

# **Responses to tuberculin among Zebu cattle in the transhumance regions of Karamoja and Nakasongola district of Uganda**

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## **Abstract**

Responses to tuberculin in Zebu cattle of the transhumant pastoral farming system in Karamoja region and Nakasongola district in the north-eastern and mid-central regions in Uganda, respectively, were investigated using a comparative intradermal tuberculin skin test. Of the 1864 cattle tested from 30 large units (superherds) in Karamoja and 7 herds in Nakasongola, a total of 28 animals from 19 herds (51.4%) tested positive. Inter-district tuberculin reactor prevalence variations seemed to be influenced by climate, with impact on both the management patterns and transmissibility of agent. High herd tuberculin reactor prevalence (51.4%) was attributed to widespread contacts and mixing of animals between herds. Low individual animal tuberculin test positivity (mean=1.4%) was attributed to low transmissibility of the agent under the Karamoja climate, which is semi-arid, and to increased resistance due to non-specific response to environmental mycobacteria and natural selection, since there was no active control against bovine tuberculosis. Owing to similarities in management practices in Karamoja and widespread risk factors, it was difficult to identify which were more important, but variations in sources of drinking water pointed to provision of lake and borehole water during dry season as reducing the risk. Positive bovine tuberculin reactor prevalence and skin reactor status were related to age.

**Keywords:** Prevalence, Bovine tuberculosis, Transhumance, Zebu cattle, Uganda

## **Abbreviations**

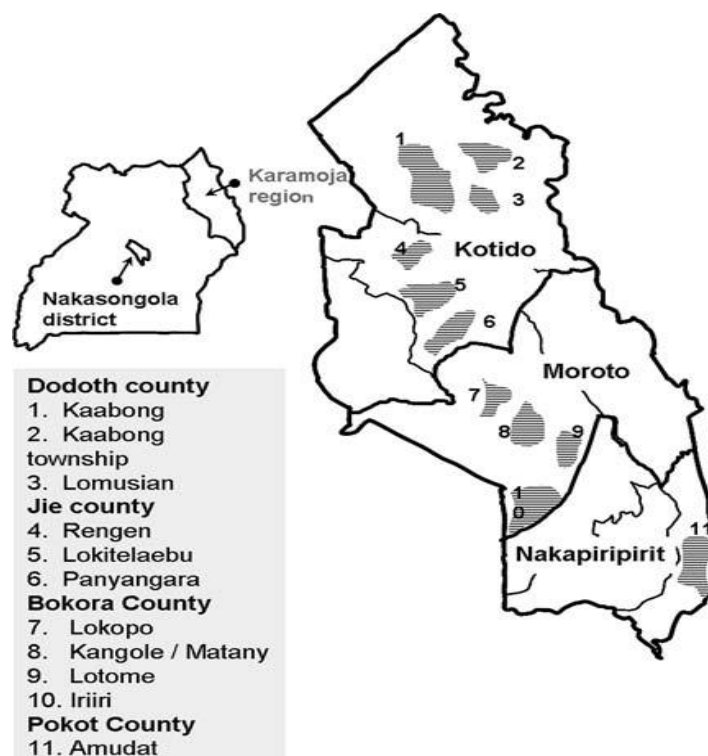
BTB bovine tuberculosis  
PPD purified protein derivative

## Introduction

*Mycobacterium bovis* infection is of major importance among cattle and other domesticated animals in many developed and developing countries and *M. bovis* has become an important opportunistic pathogen in humans with acquired immunodeficiency syndrome (AIDS) (WHO, 2001).

In Uganda, bovine tuberculosis (BTB) was reported to be common among the long-horned Ankole cattle of the western part of the country (Opuda-Asibo, 1995). Previous studies (Acen, 1991) revealed a prevalence of 19.7% in pastoral cattle in that region. Abattoir slaughter reviews (Ministry of Agriculture Animal Industries and Fisheries, 1990) showed that 1.8% of slaughter animals originating from the eastern region (including Karamoja) showed a generalized tuberculosis based on gross pathological lesions.

Karamoja region (33°30'E to 35°E and 1° 30' to 4°N), located in north-eastern Uganda (Fig. 1), is characterized by semi-arid wide plains (1500m above sea level), low rainfall (500–700 mm) and a prolonged dry season lasting 5–7 months from September to March (Karamoja-Data-Centre, 2004). Nakasongola is situated in the central region of Uganda and experiences bimodal rainfall pattern of moderate quantities (875– 1000 mm) with long dry period of 5 months. The climatic conditions of Karamoja region are harsh and unpredictable and make transhumance the only possible livestock management system to be practised. This type of transhumance is characterized by well organized mobile herding groups, which traditionally move together under the guidance of kraal leaders in search of better grazing and watering areas (Karamoja- Data-Centre, 2004). The main livestock species kept include short-horned Zebu cattle, sheep, goats and a few donkeys. There is no supplementary feeding and very limited veterinary care is provided.



