Bovine tuberculosis and brucellosis in Livestock at the Greater Ruaha Ecosystem

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SUMMARY

A cross-sectional study was conducted to determine the prevalence of bovine tuberculosis (BTB) and the seroprevalence of brucellosis in livestock at the Greater Ruaha Ecosystem in Tanzania. The study further characterized the *Mycobacterium* spp. from the slaughtered livestock. A questionnaire survey conducted to assess potential herd-level risk factors for BTB and brucellosis revealed that the respondents' ethnicity and herd mixing were the significant risk factors. Twenty-eight percent of 102 cattle herds had at least one positive or suspect BTB reactor. The overall prevalence of BTB infection in the cattle was 1.32% (18/1368). Forty-two percent of 93 flocks of the small ruminants had at least one brucellosis seropositive animal. The overall seroprevalence of brucellosis in the cattle and small ruminants was 6.6%. Although the prevalence of both diseases was relatively low for individual animals, herd-level prevalence was high suggesting that infection is widespread in the study area and a significant number of households are at risk. *Mycobacterium bovis* strain identified via polymerase chain reaction (PCR) was confirmed by spoligotyping as spoligotype SB0133. This cattle strain of *M.bovis* was similar to previously reported involving wild animals in adjacent protected areas. Isolation of identical *M. bovis* from wildlife and livestock and the demonstration of *Brucella* spp. seroprevalence in livestock in the same interface, strongly suggest livestock-wildlife interspecies sharing of these pathogens. Occurrence of the microorganisms poses a serious challenge to disease management strategies in pastoralist communities in the interface area.

Keywords: Bovine tuberculosis, Brucellosis, *Mycobacterium* spp., Risk factors, Zoonoses

INTRODUCTION

Traditional livestock keeping is an important socio-economical enterprise in many developing countries with large grazing areas of arid and semi-arid land. However, pastoralist communities are often economically and politically marginalized (Lane 1994), and face many human and animal health challenges due to their mobility and limited access to medical and veterinary services (Schelling *et al*., 2007).Additionally, cultural practices of pastoralist and agro-pastoralist communities, including the consumption of blood, unpasteurized milk, or under-cooked meat, and housing of livestock within human dwellings pose the risk of exposure to zoonotic diseases from livestock herd to humans (Cleaveland *et al*., 2007). Drought may also enhance zoonotic disease transmission in pastoralist communities because pastoralists, livestock and wildlife are forced to share the same grazing areas and water holes (Mazet *et al*., 2009).

Bovine tuberculosis (BTB) and brucellosis are two zoonotic diseases whose transmission from livestock to humans

may be enhanced by pastoralist practices and environmental change. Bovine tuberculosis, is an important disease of livestock and an emerging threat to public health (Cosivi *et al*., 1995) caused by *Mycobacterium bovis* (*M. bovis*); a gram positive acid fast bacillus closely related to the causative agent of human tuberculosis, *M. tuberculosis*.

Although most prevalent in cattle, *M. bovis* can infect and be transmitted among multiple livestock and wildlife species (Clifford *et al*., 2013). Over 95% of BTB infections are transmitted via respiratory secretions, however,1-5% of infected cattle may shed *M. bovis* in milk, feces or urine (Menzies and Neill 2000), thereby infecting humans through contact or consumption of contaminated milk, blood or meat (Cosivi *et al*., 1995).Furthermore, transmission risk is greater in countries with high HIV/AIDS prevalence, as tuberculosis is a major opportunistic infection in HIV/AIDS infected persons.

The vast majority of people with dual HIV and tuberculosis infections live in African countries (Raviglione *et al*., 1995). Nearly 40,000 new cases of tuberculosis in humans and cattle are diagnosed per year in Tanzania (Jiwa *et al*., 1997)and up to 77% of Tanzanian tuberculosis patients are also infected with HIV (Range *et al*., 2001). The extra-pulmonary form of tuberculosis (EPTB) in humans, often associated with BTB infection from animal products, accounts for 17% of the cases reported in Tanzania (Jiwa *et al*., 1997). M*ycobacterium bovis* has been isolated from seven of 31 (16%) TB and EPTB human cases from two regions of Tanzania (Kazwala *et al*., 2001a).

Brucellosis is another bacterial disease of public health and economic importance for livestock. Infection with the gram negative intracellular *Brucella abortus* (bovine), *B. melitensis* (ovine and caprine), and *B. suis* (porcine) cause abortions, placentitis and orchitis, with serious economic impact for livestock keepers. Multiple *Brucella spp.* infect wildlife and can be transmitted between wildlife and livestock (Godfroid*et al*., 2011). *Brucella spp.* is transmitted to humans via consumption of raw milk or upon direct contact with placental fluids, aborted fetuses, and uterine discharges from infected animals. Symptoms in humans include: undulating fever, lethargy, night sweats, headaches, and arthralgia (Franco *et al*., 2007). Despite being the most common human zoonotic bacterial infection worldwide, brucellosis remains under-diagnosed and under-reported, especially in developing countries. Brucellosis prevalence in pastoralist livestock from livestock-wildlife interfaces in Tanzania ranged from 1.6 to 6.9% (Karimuribo *et al*., 2007) and that of the livestock in agro-pastoralist and dairy production systems was found to range from 1.6 to 15.6% (Shirima and Kunda, 2016). Studies quantifying human brucellosis prevalence are limited in Tanzania and physicians do not have adequate the expertise needed to recognize such cases (John *et al*., 2010).

