Seroprevalence of brucellosis in domestic ruminants in livestock-wildlife interface: A case study of Ngorongoro Conservation Area, Arusha, Tanzania

L.S.B. Mellau[1], S.L. Kuya[2] and P.N. Wambura[3]

[1]Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture P.O. Box 3201, Chuo Kikuu, Morogoro, Tanzania, [2]Ngorongoro Conservation Area Authority, P.O. Box 1, Ngorongoro, Arusha Tanzania, [3]Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture, P.O. Box 3016, Chuo Kikuu, Morogoro, Tanzania.
E-mail: mellau@suanet.ac.tz; lesakit@yahoo.com

SUMMARY

A limited study was conducted to determine prevalence of brucellosis in domestic ruminants kept in a free range grazing system in Ngorongoro Conservation Area (NCA) which is a world heritage site in which pastoralists communities have been living harmoniously with wildlife for decades. Blood samples from 200 cattle, 87 goats and 13 sheep were collected by venipuncture into plain vacutainer tubes. Rose Bengal Plate Test (RBPT) and Microagglutination Test were used to detect antibodies against brucellosis in sera obtained from sampled blood. It was observed that 14.28% adult cows, 7.54% heifers, 2.38% bulls, 11.9 does, 10.7% bucks, and 10% ewes showed positive reactions to RBPT. When same samples were tested with MAT, 10.05% adult cows, 7.54% heifers, 2.38% bulls, 13.8% does, 14.3% bucks, and 3% ewes tested positive. Based on these serological tests it was concluded that brucellosis is endemic in pastoral livestock in NCA and that the reported increase in human brucellosis among pastoralists living in the NCA might be associated with domestic ruminants which are the sole source of food and income for the pastoralist in the area. Wildlife-domestic animals interaction phenomenon in NCA can as well be viewed as a significant means with which zoonoses are maintained in such ecosystem.

Key words: Prevalence, brucellosis, livestock-wildlife interface, other zoonoses

INTRODUCTION

The Ngorongoro Conservation Area (NCA) is recognized as world heritage site where pastoral communities and wildlife have been living side by side for decades. The NCA is situated in Arusha region in the northern part of Tanzania. The NCA is about 170 km from Arusha town, covering 8,293 sq km and with human population of about 65,000 inhabitants, about 163,000 cattle and 250,000 sheep and goats. The majority of inhabitants are pastoralist whose livelihood depends on livestock for food and income. The famous Ngorongoro crater which is actually a caldera is situated within NCA. All villages where pastoral families live are around the crater rim. Livestock obtain drinking water and salt licks in a salty lake at the crater base where many species of wildlife are found. Villages far from the crater are supplied with salt licks obtainable from the crater by the Ngorongoro Crater Authority. The interaction between livestock and wildlife is thought to complete a vicious cycle of diseases transmissible between domestic ruminants and wild ungulates. According to Endulen Hospital annual report (personal communication) on a limited

number of samples, human cases of brucellosis have increased from 35.6% in 2004 to 58.1% in 2005. Unfortunately in Tanzania, studies on brucellosis in domestic ruminants have been conducted in commercial farms where records could be available (Stack and Protz, 1973; Kitaly, 1984, Minga and Balemba, 1990; Swai, 1997). There have been few studies that have reported prevalence of brucellosis among pastoral livestock and none has ever reported prevalence of brucellosis in domestic ruminants in livestock-wildlife interfaces where animal diseases are likely to circulate among both categories (Shirima, 2005). Fyumagwa (personal communication, 2005) observed brucellosis prevalence of 10% in buffaloes and 1% in zebras in Serengeti National Park which borders NCA to the west. It is therefore likely that an increase of human brucellosis cases in NCA pastoral communities could have been triggered by transmission of the causal agent between wild ungulates and domestic ruminants. Since pastoralists consume milk and meat from their animals there are chances that livestock brucellosis might have also increased in the area.

Different reports have shown that in parastatal beef farms brucellosis prevalence was 13.5 % (Mahlau and Hammond, 1992), 12.3% (Weinhupl *et al.,* 2000), 4.2% (Mtui-Malamsha, 2001), where as in dairy farms prevalence of brucellosis was 15.2% (Ottaru, 1985), 5.2% (Kitaly, 1984), and 14.1% (Weihaupl *et al*., 2000).

Brucellosis is a problem in several wild animals in developed and developing countries. Some of the wild species affected includes; African buffalo, impala, hippopotamus, zebra, wildebeest and hartebeests (Madsen and Anderson, 1995). In Zimbabwe a survey showed that the prevalence was 6.5% in buffalo, 1.4% in eland, 0.9% in antelope and 0.05% giraffe (Madsen and Anderson, 1995). This implies that the prevalence in buffaloes is higher probably due to anatomical and physiological resemblance with cattle. Buffaloes constitute a great proportion of wild animals in Ngorongoro crater and have frequent contacts with domestic ruminants than other wildlife species. This study was therefore aimed at determining seroprevalence of brucellosis in domestic ruminants in the livestock wildlife interface and to try to stratify the rate of infection according to domestic ruminants' species which have various roles in the livelihood of pastoralists.

