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Risk factors for herd-level bovine-tuberculosis seropositivity in transhumant cattle in Uganda

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Abstract

We investigated the prevalence and risk factors to positive herd-level tuberculin reactivity between October 2003 to May 2004 to bovine tuberculosis (BTB) in the four transhumant districts of Uganda: three districts (Karamoja region) of nomadic transhumance cattle rearing (30 superherds and 1522 cattle), and one district (Nakasongola) of fixed-transhumance (7 herds and 342 cattle). We used the comparative intradermal skin-test, sampled 50 animals per superherd/herd, and considered herd positive if there was at least one reactor. Of the 30 superherds under nomadic transhumance, 60% (95% CI 41.4, 79) were tuberculin-test positive; of the 7 fixed herds, 14.3% (95% CI −20.7, 49.2) were tuberculin test positive. The true herd prevalence was estimated at 46.6%. Many risk factors were collinear. The final multivariable logistic-regression model included: recent introductions from market (OR = 3.4; 95% CI 1.1, 10.3), drinking water form mud holes during dry season (OR = 49; 95% CI 9.1, 262), and the presence of monkeys (OR = 0.08; 95% CI 0.0, 0.6) or warthogs (OR = 0.1; 95% CI 0.0, 0.3). No association was found between herd size or number of herd contacts with reactors; it was probably masked by the effect of high between-herd interactions. Provision of

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water from mud holes in dry river beds and introductions of new animals are risk factors that might be targeted to control BTB in transhumance areas.

Keywords: Bovine tuberculosis; Nomadic; Transhumance; Risk factors; Epidemiology; Uganda

1. Introduction

Bovine tuberculosis (BTB), a zoonotic disease of cattle, still remains a cause of concern for livestock and human health in the developing world (Cosivi et al., 1998). Although there are reports of increasing opportunistic infections with zoonotic and environmental mycobacteria in developing countries as a result of HIV (Biet et al., 2005; Cosivi et al., 1998; Daborn and Grange, 1993), the exact contribution of BTB to this is not known. A direct correlation exists between *Mycobacterium bovis* infection in cattle and disease in the human population (Cosivi et al., 1998). This information in Uganda, however, in sketchy because the basic surveillance and national public control programs on BTB (Cosivi et al., 1995, 1998) are inadequate or unavailable and therefore, many epidemiologic and public health aspects of disease remain largely unknown.

Information on the impacts of different farming systems on the prevalence of BTB in Uganda is equally limited. The available information is based on passive-surveillance data from abattoirs from regions that are active in recording and submitting annual cattle-slaughter reports. In pastoral areas, the accuracy of this information is contestable because, undocumented home slaughters which often involve sick or emaciated unsaleable animals, run in parallel. This means that national records on BTB are representative of neither the different farming systems, nor the true status of the country's different regions. Abattoir slaughter reviews (Anon., 1990) based on gross pathological lesions, found 1.8% of slaughter animals originating from the pastoral eastern regions (including Karamoja) showing generalized tuberculosis. These figures are only underestimates because of limitations of sensitivity of gross examination at post mortem (Acen, 1991).

A recent study (Faye et al., 2005) in the mixed livestock-farming districts of western Uganda reported 6.0% and 74.1% individual-animal and herd prevalence, respectively. No similar work has been done in other districts or farming systems, despite abattoir evidence of presence of tuberculous lesions in slaughter animals (Anon., 1990). A public-health burden of BTB transmitted from cattle to humans has been documented, and reports from various countries show the proportions of *M. bovis* in sputum-positive TB patients to vary between 0.4 and 6.4% (Cosivi et al., 1998; Kazwala et al., 2001; Mfinanga et al., 2003). There is also extensive literature on transmission of BTB between and within cattle herds (Phillips et al., 2003) and livestock and wild life (Parra et al., 2003; Phillips et al., 2003; Schmitt et al., 2002; Simpson, 2002) and livestock and man (Cosivi et al., 1998; O'Reilly and Daborn, 1995; Organ, 1994). Pastoral communities are therefore at high risk because they live in extremely close contact with their animals and raw milk forms major part of their daily diet (and rare or raw meat, occasional part).