Brucellosis seroprevalence in a small sample of volunteers from the Tanga region of Tanzania was 5.5%, with abattoir workers more likely to be exposed (Swai and Schoonman 2009). A recent study in the human-wildlife-livestock interface in the Katavi-Rukwa ecosystem in Tanzania, has reported comparatively lower seroprevalence of brucellosis in volunteers from the community (Assenga *et al*., 2015). Assisting livestock parturition was identified as a major risk factor for human transmission of brucellosis in northern Tanzania (John *et al*., 2010).

The Greater Ruaha ecosystem (GRE) encompasses vast wildlife protected areas bordered by rural settlements in south-central Tanzania. In the GRE, rapid increase in land use and consequent scarcity of water have altered the distribution of humans, livestock and wildlife, creating greater overlap among these populations and potential increase of zoonotic disease transmission, including

BTB and brucellosis (Lankford *et al*., 2004). Estimated BTB prevalence in cattle within and adjacent to the GRE from 1994-1997 was 13%, with 51% of sampled herds containing positive reactors (Kazwala *et al*., 2001b). *Mycobacterium bovis* has also been isolated from milk samples of pastoralist cattle in the GRE (Kazwala *et al*., 1998). Brucellosis prevalence estimates for livestock grazing in the GRE have not been reported to date, although seroprevalence ranging from 0.6 to 15.2% have been reported from the region around GRE (Karimuribo *et al*., 2007). This study investigated the prevalence and individual animal and herd level risk factors for BTB and brucellosis in livestock owned by pastoralist communities in the livestock-wildlife interface of the GRE, in south-central Tanzania. In addition, the study further characterized the *Mycobacterium* spp. recovered from the slaughtered livestock from the study area.

MATERIALS AND METHODS

Study area

This study was approved and carried out in accordance with Sokoine University of Agriculture institutional guidelines (Reference: SUA/CVMBS/R.1/2007/3).
The study was conducted from 2007 to 2009 in 21 rural villages located along the southeastern border of the community-based Pawaga-Idodi Wildlife Management Area (PIWMA), and in Ruaha National Park - south-central Tanzania between 7º 19’ S to 07º 36’ S longitudes and 35º 05’ E to 35º 29’ E latitudes (Kiwango *et al*., 2018). Study area consists of patchily distributed semi-arid woodland and bushland, and active and fallow agriculture fields. Lands are heavily grazed, as evidenced by denuded vegetation, bare patches of soil, and the presence of many livestock and livestock feces.

The Great and Little Ruaha Rivers are the major sources of surface water in the area, however, the Great Ruaha River dries and floods seasonally each year. The study villages were selected because of (a) presence of domestic animals and wildlife susceptible for BTB and brucellosis; (b) previous reports of BTB (Muma *et al*., 2006); (c) being located in close proximity to the established community-based wildlife management area and Ruaha National Park; and (d) illegal livestock grazing of cattle in the wildlife management area (Kiwango *et al*., 2018).

Study population

The study population consisted of cattle and small ruminants (owned by Barabaig, Maasai or Sukuma pastoralist and agro-pastoralist households) that had not been previously tested for BTB or vaccinated against brucellosis which grazed within the study area. Each ethnic group has different cultural practices and degrees of nomadicity. For example, Barabaig pastoralists are highly nomadic, keep cattle as their core livelihood activity, and do not grow crops. Maasai pastoralists regard cattle keeping as their core economic activity, but have diversified into other activities, including crop farming and jewelry work (Arnold 2001). Sukuma agro-pastoralists however, practice limited transhumance (most of the family is settled, but cattle may be grazed far away by a family member), and grow crops as part of their livelihood.

Sampling Design

Bovine tuberculosis was the primary disease of concern, therefore, sample size considerations were based on cattle herd size and BTB prevalence data from previous studies (Kazwala *et al*., 2001b). The target number of cattle and households to be sampled was calculated with the following goals: (a) to sample enough individual cattle within each herd so that the entire herd could be classified

as BTB positive or negative; (b) to sample enough households to get a good estimate of the herd-level prevalence and to examine individual animal and household level risk factors; and (c) to have representative samples to capture variation in risk factors. Sample size goals were balanced with logistical constraints associated with working in remote areas (vehicular access, cold chain limitations and absence of livestock restraint facilities).