**Fig. 1** Map of Uganda showing study areas in the three Karamoja districts. Study areas are shaded

Although *M. bovis* transmission is reportedly low under such extensively managed systems compared to intensive ones (Francis, 1947; Steele and Ranney, 1958), documented practices that favour transmission, such as sharing of communal grazing and watering areas by livestock from different areas or herds (Phillips *et al.*, 2003), heavy faecal contamination of a few stagnant water sources (Faries and Davis, 1997) and overcrowding in night enclosures (O'Reilly and Daborn, 1995), are routine and common in the transhumance system. Long-distance migrations, large herds ( $n > 35$  cattle) and overcrowding in cattle camps during the dry season increase herd-to-herd contacts and create an ideal environment for transmission (Neill *et al.*, 1989; Omer *et al.*, 2001).

The prevalence of clinical BTB is low in pasture beef cattle due to their short lifespan (Barlow *et al.*, 1997). It is also low in countries where active control and removal of positive cases is practised (Wilesmith and Williams, 1986). Lack of a test and slaughter policy for test positive animals, together with a culture in pastoral farming systems of keeping animals until they die of disease or old age, allows the infection to progress to advanced stages of clinical disease and long-term excretion of *M. bovis*. This ensures that the infection is maintained in herds within extensive grazing conditions.

The effect on human health of even a low prevalence of *M. bovis* infection may be severe in a system with the intense level of contact between cattle and humans that is seen in pastoral groups in this part of Africa. Notably, habits such as drinking raw milk mixed with cow's urine or blood, consumption of raw meat and close contact with cattle may be important risk factors for the spread of *M. bovis* from cattle to humans. Besides, no active BTB surveillance programme exists in Uganda, and little is known about the magnitude of the problem of bovine tuberculosis in this farming system despite risky practises (FAO/OIE, 2000; Kazwala *et al.*, 2001).

The aim of this study was to provide estimates of the prevalence of BTB in transhumant cattle in Karamoja and Nakasongola region of Uganda and to use this information to investigate the possible role of BTB as a source of infection with human TB in the same areas.

## Materials and methods

### Study area

The study was carried out in two regions: Karamoja in the north-eastern part of Uganda and Nakasongola district in the mid-central region (Fig. 1). Karamoja region was chosen following reports of cases of slaughter animals with tuberculous lesions from the abattoirs in the regions (Ministry of Agriculture Animal Industries and Fisheries, 1990). Secondly, a strong cultural practice of drinking raw milk mixed with cattle blood or urine and raw meat is common in this region. Nakasongola was chosen for comparative purposes since such risk factors are lower. Assessment of the prevalence of BTB in these cattle as a measure of their exposure to BTB was therefore necessary.

### Study design

The survey was planned to cover all three districts (Kotido, Moroto and Nakapiripirit) of Karamoja region to control for variations in agroecological zones and the influence of migration on the occurrence of BTB in all resident cattle population in domestic grazing areas. Owing to the high level of herd interactions, a two-stage sampling was planned. Larger units or superherds (having between 16 and 65 herds each) were considered as primary sampling units, while herds within them (mean herd size 95 head) were secondary sampling units. A superherd was defined as a group of herds belonging to the same cattle camp in the dry season or those identified as sharing communal grazing and watering areas during the wet season and belonging to the same camp, usually identified by the name of a kraal or camp leader, during the dry season.

To obtain necessary power to detect presumed risk factors and to allow for adjustment of between-herd and within-herd variations, 30 superherds were considered as primary units for the

study. A plan for a secondary sample size of 50 animals per superherd was calculated from an expected prevalence of 3.5%, a desired absolute precision of 5% and a 95% confidence interval (Dohoo, 2003).