Although *M. bovis* transmission is reportedly low under such extensively managed systems compared to intensive ones (Costello et al., 1998; Menzies and Neil, 2000; Morris et al., 1994; Phillips et al., 2003), practices that favour transmission, such as sharing of communal grazing and watering areas by livestock from different areas or herds (Phillips et al., 2003), provision of water from stagnant sources heavily contaminated with animal waste (Biet et al., 2005; Dailloux et al., 1999), and overcrowding in night enclosures (O'Reilly and Daborn, 1995) are routine farm-management practices 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).

Our aim was to identify and quantify risk factors for herd reactivity to tuberculin in transhumant cattle in the important transhumant communities in Karamoja and Nakasongola districts of Uganda.

2. Materials and methods

2.1. Description of study area

This study was conducted from October 2003 to May 2004 in four transhumant districts in two geographical areas: The Karamoja region comprising of Kotido, Moroto and Nakapiripirit districts (north-eastern) and the Nakasongola district (mid-central region) in Uganda. The details of the study areas are given elsewhere (Oloya et al., 2006). The Karamoja region was chosen following reports of cases of TB-like lesions (tubercles) in slaughtered animals in the abattoirs. Furthermore, many documented risk factors for BTB transmission and persistence (Phillips et al., 2003) are common in the region's nomadic transhumance farming system, besides a strong cultural tradition of pastoralists consuming raw meat and unprocessed milk mixed with blood. An assessment of risk factors for BTB as a prerequisite for better understanding of the disease dynamics in pastoral cattle was deemed necessary. Nakasongola District, an example of a fixed transhumance system, was chosen for comparison.

2.2. Management system

The Karamoja farming system is nomadic transhumance due to harsh and unpredictable climatic conditions (Anon., 2004). Nomadic transhumance involves movement of livestock to follow grazing and water over considerable distances following set seasonal patterns (with the part of family of herders living in temporary shelters which move with the herds all the year round). The major animal species kept include short horned zebu cattle, sheep, goats and donkeys. Two phases of grazing management are recognised: the residency and migratory phases. In the residency phase in the wet season, animals are kept in night kraals that in most cases are shared between three to seven close relatives or clan members. In areas with many cases of armed cattle raids, night kraals (7–35 m in diameter) may be shared between two to seven herds (mean herd size = 98; 95% CI 81, 114) to increase security. The animals are released in the morning and the herds graze separately during the day in communally owned rangelands of 14–170 km^2 around homesteads. During the migratory phase in the dry season, tens of herds (15–46 herds) are organized into mobile herding groups, called superherds. Superherds move together under the leadership of kraals leaders and seers, migrate together as a unit, and share grazing areas and watering sources along the way. About 10% of the milking animals might be left behind to provide milk for home upkeep.

Migratory distances vary from 10 to 120 km (Paul Lochap, Veterinary Officer, Kotido, personal communication) and the routes follow rivers or water sources. Once an area is chosen for settlement, a camp is constructed. It consists of one or several large enclosures (50–100 m in diameter) and containing small thorn partitions of (7–10 m in diameter) for individual herds Animals are not supplemented and they receive little veterinary attention.

In Nakasongola, a fixed transhumance is practiced in which seasonal livestock movement to lowlands or lake shores in the dry season in search of water and grazing and back to settlements at the start of rainy season, are practiced. The herders have permanent homes and only the herds and a subset of people necessary to tend them travel. Although relatives pool animals, few families (2–3) are usually involved because of large herd sizes (mean = 105, 95% CI = 38–173) and the cattle do not form camps during migration. Grazing is usually limited to an area of about 4 km^2, and restricted to owned or rented land.