The target sample size was calculated using *Epi info* version 6 (Dean *et al*., 1994) and the program *Herdacc*^{TM} version 3 (Jordan 1995) using previous survey data estimating the number of pastoralist/ agro-pastoralist households and cattle in the study area (Wildlife Conservation Society-WCS 2005, unpublished data), and previously published studies on diagnostic test sensitivity and BTB prevalence.

Specifically, the following were utilized; (a) a sampling frame of 295 known Maasai, Barabaig and Sukuma households in the study area; (b) a mean cattle herd size for these ethnic groups of 62 cattle; (c) a diagnostic sensitivity of 88-90% and specificity ≥ 99% for the Single Comparative Intradermal Tuberculin Test (SCITT) for BTB (Monaghan *et al*., 1994); and (d) an estimated SCITT reactor prevalence in cattle of 13.2% and herd prevalence (i.e. proportion of herds with at least one positive cow) of 51% (Kazwala *et al*., 2001b); and (e) a herd cut-point (i.e. the number of reactors within a herd required to classify the whole herd as positive) of a single positive reactor. While the study did not test cattle from all ethnic groups that possessed livestock, the three ethnic groups sampled collectively owned approximately 72% of the cattle in the area (Wildlife Conservation Society-WCS 2005, unpublished data).

The program *HerdAcc*^{TM} was also used to determine target cattle sample sizes within herds that would maximize herd level sensitivity (HerdSe) and herd level specificity (HerdSp) over a range of 10 to 1000 cattle per herd. The goal was to sample all individuals in herds with 10 or fewer cattle, 10 to15 individuals for herds of 11to 99 cattle, and 15to 20 individuals in herds with 100 or more cattle to maintain85 to 95% HerdSe and 80 to 90% HerdSp over all possible herd sizes and to sample 108 herds in order to estimate herd prevalence within 10% tolerable error.

Risk factor survey

Potential participants were informed about the goals and purpose of the study and verbal informed consent was obtained for each participating household. A questionnaire survey was conducted in Kiswahili by a trained interviewer to assess the following potential herd-level risk factors for BTB and brucellosis: cattle herd size, ethnic group, whether or not cattle were allowed to mix with other herds, and whether or not cattle were allowed to graze beyond the village boundaries. The geo-political division (Pawaga or Idodi) and village were recorded for each household.

Animal sampling and Tuberculin skin testing

Cattle were caught in the boma using a rope then manually restrained, while small ruminants i.e. sheep and goats were manually restrained for sampling. About 100 ml of milk samples were collected into sterile vials by gloved hand from lactating cows and up to 10 ml of blood was collected via jugular venipuncture. Blood samples were kept in a cool box to clot, and then centrifuged at 2500 rpm for 15 min to obtain serum. The milk and serum samples were stored frozen at -20°C in the gasoline powered refrigerator until laboratory testing at Sokoine University of Agriculture (SUA). For cattle, the Single Comparative Intradermal Tuberculin Test

(SCITT) was performed as described by (Lesslie and Herbert 1975).

Briefly, about 0.1 ml of avian and 0.1 ml of bovine purified protein derivative, PPD (tuberculin; Veterinary Laboratory Agency Weybridge, New Haw, Addlestone, Surrey KT153NB, U.K.) were injected intradermal in the neck at two sites spaced 12.5 cm apart (Monaghan *et al*., 1994) and measurement of skin fold thickness was performed initially and 72 hrs post injection (Shirima *et al*., 2003).

The difference of the increase in skin fold thicknesses between the bovine and avian PPD injection sites was calculated. Cows with a skin thickness difference of ≥ 4mm were classed as positive reactors for BTB (OIE 2008). Cows with < 3mm skin thickness difference were considered negative, while cows with an intermediary skin thickness difference (≥ 3mm but < 4mm) were considered suspect reactors for BTB (Shirima *et al*., 2003). All test interpretation was conducted by two trained veterinarians or by a single trained animal health technician. Age [young (< 2 years) or adult (≥ 2 years)], sex (female or male), and an ordinal body condition score (1= thin, 2= average, 3= fat) were recorded for each sampled cattle. Sheep and goats were not the main focus in the study and therefore their demographic data was not collected.

Additionally, pooled lungs and lymph node specimens from slaughtered cattle, sheep and goats from the study area were opportunistically collected for *M. bovis* culture. Presence or absence of lesions at slaughter was recorded. Tissue specimens were frozen at -20°C until culture.

Mycobacterium species Isolation and Identification

Frozen milk and tissues were thawed to room temperature, homogenized, decontaminated, and neutralized using standard methods (Watt *et al*., 1993). Resulting sediments were inoculated onto Lowenstein-Jensen medium with pyruvate and Lowenstein-Jensen medium with glycerol and incubated at 37 °C for up to 12 weeks.