However, problems were encountered owing to lack of comprehensive information on distribution of animal populations in transhumant communities, consequent on the nomadic lifestyle and 'on and off' cattle camp system. The unpredictable inflow and outflow of cattle from rustling and erratic reductions due to diseases and drought also made it difficult to make reasonable herd estimates. Cultural belief that does not allow one to count and reveal the exact number of herds or animals in a herd also made estimation difficult. It was also not possible to ascertain independence of superherds as discrete units. It was thus difficult to construct a complete sampling frame for this study. A system that took into consideration the independence of grazing areas was adopted. A total of 315 kraal leaders in charge of grazing management were identified, together with their grazing areas, from the list of previous (year 2002) contagious bovine pleuropneumonia (CBPP) vaccination programmes in the three districts. These were used as proxies for superherds and all the herds therein were considered to belong to those groups. Efforts were made to identify grazing and watering limits to minimize double sampling of interacting herds (Fig. 1).

### Selection of herds and animals

A proportional allocation of the required number of superherds to three districts was done according to number of kraal leaders listed in each. A total of 14 out of 147 superherds listed were allocated to the Dodoth and Jie counties of Kotido district, 9 out of 94 to Bokora in Moroto district, and 7 out of 74 to Pokot in Nakapiripirit District. In total, 30 non-apposing or independent grazing areas for superherds, together with the names of their kraal/camp leaders, were identified and sampled. It was not possible to obtain a complete list of superherds owing to non-compliance of some kraal leaders during the previous vaccination programme. In Nakasongola, 7 herds from 7 different villages were selected for purposes of comparison.

Individual herds were identified within the superherds. To represent the superherd, one herd was selected by a simple random process. A total of 50 animals were then randomly selected from a single herd. Overall, 1522 and 342 animals were tested in the four counties of Karamoja region and Nakasongola district, respectively. In all areas, calves below 6 months of age and recently calved cows (2 months post partum) were exempted from the study because the presence of high levels of maternal antibodies and immunosuppression that desensitize them to tuberculin (Kazwala *et al.*, 2001).

### Tuberculin testing

TB testing was done using purified protein derivative (PPD) (Animal Sciences Group, Wageningen, Netherlands), using 0.1 ml of 30 000 IU/ml and 25 000 IU/ml of bovine and avian PPDs respectively. The technical details of the test procedure and the interpretation of the comparative intradermal test readings were as described (Monaghan *et al.*, 1994). The differences in the increase of skin thickness between bovine and avian antigens for individual animals were interpreted as negative (< 2 mm), doubtful or inconclusive (2– 3.9 mm) and positive ( $\geq 4$  mm). Herds were classified as tuberculosis-positive if they had at least 1 positive bovine reactor animal (Kazwala *et al.*, 2001).

### Epidemiological information

Information about animal identity, sex, age, breed, lactation and BTB test status was recorded for each animal during testing, while herd/flock-level information was collected using a structured questionnaire to include possible management practices affecting the epidemiology of BTB.

## Statistical analysis

Raw data from individual farm testing, individual animal information and herd-level information from questionnaires were merged and handled using Epi-Info (2002) and Microsoft Excel. The validated data were then exported to Stata (Stat SE / 8.2, Stata Corp, College Station, TX, USA) for analysis.

For estimating individual prevalence of BTB across age groups, sex and study areas, the survey procedure in Stata was used. Data were defined by selecting strata (district), primary sampling unit (herd), and weighting by the inverse of the sampling fraction from each herd. Tabular analysis was used to test groups' skin test patterns (Table 1). To estimate the possible independent effects of sex and age for the odds of being BTB positive, the logistic regression procedure for survey data was used. Age grouping was undertaken using the quartiles and group ranges of the whole population sampled. Superherd level data were analysed using the same approach, but weighting was not done.

## Results

A total of 1864 animals were tested from 30 superherds in Karamoja region and 7 herds in Nakasongola. Twenty-eight animals from 19 herds tested positive on tuberculin testing. The skin tuberculin reactor patterns according to district, adjusted for primary sampling units and weight, are shown in Table 1. Classifying only animals with a reaction of  $\geq 4$ mm as BTB-positive, the adjusted tuberculin prevalence estimates for the different districts are shown in Table 2. As seen, there was a distinct difference in the prevalence of positives between the districts, ranging from 0.1% (Nakasongola) to 2.6% (Kotido).

