Spatial distribution and risk factors for brucellosis in domestic and wild animals at livestock-wildlife interface in Mikumi-Selous ecosystem, Tanzania

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SUMMARY

A study on seroprevalence, spatial distribution and risk factors of brucellosis in livestock and wild animals was carried in Mikumi-Selous ecosystem from September 2010 to August 2011. Rose Bengal Plate Test (RBPT) and Competitive Enzyme Linked Immunosorbent Assay (cELISA) techniques were applied for the disease diagnosis. A total of 747 cattle, 198 goats, 168 sheep and 88 wild animals were tested for *Brucella* infection. Serological survey showed that 14.1%, 0.5% and 0.6% of cattle, goats and sheep were seropositive, respectively. The study showed that domestic animals in Kilosa, Kilombero, Mvomero and Ulanga districts were equally infected by *Brucella*. A proportion of 7.7% and 13.6% of buffalo tested positive by RBPT and cELISA, respectively. Animal-to-animal contact was the risk factor associated with the spread of the disease in the interface areas (P=0.02, OR=2.34). This study showed that brucellosis has spread amongst animals due to shared grazing land and habitat. However, could not identify the source of infection between the two animal populations. The study calls for more studies on molecular epidemiology of the disease in order to establish the dynamics of *Brucella* spp. in the study areas and other livestock/wildlife/humans interface areas. Such knowledge is vital for effective intervention of brucellosis.

Keywords: Neglected zoonoses, pastoral cattle, Seroprevalence

INTRODUCTION

Brucellosis is one of the neglected bacterial zoonoses, affecting livestock, humans and wild animals worldwide (Lopes *et al*., 2010; Swai and Schoonman, 2010; Ghodasara *et al*., 2010). Brucellosis is caused by Gram-negative, coccobacilli, bacteria of the genus *Brucella.* Clinically the disease is manifested by abortion at third trimester, retention of placenta, metritis, infertility, reduced milk production, orchitis and stillbirth (WHO, 2006). In Tanzania, seroprevalence studies show that brucellosis in cattle vary widely in different regions and zones (Swai and Schoonman, 2010; Swai *et al.,* 2005; Karimuribo *et al.,* 2007; Temba, 2012; Chitupila *et al.,* 2015; Assenga *et al*., 2015). *Brucella* infection in wildlife has been reported in Serengeti-Ngorongoro ecosystem (Fyumagwa *et al*., 2009; Mellau *et al*., 2009). Presence of brucellosis in animals can be as a natural sustainable infection within susceptible

population or influenced by grazing strategy (Muna et al. 2006). Previous studies showed that herd size, sex, location and age are the risk factors (Mohammed *et al.,* 2011; Adugna *et al.,* 2013).

The aim of this study was to determine the burden, spatial distribution and identify risk factors associated with spread of brucellosis between domestic and wild animals in livestock-wildlife interface in Mikumi-Selous ecosystem.

MATERIALS AND METHODS

Study area

The study was carried out in four districts namely Kilombero, Ulanga, Kilosa, and Mvomero in Morogoro region. The area is located between 6º 49´ 0´´-10° 0´ 0´´ S and 35º 40´ 0´´-38º 60´ 0´´E. The Mikumi-Selous ecosystem is situated within Kilosa, Kilombero and Ulanga, it comprises of Mikumi National Park and Selous game reserve. The study area was selected because there was high interaction between wild animals, livestock and humans. The livestock (cattle, goats and sheep) kept were local breed managed under extensive grazing system. There was no history of vaccination against brucellosis in the study area.

Study design and sample size

A cross-sectional study was conducted and a combination of Cluster and random sampling methods (Bennet *et al*., 1991) were applied to select study villages, households and animals. The sample size was estimated based on prevalence of 12% reported in eastern previously (Swai and Schoonman, 2010). A total of 35 villages and 175 households randomly selected were involved in the study. Six animals (cattle aged >12 months, goats and sheep aged >6 months) were randomly selected from each household. A total of 747 cattle, 198 goats, and 168 sheep were tested for *Brucella* infection. Also, a total of 88 wild animals of 9 different species Mikumi national park and Selous game reserve were tested, including 66 African buffaloes (*Syncerus caffer*), two zebra (*Equus burchellii),* six Wildebeest (*Connochaetes taurinus)*, four Elephant (*Loxodonta africana*), four Bushbuck (*Tragelaphus sylvaticus*), one Reedbuck (*Redunca arundinum*), two Impala (*Aepyceros melampus*), one Sable (*Hippotragus niger*), two Hartebeest (*Alcelaphus buselaphus*).