2.3. Study design

The survey was planned to cover all three districts (Kotido, Moroto and Nakapiripirit) of Karamoja region to control for influence of residency and migratory areas on the occurrence of BTB in all resident cattle population in domestic grazing areas. Due to the high level of herd interactions, a two-stage sampling was planned. Larger units or superherds were considered as primary sampling units while herds within them were secondary sampling units. A superherd was defined in this study as groups of herds belonging to the same cattle camp in the dry season (September to March) or those identified as sharing communal grazing and watering areas during wet season and belonging to a same cattle 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-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 with a 95% confidence interval (Dohoo et al., 2003). However, the sampling plan encountered problems due to lack of comprehensive information on distribution of animal populations in transhumant communities, owing to seasonal movements, multilevel herd contacts and ‘on-and-off’ cattle-camp system. The unpredictable inflow and outflow of cattle from raids, erratic reductions due to diseases and drought also made it difficult to give reasonable herd estimates. Cultural belief that does not allow one to count and reveal the exact number of herds or animals in herd was further seen to make estimates 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 of Karamoja. 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 minimise double sampling of interacting herds.

2.4. Selection of herds and animals

A 10% proportional allocation of the required number of superherds to three districts was done according to number of kraal leaders listed in each. Out of 315 superherds listed, 14 out of 147 superherds listed were selected from Kotido district, 9 out of 94 from Moroto district and 7 out of 74 from Nakapiripirit District. Independent grazing areas were identified from grazing maps and information provided by the respective District Veterinary Offices. In total, 30 non-apposing or independent grazing areas (Oloya et al., 2006) for superherds together with the names of their kraal/camp leaders were identified. It was not possible to get the whole list of superherds due to non-compliance of some kraal leaders during the previous vaccination programme. In Nakasongola, seven herds from seven different villages were also selected by a table of random numbers at each village level for purposes of comparison. Fifty animals per herd were selected by numbers generated by a table of random numbers.

Within the superherds, individual herds were identified. A list was drawn and one herd was selected by lottery system to represent the superherd. A total of 50 animals were selected as mentioned above. Overall 1522 and 342 animals were tested in district(s) of Karamoja and Nakasongola, respectively. In all the study areas, calves $\leq$6 months and recently calved cows (2 months post-partum) were exempted from the study because of high maternal antibodies and immunosuppression that desensitises them to tuberculin, respectively (Kazwala et al., 2001).

2.5. Tuberculin testing

TB testing was done using purified protein derivative (PPD) (Animal Sciences Group, Wageningen, The Netherlands). Volumes of 0.1 ml of 30,000 and 25,000 IU/ml of Bovine and Avian PPD, respectively, were used. The technical details of the test procedure of the comparative intradermal test readings were as described (Monaghan et al., 1994). An animal was classified as positive if the swelling of the skin fold at the bovine site was greater than at the avian site by 4 mm. Herds were classified as tuberculosis positive if they had at least one positive bovine reactor animal (Kazwala et al., 2001).

2.6. Herd sensitivity and specificity

The sensitivity (Se) of 0.80 (Faye et al., 2005) and specificity (Sp) of 0.96 (Monaghan et al., 1994) of the tuberculin test were used to calculate Herd sensitivity $(\text{HSe}) = 1 - (1 - \text{AP}_{\text{pos}})^n$ and Herd specificity $(\text{HSp}) = \text{Sp}_{\text{ind}}{}^n$ as described (Dohoo et al., 2003). AP_{pos} and Sp_{ind} are apparent individual-animal prevalence and individual specificity, while n is number of animals (50 cattle) tested per herd. A low Sp was used in the calculation owing to high proportion of non-specific reactors reported in similar farming system (Faye et al., 2005). The approximate unbiased estimate of true Herd prevalence (P) was determined using the Rogan–Gladen estimator (P_{RG}) (Greiner and Gardner, 2000): with HAP, HSe and HSp substituted for AP, Se and Sp:

$$P(\text{herd}) = \frac{\text{HAP} + \text{HSp} - 1}{\text{HSe} + \text{HSp} - 1}.$$