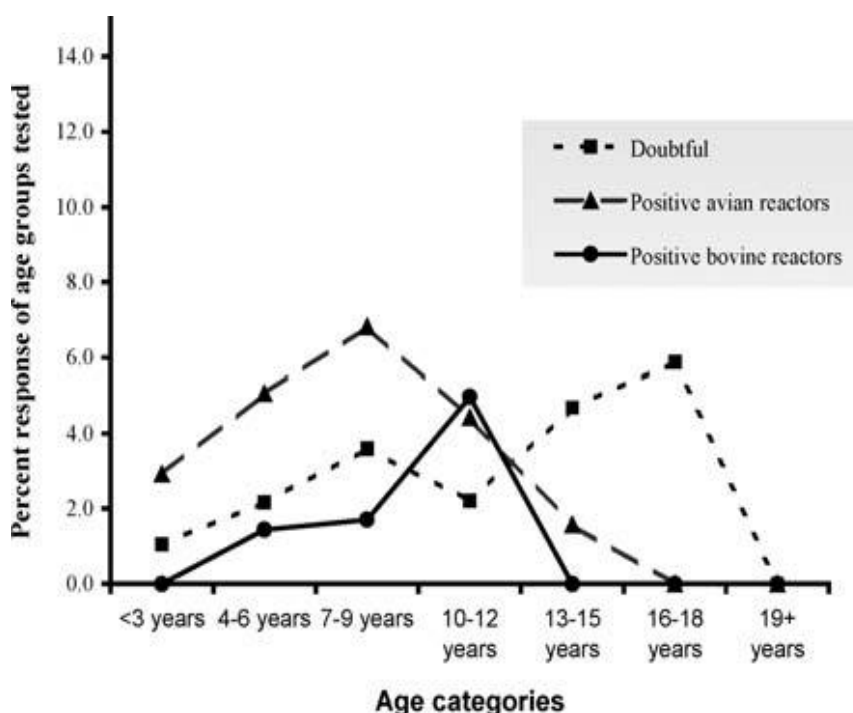
**Table 1** Variations in the skin reactions to tuberculin in different districts showing prevalence adjusted for primary sampling units and weighted using the survey procedure in Stata

District	Avian reactors ( $\leq -3$ mm)		Negative reactors ( $-2.9$ to $1.9$ mm)		Doubtful reactors ( $2-3.9$ mm)		Positive reactors ( $\geq 4$ mm)	
	No.	% (SE)	No.	% (SE)	No.	% (SE)	No.	% (SE)
Kotido	46	6.3 (1.11)	637	89.2 (1.33)	16	1.8 (0.46)	18	2.6 (0.65)
Moroto	9	1.8 (0.97)	436	96.0 (1.03)	6	1.2 (0.38)	4	1.1 (0.49)
Nakapiripirit	10	3.0 (0.88)	319	91.5 (1.42)	16	4.3 (0.87)	5	1.3 (0.64)
Nakasongola	26	7.3 (1.45)	307	91.5 (1.69)	8	1.1 (0.70)	1	0.1 (0.12)
Total	91	5.3 (0.66)	1699	91.4 (0.78)	46	1.9 (0.36)	28	1.4 (0.32)

No., number of reactors

SE, standard error

The difference of BTB reactors in different age groups is shown in Table 2. Variations in skin reactions to avian and bovine tuberculins with age group are depicted in Fig. 2. No positive reactors were recorded beyond 12 years and doubtful reactions were seen to increase with age (Table 2 and Fig. 2).



**Fig. 2** Variations of skin reactions to comparative intradermal tuberculin test with age

**Table 2** Distribution of individual bovine tuberculosis reactors by district, age group and sex in cattle in the study areas, with prevalence adjusted for primary sampling units and weighting using the survey procedure in Stata

Variable		Number tested	Positive	Prevalence (SE)
District	Kotido	717 (14 S/herds) <sup>a</sup>	18	2.6 (0.65)
	Moroto	455 (9 S/herds)	4	1.1 (0.49)
	Nakapiripirit	350 (7 S/herds)	5	1.3 (0.64)
	Nakasongola	342 (7 herds)	1	0.1 (0.12)
Age	<4 years	377	0	0.0 (0.0)
	4–6 years	692	10	1.5 (0.48)
	7–9 years	528	9	1.6 (0.48)
	10–12 years	181	9	3.5 (1.43)
	13–15 years	64	0	0.0 (0.0)
	16–18 years	17	0	0.0 (0.0)
	19–21 years	4	0	0.0 (0.0)
Sex	Female	1504	20	1.3 (0.40)
	Male	360	8	2.0 (0.89)
Total		1864 (337 herds)	28	1.4 (0.32)

<sup>a</sup>S/herd, superherd;  
mean = 26 herds; mean herd size = 95 cattle

Further statistical analysis of individual animal data with adjustments for sampling stratum (district), showed a clear effect of age group on occurrence of positive reactions for BTB. The odds ratios of an animal being a positive reactor to BTB test in different age groups compared to that in the first quartile (1–3 years) are shown in Table 3. More males were positive (OR = 1.99), but the statistical effect of this was weak owing to the small number of males tested (Table 3).