Positive cultures with suggestive colony morphology were purified by subculturing onto another set of the same media for 3 to 4 weeks and then examined under the microscope for the presence of acid-fast-bacilli (AFB) using Ziehl-Neelsen stain (Vestal and Kubica 1966). Heat-killed AFB-positive samples were further characterized by multiplex polymerase chain reaction (PCR), using primers to the *16S rRNA* gene specific for the *Mycobacterium* genus and able to distinguish between *M. avium* and *M.intracellulare*.

The primers targeted the MPB70 gene of *M. tuberculosis* complex (Wilton and Cousins 1992). Samples with an amplification product of 1030 bp indicative of the genus *Mycobacterium* and of 372 bp were considered positive signals for *M. tuberculosis* complex (MTBC). For animals with MTBC, spoligotyping was used to delineate the MTBC and determine the strain type as previously described (Dale *et al*., 2001).

Rose Bengal Plate Test (RBPT)

Serum samples from cattle and small ruminants were tested for antibodies to *Brucella* spp. using the RBPT (Alton *et al*., 1975). 30 μl of RBPT antigen and 30 μl of the test serum were placed alongside on a white porcelain plate, and mixed thoroughly with a sterile stick. The plate was shaken for 3 to 4 min and the degree of agglutination reactions was recorded. The sample was classified positive if any agglutination was observed and negative if no agglutination was observed. Positive and negative controls were included for comparison.

Statistical analysis

The prevalence and associated exact 95% binomial confidence intervals for BTB positive and suspect reactor cattle, *Brucella* spp. exposure in live cattle and small ruminants, and BTB infection in slaughtered livestock were calculated using standard methods (Brown *et al*., 2001). Associations between cattle BTB infection or *Brucella* spp. exposure, and age (young: old), sex (male: female), body condition (thin: average: fat), and the presence of concurrent BTB infection or *Brucella* spp. exposure were evaluated in a multivariate logistic regression model incorporating "herd" as a random effect variable to account for the fact that cattle belonging to the same herd may be more similar due to unmeasured location and management factors.

Regression model variables were selected based on biological considerations and univariate analysis and likelihood ratio (LR) tests. The strength of associations were estimated using logistic odds ratios (OR) and 95% binomial confidence intervals (Long and Freese, 2001). Overall model fit was assessed as described by Hosmer *et al*. (1997).

Any herd containing >1 positive BTB reactor or *Brucella* spp. seropositive cow, was considered a positive herd for bovine tuberculosis and brucellosis, respectively. Any herd containing $\geq$ 1 suspect BTB reactor (but no positive reactors), was considered a "suspect" herd.

The herd prevalence (proportion of positive herds/ number of herds tested) and associated 95% CI for BTB infection and *Brucella* spp. exposure was then calculated and a multivariate logistic regression model (as described above) was used to determine if BTB infected or *Brucella* exposed herds were associated with ethnic group, herd size [small (< 100 cattle) or large (≥100 cattle)]. Additional assessment included whether cattle were allowed to mix with other herds, or whether they were allowed to graze beyond the village boundaries. Concurrent presence of BTB infection or *Brucella* spp. exposure was also determined (STATA ver. 8.0, Stata Corporation, College Station, TX, USA).

For BTB, two outcome groups were evaluated at both the individual animal and herd level. The first BTB outcome group included only positive reactor cattle and herds, respectively. Separate logistic regression models were run for a second outcome group comprising of both BTB positive and suspect reactor cattle or herds.

RESULTS

Tuberculin Skin Testing

The tuberculin skin tests were administered to 1366 cattle from 102 herds. Results were recorded 72 h later for 1347 cattle and 19 animals were reported lost, sold, or slaughtered during the 72 h period and therefore were excluded from the study. On average, 14 cows/ herd were sampled, meeting the individual animal sample size goal of 10 to 15 individuals/ herd for herds with < 100 cattle, but below the target sample size of 15 to 20 individuals/ herd for herds with ≥ 100 cattle. The final sample size of 102 herds was close to the target sample size of 108 herds.

Bovine tuberculosis and brucellosis infection in cattle and associated risk factors

The overall prevalence of BTB positive and suspect reactors was 1.6% and 1.5%,

respectively (Table 1). A total of 43% of sampled cattle originated from Idodi and 57% originated from Pawaga division. The prevalence of BTB positive and suspect cattle was similar in each division (Table 1).

Risk factor analysis and stratified prevalence estimates were calculated for 970 cattle with BTB SCITT results and 985 cattle with *Brucella* spp. RBPT results whose demographic data were obtained through administered questionnaire (Table 1). Female and adult cattle were most commonly sampled, and sampled females were 9.4 times more likely to be adults than sampled males (χ^2= 66.91, p< 0.001). Female cattle were also 2.6 times more likely to be classified as thin compared to male cattle (χ^2= 18.94, p< 0.001).