Ethical clearance

A Free Permit (Ref. # TNP/HQ/C.10/13) was acquired by Tanzania Wildlife Research Institute.

Livestock and wildlife sampling

Selected domestic animals were physically restrained while buffalo were chemically immobilized using a combination of Etorphine hydrochloride 8-10 mg/ml and Azaperon 80-150 mg/ml prior to sampling, Diprenorphine 16-15 mg were used as antidote. Sampled buffaloes were marked with visible ink to avoid re-sampling. For hunter killed animals, heart blood was used for test. Sera were harvested after 12hr and stored in freezer at -20°C before analysis at the College of Veterinary Medicine and Medical Sciences. Geo-references of all sampled households and location of tested wildlife were recorded using Global Positioning Systems (GPS). A

Rose Bengal Plate Test (RBPT) and competitive Enzyme Linked Immunosorbent Assay (c-ELISA) (WHO, 2006) were applied to analyse the samples. A village and household (herd) was considered positive if at least one animal tested positive by c-ELISA while an animal was considered positive if tested positive to both RBPT and c-ELISA.

Questionnaire survey

Pre-designed questionnaires were administered at selected households to address the risk factors for *Brucella* infection. A total of 148 respondents were interviewed after the questionnaires were pre-tested to 17 pastoralist households. The survey was designed to gather information on the knowledge of brucellosis, herd management practices, livestock and wild animals grazing areas, contacts between wild and domestic animals and livestock transfer movements. Households' leaders were interviewed for 10-15 minutes by the researchers after animal sampling.

Statistical analysis

Data were entered and analysed using Epi Info® statistical software. Proportions and comparison of variables were analysed using Chi-square test. The Association between seropositivity, categorical and ordinal risk factors was assessed using Fisher's exact test and contingency table analysis. Agreement between the tests was determined by Kappa (κ) Statistics. A value of $P<0.05$ was considered indicative for statistically significant difference.

RESULTS

Sampling of livestock and wildlife was carried out from September 2010 to August 2011. A total of 88 animals were sampled from Mikumi-Selous ecosystem. The status of brucellosis in livestock from the districts is shown (Table 1). There was no significant difference ($p<0.05$) in seroprevalence among the districts. Overall, 107 (14.3%) of cattle were seropositive while only 2 (0.5%) of small ruminants (goats and sheep) tested positive in the study areas. The number of infected cattle were significantly higher ($P=0.001$) compared to the infected small ruminants. Positive reactors in wild animals were found at Mikumi National Park while there was no positive animal at Selous game reserve (Table 2). The agreement between RBPT and c-ELISA techniques for detecting was good ($k=0.93$), suggesting that the results were reliable.

Out of 148 respondents reported to have sold or purchased animals from other herds while 98.6% of respondents reported livestock contacts between different herds. Also 92.6% of respondents reported to apply communal grazing, while 91.2% and 77.7% respondents reported to use communal water which is shared between livestock and wild animals. Animal contacts was significantly associated with seropositive herds in both buffalo and livestock herds at Mikumi National Park and its adjacent livestock-wildlife interface rangeland ($P=0.02$, OR=2.34).

The spatial distribution of the disease at village level is shown (Figure 1). Out of 36 villages, 30 (83.3%) villages had

seropositive livestock while 6 (16.7%) villages had none. Proportions of infected villages in the districts were 8 (88.9%), 8 (88.9%), 6 (85.7%) and 8 (72.7%) in Ulanga, Kilombero, Mvomero and Kilosa, respectively. There was no significant different ($p<0.05$) in prevalence between villages in the districts. Also 81 (54.7%) herds reported incidences of pregnancy abortion at third trimester.

