Asseged et al. 2000; Cook et al. 1996) Cattle of all ages are susceptible to TB, but the probability of becoming infected generally increases with age due to the proportionate increase in exposure time, with older animals being said to have a greater risk on account of their longer life spans (O'Reilly and Daborn 1995).

Results from the model indicated that body condition score, age and grazing strategy as important predictors for cattle BTB. It is likely that emaciation was an indicator of tuberculosis occurring in an animal. It is also possible that tuberculosis positive animals have poor body condition score as a result of being infected, i.e. a clinical sign that typically follows an active infection with *M. bovis* (Kazwala et al. 2001; Pritchard 1988).

Grazing strategy apart from being a major predictor variable for BTB status, it is also a proxy variable for other risk factors. For example, interface cattle herds are known to be in constant contact with lechwe herds, previously described as sources of residual infection of tuberculosis (Cook et al. 1996; Pandey 1998; Stafford 1991). Interface herds are also known to be large thus facilitating for close contact within and between herds and thus favouring the transmission of respiratory diseases including tuberculosis (Radostist et al. 1994)

It was also noted that there was more than one animal per herd that gave a positive result, giving rise to a certain degree of clustering. However, these numbers were very small and it was considered very unlikely that a clustering effect would influence the results of the study, and it was not included in the analysis.

This study has shown that BTB is relatively high in cattle in the livestock/wildlife interface areas of Lochinvar and Blue Lagoon compared to Kazungula raising concerns in terms of animal productivity and public health. The study further showed that old and emaciated animals raised in the interface areas where more likely to test positive for BTB. Overall, the study has been able to establish geographical area attributable differences in prevalence coupled with potential risk factors of its occurrence at animal level.

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