The prevalence of BTB positive and suspect reactors was similar among sex, age and body condition strata (Table 1) and there were no statistically significant associations between sex, age, body condition, or *Brucella* spp. exposure and BTB infection. There was no change in risk factor associations when suspect cattle were included in the BTB positive group. Inclusion of a random effects term to account for heterogeneity between herds did not alter the outcome of the logistic regression model and the likelihood ratio for inclusion of the herd variable was not significant (p> 0.21).

Brucella spp. seroprevalence for cattle was 7.1%, and was similar among geo-political divisions, sex and age groups (Table 1). A univariate logistic regression model indicated potential significance of body condition (z= 1.71, p= 0.09) as a predictor of *Brucella* spp. exposure (with "fat" cattle at higher risk of exposure), however, inclusion of a random effects term for herd greatly altered the model. The herd random effects variable was highly significant (χ^2 boundary = 36.02, p< 0.001), while the significance of body condition as a predictor of *Brucella* spp. exposure decreased substantially (z= 0.93, p= 0.354).

Examination of the data indicated that the high prevalence of *Brucella* spp. exposure observed in fat cattle, originated from two exposed fat cattle belonging to the same herd, and that the five cows classified as fat belonged to only three herds. Age, sex, BCS, and BTB positive status were evaluated further in a multivariate model, with no risk factors emerging as significant; the herd random effects variable, however remained significant (p< 0.001) indicating that multiple *Brucella* spp. exposed cattle often belonged to the same herd.

Brucella spp. seroprevalence for sampled small ruminants was 1.7% [2/116; 95% CI (0.5-6.1)]. The two exposed individuals were adult female goats from two separate herds: one located in Pawaga division, one located in Idodi division. Both households with *Brucella* spp. seropositive goats also had seropositive cattle in the herd.

Table 1. Prevalence of BTB by the SCITT and *Brucella* spp. exposure by RBPT in individual cattle

Study Group	Bovine Tuberculosis					Brucellosis		
	No. of cattle tested	SCITT positive reactors	% Positive reactor prevalence (95% CI)	SCITT suspect reactors	% Suspect reactor prevalence (95% CI)	No. of cattle tested	RBPT positive	% Seroprevalence (95% CI)
Overall	1347	21	1.6 (1.0-2.4)	20	1.5 (1.0-2.3)	1218	86	7.1 (5.8-8.6)
Study areas								
Pawaga	766	13	1.7 (1.0-2.9)	9	1.2 (0.6-2.2)	721	55	7.6 (5.9-9.8)
Idodi	581	8	1.4 (0.7-2.7)	11	1.9 (1.1-3.4)	497	31	6.2 (4.4-8.7)
Sex								
Male	186	*2*	1.1 (0.3-3.8)	2	1.1 (0.3-3.8)	187	11	5.9 (3.3-10.2)
Female	784	15	1.9 (1.2-3.1)	7	0.9 (0.4-1.8)	798	51	6.4 (4.9-8.3)
Age								
Young	48	1	2.1 (0.4-10.9)	0	0.0 n/a	48	1	2.1 (0.4-10.9)
Adult	922	16	1.7 (1.1-2.8)	9	1.0 (0.5-1.8)	937	62	6.6 (5.2-8.4)
Body condition								
Thin	273	7	2.6 (1.2-5.2)	2	0.7 (0.2-2.6)	275	13	4.7 (2.8-7.9)
Average	692	10	1.4 (0.8-2.6)	7	1.0 (0.5-2.1)	705	47	6.7 (5.1-8.8)
Fat	5	0	0.0 n/a	0	0.0 n/a	5	2	40.0 (11.8-76.9)

BTB- bovine tuberculosis, SCITT-Single Comparative Intradermal Tuberculin Test, RBPT- Rose Bengal Plate Test, n/a- not applicable

BTB infection and Brucella spp. exposure at the herd level and associated risk factors

Eighteen of the 102 (17.6%) sampled cattle herds contained at least one SCITT positive reactor animal. Six of these BTB positive herds also contained one or more suspect reactors. Eleven additional herds contained one or more suspect reactor animals, resulting in a total of 29 herds (28.4%) that had at least one BTB positive or suspect reactor animal (Supplementary Table 1). The number of cattle herds tested for BTB infection was similar for both Idodi and Pawaga divisions and herds from all three target ethnic groups were sampled roughly in proportion to their populations in the study area, with Maasai most common (56%), Sukuma second

(33%), and Barabaig herds the fewest (11%). Although 68% of the herds sampled had less than 100 cattle, small herds were as equally likely as large herds to mix with other herds and graze beyond the village boundary.

Herd size, grazing beyond the village boundary, mixing with other herds and *Brucella* spp. exposure were not associated with BTB positive herd status, however, cattle herds belonging to Sukuma agro-pastoralists were 4.1 times more likely to have a BTB reactor than those belonging to Maasai pastoralists [p= 0.0130, 95% CI for OR (1.3-12.30)].