2.7. Epidemiological information

Herd-level information on general herd management, herd profile, breed, history of BTB and cattle movements in and out of the herd, disease outbreaks, other animal species kept, types and levels of herd contacts, water sources (during residency and migratory phase), possibility of wild-animal contacts and management practices considered potential risk factors to prevalence of BTB was captured from 37 superherds/herds with a structured pre-tested questionnaire. Herd contacts were defined as sharing kraal, grazing or watering areas and belonging to same superherd during residency or belonging to a cattle camp, sharing grazing and watering areas during the 7–9 months of the migratory phase. Area veterinarians fluent in local languages, knowledgeable on local farming practices and who had received prior training on the administration and the scope of questions supported by the author, guided farmers or kraal leaders on the interview.

The questionnaires consisted of open and semi-open questions. The questionnaire was pre-tested on three superherds in Kotido to assess the clarity of questions and adapted to capture some aspects of management initially missed. Templates were designed in Epi Info to ensure uniformity in entries after all variables were coded for all possible responses.

2.8. Statistical analysis

Herd-level data from questionnaires were merged and handled using Epi Info™ (2002) and Microsoft Excel®. The data were cleaned by checking for missing data and incorrect entries and exported to Stata 9/SE (Stata Corp., College Station, TX) for analysis. The outcome of all statistical analyses was the herd-level outcome, defined as having at least 1 positive tuberculin reactor in the herd. All independent variables were tested by tabular analyses (for categorical variables) or Wilcoxon rank-sum test (for ranked variables) versus the outcome (herd test positive or negative). All variables with either a high biological relevance (depending on current knowledge) or a p-value of ≤ 0.20 from univariable analyses were offered as candidate variables to the logistic regression for model building.

Collinearity among variables was evaluated by further graphical examination and cross-tabulation of candidate variables. Two explanatory variables with dichotomous outcomes were considered collinear if when cross tabulated had p-value ≤ 0.05. We tested the 2×2 interactions of variables from the final model as a group using the Likelihood ratio test. A final multivariable logistic model with herd status as outcome variable was constructed using a county (representing agro-ecological zone determining the migratory system) as random effect and forward-selection procedure (Dohoo et al., 2003). Models were compared using the ‘likelihood ratio test’. The model fit was assessed using Hosmer–Lemeshow statistics.

Table 1
Distribution of reactor cattle herds and other hypothesized attributes associated with tuberculin herd positivity in 37 cattle herds in Uganda

Variable	Level	No. of herds	Percent tuberculin reactors
District (three Karamoja districts)	Kotido	14	79
	Moroto	9	44
	Nakapiripirit	7	43
	Nakasongola district	7	14
Farming system	Nomadic transhumance	30	60
	Fixed transhumance	7	14
Breed[a]	Karimojong Zebu	30	60
	Long horned Ankole	7	14
Cattle herded with donkeys[b]	Yes	7	86
	No	30	43
Cattle herded with sheep[a]	Yes	23	61
	No	14	36
Straying to into other herds[c]	Yes	31	45
	No	6	83
Rearing cattle for others[b,d]	Yes	24	63
	No	13	31
Shared herd movement during transhumance	Yes	32	56
	No	5	20
Shared night cattle shelters[b,d]	Yes	25	64
	No	12	25
Communal grazing	Yes	34	56
	No	3	0
Number of herds in contact	≥18	18	56
	<18	19	47
Herd size (median)[b]	≥91	17	53
	<91	20	50
Purchase of animals	Yes	19	63
	No	18	39
Water from wells during wet season[b]	Yes	5	100
	No	32	44
Water from patches or stagnant dirty water sources or mud holes in dry river beds	Yes	19	74
	No	18	28
Presence of warthogs	Yes	22	41
	No	15	67
Presence of monkeys	Yes	13	23
	No	24	67
Presence of guinea fowls[b,d]	Yes	21	38
	No	16	69

p-Values from Fisher's exact test (categorical variables) or Wilcoxon rank-sum test (ranked variables).

[a] Variables dropped due to collinearity with water from patches or stagnant dirty water sources or mud holes in dry river beds.

[b] Variables dropped due to collinearity with market source.