**Table 3** Individual animal risk factors and odds of occurrence of positive bovine tuberculosis reaction estimated with the survey logistic regression procedure in Stata.

	O.R	95% CI
<4 years (first quartile)	1.00	–
4–6 years (second quartile)	5.91	1.25–28.03
7–8 years (third quartile)	7.29	1.53–34.65
9–22 years (fourth quartile)	8.14	1.76–37.52
Sex (male vs female)	1.99	0.86–4.59

Classifying any herd with  $\geq 1$  test positive animals as a positive herd showed a major difference between districts, with herd prevalence ranging from 14.3% (Nakosongala) to 78.6% in Kotido (Table 4).

**Table 4** Distribution of tuberculin-positive reactor herds ( $\geq 1$  test-positive animal) in different districts.

District	Herds tested	Number positive	Percent positive	SE
Kotido	14	11	78.6	11.4
Moroto	9	4	44.4	17.5
Nakapiripirit	7	3	42.9	20.2
Nakasongola	7	1	14.3	14.2
Total number of herds tested	37	19	51.4	7.6

## Discussion

The study found that the occurrence of bovine tuberculin reactors in individual cattle in transhumant areas of Karamoja region and Nakasongola district was low (Table 2) and did not differ from previous findings in the East African region of Tanzania (Kazwala *et al.*, 2001).

The odds of failing a tuberculin test were respectively 5.91, 7.29 and 8.14 times higher in the 4–6 year, 7–8 year and 9–22 year age groups than in the 1–3 year age group (Table 4). This is suggestive of the chronic nature of BTB. The observed odds ratio of disease in corresponding age groups was lower than reported in an intensive management system (Benham, 1985), further suggesting low transmissibility in an extensive system in the transhumance areas. The delayed onset of tuberculin positivity at 3.5 years of age (Table 2 and Fig. 2) could be attributed to the long incubation period (15–34 months) (Perez *et al.*, 2002), pre-allergenic status (Stabel and Whitlock, 2001) and/or partly acquired or maternal immunity (Kazwala *et al.*, 2001). Lack of response or doubtful response beyond 10 years could be due to anergic status and the presence of calcified lesions that could not stimulate the animal to levels that give a positive response to tuberculin (Benham, 1985).

High response to avian PPD was observed in cattle 7–9 years old and doubtful reactions in cattle older than 10 years of age (Fig. 2). This could be due to a non-specific immune response to environmental mycobacteria of the *Mycobacterium avium* complex in the natural water sources



from which these herds were drinking (Grange, 1987; Phillips *et al.*, 2003). The shift of the response from avian in early years to doubtful tuberculin status in later years (Fig. 2) is in accordance with the theory that environmental mycobacteria outside the *M. avium* complex induce non-specific immune responses mainly directed towards bovine PPD and thereby overwhelm the effect of contamination by *M. avium* complex with increasing age (Lauzi, 2000). Sex of reactors was found a weak risk factor in the occurrence of BTB (Table 2).

In contrast, herd prevalence in the three different districts in Karamoja was very high, from 40% in Moroto and Nakapiripirit to almost 80% in Kotido (Table 4). This was attributed to high herd interactions in the Karamoja region that could have enhanced spread of disease between herds (Lauzi, 2000). Interactions lead to more efficient contacts and to a higher degree of microbial distribution in the herds and environment. An infected animal introduced into a single herd in this region therefore becomes potentially important in seeding the population (Lauzi, 2000).

A crucial element here is the high specificity attributed to test-positive animals. With the low number of positives, many of the herds were classified as positive on the basis of one positive animal. However, previous experience with BTB testing clearly indicates that any animal group with at least one animal with test result of >4mm is a BTB group. In Nakasongola district, although big herds were equally involved, sharing of communal grazing and watering areas was limited to cattle of close relatives and nearby areas within 3–5 km<sup>2</sup>, and this could explain the lower prevalence of positive herds in this district.