Data suggested Sukuma herds were more likely to have a BTB positive reactor than Barabaig herds, but too few Barabaig herds were sampled to make meaningful statistical inferences. No herd level risk factors were associated with BTB when suspect cattle were included in the BTB positive group.

Brucella spp. exposure was common, with 38% (39/102) of herds having one or more seropositive animals (Supplementary Table 1). *Brucella* spp. exposure was not associated with herd size, grazing beyond the village boundaries, the presence of BTB positive reactors, or ethnic group, however, households reporting that they did not mix their livestock herds with other herds were three times more likely to have *Brucella* spp. exposed livestock in their herd [p= 0.011, 95% CI for OR (1.30-7.15)].

Bovine Tuberculosis in slaughtered cattle and small ruminants

Pooled tissue sets from 162 cattle and 66 small ruminants were cultured for mycobacteria. A total of 57 growths on culture were suggestive of *Mycobacterium* spp. Twenty-eight of these growths were positive for the presence of acid fast bacilli and subsequently tested via PCR. *Mycobacterium bovis* infection was confirmed via PCR and spoligotyping in a single cow [0.44%, 95% CI (0.00-2.40%)], and the isolate identified as SB0133.

Logistic regression model results for BTB and brucellosis factors at herd level

The risk factors evaluated in the current study, namely, cattle herd size, ethnic group, cattle herd mixing with others, and whether or not cattle were allowed to graze beyond the village boundaries, were individually modeled. However, one risk factor for BTB, namely, respondents' ethnicity (Table 2) and herd mixing, for brucellosis, remained significant in the model (Table 3).

Table 2. Logistic regression model results for BTB infection risk factors at herd level.

Risk Factor	BTB-positive herds				BTB-positive herds		
	n	OR	95% CI	p-value	OR	95% CI	p-value
Large herd size	102	0.37	0.09-1.50	0.17	0.69	0.25-1.93	0.484
Graze beyond village	102	1.98	0.40-9.80	0.402	1.99	0.48-8.15	0.34
Herd mixing	102	0.71	0.22-2.37	0.584	1.05	0.37-3.03	0.923
Brucella exposure	102	1.76	0.57-5.47	0.326	1.6	0.62-4.15	0.332
Ethnic group							
Sukuma	34	4.13	1.24-13.75	0.021	2.4	0.88-6.54	0.087
Barabaig	11	0.87	0.09-8.50	0.903	0.29	0.03-2.56	0.265
Maasai	57	ref	-	-	ref	-	-

Ref: reference

Table 3. Logistic regression model results for *Brucella* spp. exposure at herd level.

Risk Factor	*Brucella* spp. exposed herds			
	n	OR	95% CI	p-value
Large herd size	102	1.34	0.53-3.39	0.53
Graze beyond village	102	0.75	0.21-2.71	0.665
Herd mixing	102	0.416	0.16-1.06	0.067
BTB infection (pos only)	102	1.66	0.53-5.15	0.383
Ethnic group				
Sukuma	34	1.4	0.52-3.81	0.509
Barabaig	57	2.3	0.57-9.28	0.242
Maasai	11	ref	-	-

Ref: reference

DISCUSSION

The prevalence and driving factors of the epidemiology of the BTB and brucellosis in wildlife, livestock and humans in Tanzania is not well known. From studies conducted elsewhere, well documented examples of spread of BTB included infection in badgers in the United Kingdom and possums in New Zealand (Ramsey and Efford 2010). In GRE, the prevalence of bovine tuberculosis and *Brucella* spp. was low at the individual animal level, but a high prevalence was found at the herd-level. The BTB prevalence in sampled cattle was less than 2%, however, about one-fourth of the households sampled had at least one

infected cow. Similarly, *Brucella* spp. prevalence in sampled cattle was 7.1%, whereas more than one-third of herds contained at least one exposed individual.

According to the meta-analysis performed to derive prevalence estimates for *Mycobacterium* and *Brucella* spp. in cattle in Tanzania, the results showed an array of diversity between studies, with wide ranges in the *Mycobacterium* spp. prevalence from 0.1 to 13.2% and *Brucella* spp. from 0.3 to 60.8% (Katale *et al*., 2013).

As opposed, other previous study conducted adjacent to this study area reported a high BTB prevalence of 13.2%. This difference in the prevalence could be explained by the fact that the current study was conducted when water level of the Great Ruaha River was diminishing as a result of agriculture, overgrazing and drought, thus, livestock were dispersed in search of water (Lankford *et al*., 2004), while, in the other study, the water level was normal and livestock could congregate for water in the area previously reported as BTB hotspot (Kazwala *et al*., 2001).