[c] Variables dropped due to collinearity with warthogs.

[d] Variables dropped due to collinearity with monkeys.

Table 2
Final multivariable logistic regression model of herd-level risk factors for prevalence of BTB in 37 transhumant cattle herds in Uganda

Variable	*b*	S.E.(*b*)	*P*	OR	95% CI
Purchase of animals (yes vs. no)	1.23	0.57	0.03	3.4	1.1, 10.3
Drinking water from stagnant dirty water sources or mud holes in dry river beds vs. flowing water from streams/rivers during dry season	3.89	0.86	<0.001	48.9	9.1, 262
Monkeys (presence vs. absence)	−2.54	1.00	0.01	0.08	0.01, 0.56
Warthogs (presence vs. absence)	−2.16	0.52	<0.001	0.12	0.04, 0.32
Constant	−0.50	0.91	0.58	–	–

Deviance = 25.5 d.f. = 32 model $p = 0.00$, Model based upon variables from Table 1 with a p-value <0.05 in Fischer or Wilcoxon rank-sum test.

3. Results

Of the 37 cattle herds (including 30 proxies for superherds) tested, the computed true herd prevalence was 49%, varying between Kotido (76.6%), Moroto (42.2%), Nakapiripirit (40.9%) and Nakasongola (12.3%). The percentage of cattle herds affected per district, and other putative risk factors are given in Table 1. Univariable analysis demonstrated a large number of factors possibly associated with BTB-positive herds, as shown in Table 1. However, uniformity in the management systems caused high collinearity between many risk factors.

The final multivariable model (Table 2) identified risk factors analysed as introduction of new animals from other sources, especially purchase of animals and the provision of water from mud holes or dirty water patches in river beds during dry season as important predictors for positive herd tuberculin reactivity. Presence of monkeys and warthogs was strongly negatively correlated with the presence of BTB reactors (Table 2). The Hosmer–Lemeshow statistics indicated acceptable fit ($p = 0.8$).

4. Discussion

Our observation that 40.9–76.7% of the superherds in Karamoja studied had at least one reactor animal to tuberculin suggests that the prevalence of BTB in the nomadic transhumant cattle population of Karamoja region was fairly high and widespread compared to 14% of the herds in the fixed transhumance in Nakasongola (Table 1). The findings in Karamoja region with a low individual-animal prevalence (Oloya et al., 2006) were contrary to what has been reported in neighbouring Tanzania where both herd and individual-animal prevalence were high (Kazwala et al., 2001). Several documented risk factors reported earlier as favouring transmission between and within herds (Phillips et al., 2003) were confirmed in our study. However, their roles in positive tuberculin reactivity could not be assessed due to collinearity caused by similarities in management factors and other explanatory variables.

Our study population in Karamoja region could best be described as consisting of zonal floating sub-populations with neither spatially nor temporally fixed locations nor defined herd sizes. Differently from what has been reported (Jiwa et al., 1997; Kaneene et al., 2002; Menzies and Neil, 2000), the individual effect of herd size and contacts on BTB tuberculin reactivity were not profound ($p = 0.86$ and 0.62, respectively) and could have been lost through these high herd interactions and mergers during residence and migratory phases, respectively. In this study, the influence of composite environmental and management factors in the entire grazing cycle were probably more relevant than herd size or herds in contact.

The high stock density during the drier months of the year caused overgrazing of the limited pasturage around watering points in grazing areas without adequate time for resting and heavy contamination of water sources with animal wastes. Presence of such organic matter in muddy or stagnant water sources favors survival of mycobacteria (Iivanainen et al., 1999) and render them potential sources of BTB infection (Dailloux et al., 1999; Kaneene et al., 2002). This too, could be crucial in the establishment of endemic TB in resident cattle populations (Shirima et al., 2003).