Table 1. Seroprevalence of brucellosis in livestock in the study area

District	Animal species	Total samples	Positive	Negative	Positive 95% (CI)
Ulanga	Cattle	202	21	181	10.4 (6.6-15.5)
	Goats	36	0	36	0.0 (0.0-9.7)
	Sheep	34	0	34	0.0 (0.0-10.3)
Kilombero	Cattle	193	31	162	16.1 (11.2-22.0)
	Goats	36	0	36	0.0 (0.0-9.7)
	Sheep	42	1	41	2.4 (0.1-12.6)
Mvomero	Cattle	168	25	142	14.9 (9.9-21.2)
	Goats	50	1	49	2.0 (0.1-10.6)
	Sheep	22	0	22	0.0 (0.0-15.4)
Kilosa	Cattle	184	30	153	16.3 (11.3-22.5)
	Goats	76	0	76	0.0 (0.0-4.7)
	Sheep	70	0	70	0.0 (0.0-5.1)

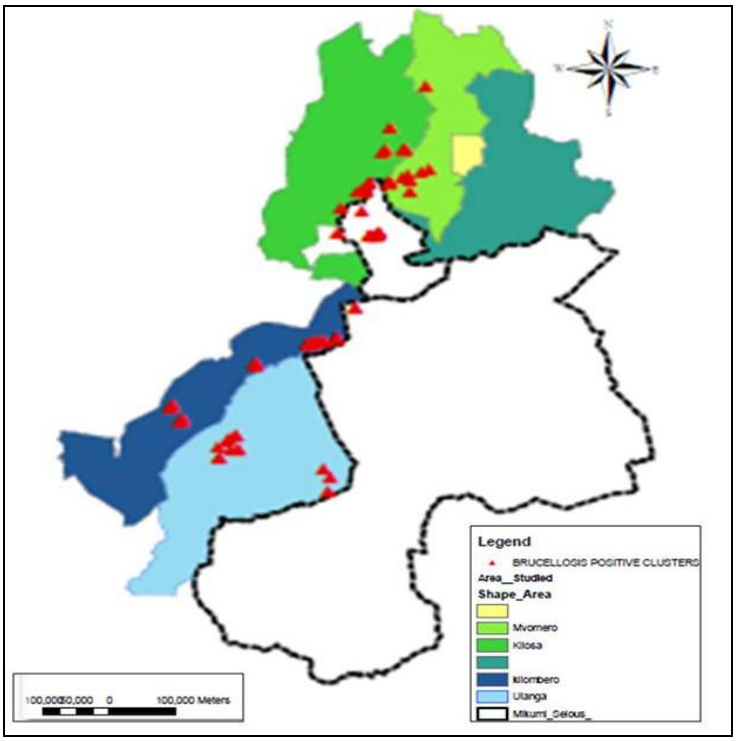

Figure 1. Spatial distribution of seropositive animals in the study area.

Table 2. Seroprevalence of brucellosis in livestock and wild animals in the study area.

		RBPT	c-ELISA
Animal species	Number tested	Number +ve (%) 95%CI	Number +ve (%) 95%CI
Cattle	747	107 (14.3) 11.9-17.1	107 (14.3) 11.9-17.1)
Goats	198	1 (0.5) 0.0-2.8	1 (0.5) 0.0-2.8
Sheep	168	1 (0.6) 0.0-3.3	1 (0.6) 0.0-3.3
Buffalo (MNP)*	66	5 (7.0) 4.7-29.5	9 (13.6) 6.5-24.7
Wildlife (SGR)**	22	0.0	0.0%

MNP* Buffaloes tested at Mikumi National Park
SGR**Wild animal carcasses tested at Selous game reserve

Animal-to-animal contact was statistically significant risk factor for brucellosis spread amongst animals (OR=2.34; 95%CI 1.01<OR<5.49). Other factors had no impact on the transmission of the disease in the study area. (Table 3).