Furthermore, the prevalence estimates of the two chronic zoonoses in the current study agreed with the prevalence range previously reported in the country at large. In light of other data reported elsewhere, the observation on the BTB and *Brucella* spp., prevalence estimates in this study, are in line with previous studies conducted in the East African region (Gumi *et al*., 2012). In this study, detection of *Brucella* infection was done using the Rose Bengal Plate Test (RBPT). There was no *Brucella* spp. confirmatory test used in this study.

Thus, the *Brucella* spp. seroprevalence reported in this study is likely to be higher. In another study, a high *Brucella* spp. seroprevalence of 6.5% was reported with RBPT. Upon confirming the *Brucella* test result using Competitive Enzyme Linked Immuno-Sorbent Assay (c-ELISA), the seroprevalence was reduced to 5.8% and the two *Brucella* infection assays were not in agreement for up to 11% (Assenga *et al*., 2015).

Previous studies conducted in Tanzania to investigate the risk factors that increase exposure to and potentiate the transmission of the two zoonoses (Cleaveland *et al*., 2007) investigated the modified risk factors considering the existing livestock-wildlife interface area of GRE.

Majority of the risk factors evaluated in the current study, namely, cattle herd size, ethnic group, whether or not cattle were allowed to mix with other herds, and whether or not cattle were allowed to graze beyond the village boundaries, were not significant in the model. However, one risk factor for BTB, namely, respondents' ethnicity; and for brucellosis, herd mixing, remained significant in the model (Tables 2 and 3).

Similar observation on the statistical significance of respondents' ethnicity resulting from the analysis of the disease associated risk factors has been shown elsewhere (Tschopp *et al*., 2015). In this study, the significant difference could be attributed to the existence of different lifestyles between the main ethnic groups (Sukuma, Maasai and Barabaig) within the divisions. These ethnic groups have different lifestyles, in which Maasai and Barabaig are intermediate to highly migrating nomads, respectively, while, Sukuma are sedentary and agricultural nomads (Arnold 2001).

In addition, animal population size has been reported elsewhere as an important risk factor that prompts widespread distribution of chronic diseases of public health significance (Cosivi *et al*., 1998). The highest incidence of BTB has been observed where intensification of dairy production is common. Citing the United

States, as an example, where BTB is close to elimination, large dairy herds where cattle are crowded were reported to represent the main source of infection (Cosivi *et al*., 1998).

Most of the cattle herds in the study area were owned by the Maasai, but the most cattle were sampled from the Sukuma herds, followed by Maasai and Barabaig communities. Most of the Sukuma in the study area were those from the BTB hotspots of the Usangu plains who were evicted from the eastern Usangu water catchment area encompassing *Ihefu* wetlands (Kihwele *et al*., 2012). These BTB hotspots were those previously reported in a study conducted adjacent to the study area that had shown the distribution of BTB reactors in cattle herds with an overall and herd-level prevalence of 13.2% and 51%, respectively (Kazwala *et al*., 2001).

As it is also reported elsewhere, the BTB positive reactor cattle in the study area could be a potential source of cross-species transmission of BTB as previously reported. Thus, in this study, the high stocking densities could explain a possible cross-species spillover effect in which two adult female goats were exposed to brucellosis with each goat originating from separate herds both herds having *Brucella* spp. seropositive cattle as well (Tschopp *et al*., 2015).

The current study investigated a different set of risk factors as opposed to the previous study conducted adjacent to the study area in which almost all risk factors studied were significant. Therefore, in this study, the finding that few of risks factors assessed were significantly associated with infection status could be explained by the low sample size within a herd. The proportion of the pastoralist cattle and cattle herds tested was 7.5% (n= 1368 and N= 18,239) and 34.6% (n= 102 and N= 295) (Wildlife Conservation Society-WCS 2005, unpublished data) respectively. The proportion of cattle included was 1%of the indigenous cattle in the southern highlands of the country and a much larger proportion of each herd was included in the study (Kazwala *et al*., 2001).

Previous studies conducted have shown several factors that can influence the sensitivity of tuberculin skin test, including the age and physiological status of an animal (Dejene *et al*., 2016). This study excluded calves and pregnant cows near the term, because the skin test reaction is significantly more likely to be positive. Female cattle were most commonly sampled, and were 9.4 times more likely to be adults, than sampled males ($p< 0.001$). In total, there were fewer male cattle in the herds than the females. Besides their number, the owners were reluctant to sample large males because of fear of injuries. Thus, absence of livestock restraining facilities and possibility for physical injuries were some of the study limitations. The similar encounter of sampling fewer males than females commonly occurs in African fieldwork settings (Awah-Ndukum *et al*., 2016).