Factors such as congregation of herds in grazing and watering areas, merging of different cattle herds during migration, high cattle density in cattle camps, overcrowded night kraals, large herd sizes (mean = 95 cattle) and cattle rustling could explain the high prevalence of positive herds in Karamoja districts (Neill *et al.*, 1989; O'Reilly and Daborn, 1995). Of special importance are the rampant armed cattle raids between ethnic groups for enhancement of socioeconomic status, which move cattle from one district or area to another. Between January 2003 and June 2004, 11 380 cattle were raided within and between Kotido and Moroto districts. These animals are normally distributed among raiders, who will in turn take them to their own herds. Stolen cattle can be spread over an area of 1200 km<sup>2</sup>. The victims also raid other less-protected kraals. Under this pastoral system and human activity, if *M. bovis* is present, the disease would soon be widely distributed (Neill *et al.*, 1989), and this could partly explain a high herd-level and low individual cattle prevalence in Karamoja region.

Adverse weather conditions such as high environmental temperatures (30–35°C), strong direct sunlight (specifically ultraviolet light) and dry weather conditions as experienced in Karamoja (500–700 mm) (Karamoja-Data-Centre, 2004) and Nakasongola (875 mm) are capable of reducing the survival of the bacilli in the environment (Menzies and Neill, 2000; Phillips *et al.*, 2003) and the likelihood of its transmission (King, 1999; Phillips *et al.*, 2003). Dry conditions also affect the critical size of aerosol droplets necessary to establish an infection (Chambers *et al.*, 2001) and diminish the efficiency of infections by the respiratory route (O'Reilly and Daborn, 1995), which is of most importance in BTB transmission. Under these conditions, few *M. bovis* organisms would survive for long at concentrations above the minimum number of particles required to establish infection (Menzies and Neill, 2000). Under such conditions, low-dose exposure may be common; with the animals' non-specific immune mechanisms eliminating the mycobacteria before infection becomes established and cell-mediated immunity is activated (O'Reilly and Daborn, 1995). A substantial proportion of cattle are able to mount an effective protective response to *M. bovis* exposure (Smith and Wiegesshaus, 1989; Dannenberg, 1991). This might explain the low individual cattle tuberculin reactor prevalence (Table 2) despite the lack of any control programme and the presence of several different risk factors (Neill *et al.*, 1989; O'Reilly and Daborn, 1995).

It appeared that the agroecological zones influenced the spatial distribution of grazing areas in different districts. This in turn seemed to influence the migratory patterns and grazing behaviour, leading to clustering of animals in low-lying areas during dry months of the year. Tuberculin reactor distribution appeared to follow this clustering, which was closely linked to availability

of water (Karamoja-Data-Centre, 2004) and grazing areas in the districts studied (Table 4 and Fig. 2). The influence of water sources on the reactor status was apparently not observed in Bokora County study area in Moroto and Nakasongola, where animals drank water from boreholes and lakes, respectively during the dry season (Table 1). These areas recorded lower herd and individual cattle tuberculin reactor prevalence compared to other animals that drank from valley dams and isolated stagnant water sources or mud holes in dry riverbeds (Tables 1, 2 and 4 and Fig. 1). Such stagnant water sources are a potential source of infection (Dailloux *et al.*, 1999), but exposure to environmental mycobacteria from these sites could also have the beneficial effect of inducing non-specific reactions to bovine and avian tuberculin, which could offer some protection (Phillips *et al.*, 2003).

A high stock density in Karamoja was also observed to cause overgrazing of limited pasturage around watering points in communal grazing areas without adequate time for resting. It also caused heavy contamination of water sources with faeces. These practices could increase the chances of exposure to infective materials, including *M. bovis* bacteria that may not be destroyed by sunlight under these conditions (Faries and Davis, 1997; Sheffield *et al.*, 1997; Phillips *et al.*, 2003). High herd contacts also allow susceptible animals within their populations to become exposed. Alternatively, contact may also expose animals at critical times when they are susceptible, e.g. during drought periods when nutrition is very poor (Griffin *et al.*, 1993). This could be contributory to a high herd prevalence.

The contribution of feral reservoirs in maintaining the infection in this environment (Menzie and Neill, 2000) cannot be ruled out. Several feral species, including antelopes, buffaloes and bush pigs, were sighted in the grazing areas and were often hunted for meat, but indirect contacts through limited water sources in dry season could play an important role.

In conclusion, despite the presence of several documented risk factors favouring transmission of BTB coupled with lack of attempts to control the tuberculosis in cattle in the transhumance system in the study area, the prevalence remains low. This could be attributed to unfavourable environmental conditions for transmission of the agent, increased host resistance and probably low agent virulence. It contrasts with the tuberculosis infection amongst the Ankole long-horned cattle, which had higher individual animal prevalence rates (Benham, 1985). However, determination of the prevalence of tuberculosis lesions in slaughter cattle and characterization of mycobacterial isolates need to be done.

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