We observed that within Karamoja region, Kotido district with most of their herds drinking water from stagnant dirty water sources in mud holes in the riverbeds or heavily soiled valley dams in dry seasons were more likely to be tuberculin seropositive (Table 2) in comparison to Moroto and Nakasongola districts whose herds drank water from boreholes and rivers, respectively. These areas also recorded lower individual animal-level prevalence (Oloya et al., 2006) compared to others.

Although exposure to environmental mycobacteria from these contaminated water sources could have beneficial effect of inducing some protection (Phillips et al., 2003), in our study, it could have interfered with the specificity of tuberculin skin-test diagnosis (Monaghan et al., 1994). This was evident in our study, where 5.3% ($n = 91$) and 1.9% ($n = 46$) of animals tested were classified as avian and suspicious reactors, respectively.

High stock density observed in Karamoja region could increase the chances of exposure to infective materials before the *M. bovis* bacteria are destroyed by sunlight (Faries and Davis, 1997; Phillips et al., 2003; Sheffield et al., 1997). In Nakasongola districts, although big herds were equally involved, level of herd interactions were comparatively limited. Sharing of communal grazing and watering areas were limited to close relatives and areas varied from 3 to 5 km^2.

Introductions of new animals was one important risk factor identified in our study, and many cattle owners reported new purchases, even though we believe that purchase was not a common practice. Information from local area veterinary sources, attributed use of word 'purchases of cattle' as a euphemism for animals stolen in raids. The effect of armed cattle raids was difficult to ascertain due to its sensitive nature, but raids between ethnic groups for enhancement of socioeconomic status were common practices. Raided animals are distributed amongst raiders. In such situations, if present, the infection would be introduced to new areas and herds (Phillips et al., 2003).

High levels of stress and herd contacts coupled with poor nutrition during the migrations in dry season could further allow susceptible animals to get infected (Griffin et al., 1993).

We did not investigate the role of wild animal populations in the epidemiology of BTB in the study areas, however, species such as antelopes, buffaloes and wild pigs, that are reported as potential reservoirs for *M. bovis* (Michel et al., 2006; Phillips et al., 2003), were sighted in the grazing areas. Their level of contact with infected herds could not be qualified but infection of domestic stock through contaminated pasture and water has been documented (Phillips et al., 2003). Our study only revealed a positive BTB-seropositivity link to herds in areas characterised by absence of monkeys and warthogs during dry season, a dominant species during rainy season, an association we cannot explain, but their presence during the wet season and strong preference for lowlands and watering places for herds could be suggestive of existence of sets of local conditions in those areas that are both conducive for their survival and transmission of the agents or are proxies for unidentified factors.

It should be mentioned that diseases of long incubation period and chronic nature (like BTB), are easy to quantify in cross sectional studies (Kelsey et al., 1996). However, it may be difficult to fully explain the role of individual risk factors observed, due to wide temporal and spatial variations in the exposure risk (Iivanainen et al., 1999). The design and objectives of our study did not allow for a detailed evaluation of the effect of environmental factors, e.g. temperature humidity, etc., on herd prevalence. However, details of the effect of this on individual-animal level are discussed elsewhere (Oloya et al., 2006). One weakness of the present study is the small data set of 37 herds. This arose mainly because large, highly clustered populations with uniform management practices were involved. Not much variable or additional information would have been generated by inclusion of more superherds. We anticipated more variations in the geographical location than aspects of management of herds, a main reason we put emphasis on the districts. The other weakness was a failure to define an effective contact necessary for disease transmission. Though broadly classified as belonging to the same superherd, duration and intensity of herd and individual animal contacts were highly variable and difficult to quantify and more so made difficult in chronic disease situation like BTB. The individual effect of migratory or residency areas could also not be verified due to the chronic and subclinical nature of BTB infections. The uniformity of the management system with resultant distribution of or exposure to potential risk factors made it difficult to evaluate their roles individually.

5. Conclusions

In conclusion, the herd-level true prevalence of BTB-positivity in transhumant cattle is 49%. The main findings of the study were strong associations between watering practices and positive herd reactivity with also higher odds for those introducing animals into their herds. On the other hand, a negative association was found between a few selected wildlife contacts and reactors.

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