Previous studies conducted in the study area have reported BTB and brucellosis as diseases of concern among regional pastoralist and agro-pastoralist households (Mazet *et al*., 2009). Bovine tuberculosis was confirmed in regional wildlife, and identical *Mycobacterium bovis* strains were isolated from wildlife around GRE (Clifford *et al*., 2013) which is in proximity to the current study area. The latter was conducted in the protected areas at the livestock- wildlife interface in the Ruaha ecosystem. Interestingly, livestock and wild animals share water points, pasture or territory at the interface area (Mazet *et al*., 2009; Clifford *et al*., 2013). Isolation of identical *M. bovis* strains from wildlife (Clifford *et al*., 2013) and livestock in this study indicate the

potential of both wild animals and livestock being disease reservoirs of such microorganisms in the livestock-wildlife interface area of GRE. Occurrence of identical *M. bovis* from wildlife and livestock in the same livestock-wildlife interface area, support concerns about interspecies sharing of pathogens (Clifford *et al*., 2013).

M. bovis isolates recovered from one cow had identical spoligotype patterns similar to those reported elsewhere, and were classified as SB0133 spoligotype (Biffa *et al*., 2010). The *M. bovis* SB0133 spoligotype is unique and has adapted to wildlife (Clifford *et al*., 2013; Mwakapuja *et al*., 2013) and livestock in sub-Saharan Africa (Biffa *et al*., 2010). In line with other previous studies conducted in the country (Katale *et al*., 2017), in this study, the spoligotype SB0133 was isolated from cattle at the livestock-wildlife interface of the GRE.

By comparison, the SB0133spoligotype pattern was observed for *M. bovis* strains from livestock in the study, and those from wildlife samples (Clifford *et al*., 2013), and for cattle samples from a study in Mbeya and Iringa regions (Kazwala *et al*., 2006).
Moreover, the same spoligotype has also been isolated in livestock and wildlife in other countries of Eastern Africa (Biffa *et al*., 2010) and wildlife (Katale *et al*., 2017), suggesting its wide spread distribution in wild animals and livestock. Occurrence of such microorganisms in wild animals may represent a permanent reservoir of infection that poses a serious threat to disease management (Cosivi *et al*., 1999).

Demonstration of a *M. bovis* strain from livestock in the livestock-wildlife interface with a similar pattern to those previously detected from wild animals in the protected areas adjacent to the study area, suggests the possibility of interspecies sharing of pathogens. Although this study has quantified the prevalence of the two zoonoses in the study area and demonstrated that a significant portion of households with infected animals, many pastoralists are reluctant to dispose of the infected animals, despite being aware of the risks of disease transmission if the infected animals remain in the herd.

Therefore, it is imperative to consider, among others, cultural and rural economic considerations in order to develop effective strategies for the management of zoonotic diseases in pastoralist and agro-pastoralist communities.

COMPETING INTERESTS

None were identified.

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Supplementary Table 1. Prevalence of BTB as determined by the SCITT and *Brucella* spp. exposure as determined by the RBPT in cattle herds tested.

Study Group	Bovine Tuberculosis					Brucellosis	
	No of herds tested	Positive Herds	% Positive Herd Prevalence (95% CI)	Suspect Herds	% Suspect Herd Prevalence (95% CI)	No. of RBPT positive	% Seroprevalence (95% CI)
Overall	102	18	17.6(10.8-26.5)	11	10.8(5.5-18.5)	39	38.2(28.8-48.4)
Geographic Area							
Pawaga	52	10	19.2(9.6-32.5)	4	7.7(2.1-18.5)	22	42.3(28.7-56.8)
Idodi	50	8	16.0(7.2-29.1)	7	14.0(5.8-26.7)	17	34.0(21.2-48.8)
Herd size							
Small (<100 cattle)	70	15	21.4(12.5-32.9)	7	10.0(4.1-19.5)	25	35.7(24.6-48.1)
Large (≥100 cattle)	32	3	9.4(2.0-25.0)	4	12.5(3.5-29.0)	14	43.8(26.4-62.3)
Ethnic group							
Sukuma	34	11	32.4(17.4-50.5)	3	8.8(1.9-23.7)	16	47.1(29.8-64.9)
Maasai	57	6	10.5(4.0-21.5)	8	14.0(6.3-25.8)	17	29.8(18.4-43.4)
Barabaig	11	1	9.1(0.2-41.3)	0	0.0(n/a)	6	54.5(23.4-83.3)
Degree of cattle mixing							
Nomixing with other herds	34	8	23.5(10.8-41.2)	2	5.9(0.7-19.7)	19	55.9(37.9-72.8)
Allowed to mixing	68	10	14.7(7.3-25.4)	9	13.2(6.2-23.6)	20	29.4(19.0-41.7)
Grazing practice							
Graze within the village	15	3	20.0(4.3-48.1)	1	6.7(0.2-32.0)	8	53.3(26.6-78.7)
Graze beyond village borders	87	15	17.2(10.0-26.8)	10	11.5(5.7-20.1)	31	35.6(25.7-46.6)

No- number, BTB- bovine tuberculosis, SCITT-Single Comparative Intradermal Tuberculin Test, RBPT- Rose Bengal Plate Test, n/a- not applicable