Table 3. Risk factors for brucellosis in livestock and wild animals in the study area

Variable	Level	Proportion (%)	*Brucella* herd prevalence (%)	(P- value)
Ethnicity	(i) Maasai	45.3	41.8	0.37
	(ii) Barbaig	4.7	28.6	
	(iii) Sukuma	43.2	42.2	
	(iv) Others	6.8	10.0	
Access to Veterinary services	(i) Yes	6.8	50.0	0.13
	(ii) No	93.2	38.4	
Sale or purchase of animals	(i) Yes	95.3	39.7	0.43
	(ii) No	4.7	28.6	
Aborted foetus fed raw to dog	(i) Yes	51.4	44.2	0.75
	(ii) No	48.6	50.0	
Aborted foetus thrown to the bush	(i) Yes	34.5	40.4	0.28
	(ii) No	65.5	51.0	
Placenta fed raw to dogs	(i) Yes	36.5	46.3	0.66
	(ii) No	63.5	42.9	
Placenta thrown to bush	(i) Yes	62.2	44.6	0.99
	(ii) No	37.8	44.0	
Do your animals contact other livestock	(i) Yes	98.6	39.7	0.37
	(ii) No	1.4	0.0	
Did you acquire new animal last year	(i) Yes	44.6	41.8	0.47
	(ii) No	55.4	37.0	
Graze in communal pasture	(i) Yes	92.6	40.1	0.3
	(ii) No	7.4	27.3	
Livestock contact with wild animals	(i) Yes	23	55.9	0.02
	(ii) No	77	35.1	
Grazing of livestock in conservation	(i) Yes	18.9	50.0	0.19
	(ii) No	81.1	36.7	
Use of communal water point	(i) Yes	91.2	40.7	0.2
	(ii) No	8.8	23.1	
Livestock and wild animals share water	(i) Yes	77.7	42.6	0.7
	(ii) No	22.3	27.3	

DISCUSSION

This study had shown that current status of brucellosis agree with previous studies conducted in Tanzania (Swai and Schoonman, 2010). Some variations mighty be due to different livestock farming systems, management practices and socioeconomic factors (Matope *et al.,* 2010). The current study showed that brucellosis burden was relatively higher in cattle and buffaloes compared with cattle and buffaloes tested in Katavi ecosystem (Assenga *et al.,* 2015). Furthermore, the study showed that the disease was widely distributed among villages (83.3%) in Mikumi-Selous ecosystem. This implies that the disease can easily spread to other uninfected herds and humans are at risk due to communal grazing and pasture contamination, this also has been observed elsewhere (Alemayehu, 2012).

In buffaloes, RBPT and c-ELISA recorded seropositive of 7.0% and 13.6%, respectively. The results showed that c-ELISA was superior to RBPT in detecting an infected buffalo. The performance of the two tests is in line with earlier study (Islam *et al.,* 2013). This suggests that c-ELISA is more precise and efficient diagnostic tool for brucellosis because the chances of missing an infected animal are minimal. This study indicated that there was no significant difference in seroprevalence of brucellosis between African buffalo and livestock in Mikumi National Park and the interface areas, respectively. However, the prevalence of brucellosis at the study area was lower compared to other reports from Serengeti-Ngorongoro and Tarangire ecosystems (Fyumagwa *et al.,* 2009). The difference in brucellosis burden can be associated with different factors including wildlife population density and different abiotic and biotic factors.

This study showed that there were no positive animal in Selous Game Reserve, the small sample size (27 male animals) of tested wild animals at the area could be a factor. On the other hand, the absence of positive reactors could be associated by different factors including, yearly 6 months hunting activities which decrease animal population size hence affecting the disease dynamics, and less interaction between wildlife and livestock due to large area of Selous ecosystem with plenty of water and pastures. The current studies showed that most villages were infected with brucellosis. This can be associated with extensive management system which allows maximum animal interaction and communal grazing. Furthermore, the study showed that animal-to-animal contact was a risk factor. The findings are in agreement with earlier reports (Mohammed *et al.,* 2011) from Ethiopia and western Africa.

From the findings of this study, it is concluded that *Brucella* spp is circulating amongst livestock and wildlife populations due to direct contact, water and rangeland sharing. However, source of the *Brucella* in the animals was not identified between the two populations. Thus the study calls for further investigations on the molecular characterization of *Brucella* biovars that circulate in livestock, wildlife and human populations at the study area and other area involving extensive pastoral livestock management and wildlife interaction. This will improve control and eradication strategies through vaccination programmes. Also community based intervention programmes are needed to control the disease in humans.

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