MATERIALS AND METHODS

Study Area

This study was conducted using domestic ruminants kept traditionally and involved animals from three villages which were conveniently chosen at NCA in Ngorongoro district, Arusha, Region. The NCA is geographically located at latitude 03°14'715S and 35 ° 29'275 E. Livestock numbers in NCA are 163,000 cattle and 250,000 sheep and goats (National livestock census, 2000). The selected villages have 16,000 cattle and around 8,000 mainly goats, and sheep. The three villages were Oloirobi (medium interaction with wild ungulates), Misigiyo (higher level of interaction with wild ungulates) and Endulen (low level of interaction with wild ungulates). The 3 villages are about 10, 25, and about 45 km from the crater rim respectively. The Misigiyo village is within the wildlife migratory route in and out of Ngorongoro crater and to the famous Ngorongoro plains where wildebeest go for calving every year. These three villages were selected conveniently due to their accessibility by road, willingness of owners to test their animals and that most of human brucellosis cases reported in Endulen Hospital were inhabitants of these villages. Furthermore the type of grazing

system practiced is free range of livestock in the communal grazing areas where domestic animals interact with wild herbivores such as African buffaloes, wildebeests, zebra, waterbucks, and elephants among other species.

Study animals identification procedure

Study animals were obtained using a three stage random sampling. The village was regarded as primary unit, the herds as secondary units and individual animals as tertiary units. An average of 5-10 female cattle aged 9 month and above were picked randomly from each selected herd and screened for brucellosis. Beside females adult breeding bulls in the herds were also screened. Due to limited time of research, the sample size for the three villages was 200 cattle and 100 adult small ruminants.

Blood collection procedure

Collection of blood samples was done at 8.00 hr in the morning and around 16 hr in the afternoon because grazing system practiced is free range and therefore animals were not available during mid day. Blood sample was taken by jugular vein puncture using a sterile vacutainer needle and sterile plain vacutainer tubes. The blood samples were placed in a cool box in which ice packs have been put in. Samples were taken to NCA laboratory for centrifugation and decanting. Sera were decanted to the eppendorf tubes and stored frozen while waiting for laboratory test.

Sample testing for brucellosis

Each serum sample was subjected to Rose Bengal Plate test (RBPT) and Microagglutinantion Test (MAT), in Microbiology laboratory at the Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture.

The RBPT

The test was carried out according to a standard technique described by Brainley Morgan *et al*. (1978). The test was done by placing a drop of antigen equivalent to 25 micro liter on a transparent glass tile with zones of approximately 2 mm in diameter and an equal amount of test serum was placed on the slide alongside (not unto). The two parts were mixed thoroughly using a wooden applicator stick and the results read within 4 minutes. Any evidence of agglutination was regarded as positive and those with no agglutination as negative samples.

The MAT

Due to the fact that RBPT has a tendency of producing false positive and false negative results the samples were again subjected to MAT. The antigen was prepared by making a dilution of 1/10 of Rose Bengal reagent and Phosphate buffered saline (PBS) respectively. 25 micro liters of PBS were dispensed using a multi channel micropipette in all wells and then the same amount of test serum was dispensed in each first well of the rolls. The serial dilution was done from the first well of the roll to the last transferring 25 µm from the first to the last well and the last 25 µm was discarded and lastly 25 µm of prepared antigen was dispensed in all wells and the mixture was incubated at 37ºC for 24 hours. The results were read by comparing the degree of agglutination with control against a background with a source of light. Titers of 1:40 onwards were regarded as positive.

Statistical analysis

Collected data were entered in Epi-info database (Coulombier *et al*., 2001) for summarization and presentation.

RESULTS

Table 1. Bovine sera tested for brucellosis by Rose Bengal Plate test (RBPT) and Microagglutinantion Test (MAT)

Cattle	Number of Sera	Number positive (%)	
		RBPT	MAT
Adult cows	105	15 (14.28)	11(10.05)
Heifers	53	4 (7.54)	4 (7.54)
Bulls	42	1 (2.38)	1(2.38)
Total	200	20 (10)	12 (6)

Table 2. Goat sera tested for brucellosis by Rose Bengal Plate test (RBPT) and Microagglutinantion Test (MAT)

Cattle	Number of Sera	Number positive (%)	
		RBPT	MAT
Females	59	7 (11.9)	8 (13.8)
Males	28	3 (10.7)	4 (14.28)
Total	87	10 (11.49)	12 (13.79)

Table 3. Sheep sera tested for brucellosis by Rose Bengal Plate test (RBPT) and Microagglutinantion Test (MAT)

Cattle	Number of Sera	Number Positive (%)	
		RBPT	MAT
Females	10	1(10)	3(30)
Males	3	0 (0)	0 (0)
Total	13	1(7.7)	3 (23)

DISCUSSION

This study has demonstrated that domestic ruminants in NCA had brucellosis as per RBPT and MAT results. The results obtained can be regarded as reliable because both tests produced almost similar results. The observed proportions of diseased animals in this study are slightly higher compared from results reported earlier which showed that the overall prevalence in Ngorongoro district was 8.8% (Shirima, 2005). It is likely that the lower prevalence observed previously was due to a large sample size which also involved animals in villages far away from the crater where there is a higher concentration of wild animals which are thought to play a great role in transmission of brucellosis to domestic ruminants.

In this study the higher level of brucellosis prevalence among domestic animals as well as the increase of human brucellosis cases among individuals residing at NCA may be a reflection of indigenous culture and life styles which favour the existence and transmission of *Brucella* organisms

between wild and domestic animals and between domestic animals and humans. The great concern is toward the lack of proper disposal of foetal material and aborted foetuses in human settlements which play a great role in the disease transmission among domestic animals. The other reason for this higher prevalence in domestic ruminants is the coexistence of livestock and wild animals which facilitates survival and translocation of the disease causing agent. Test and slaughter policy for controlling brucellosis is not practiced by pastoralists at NCA. Therefore, the rate of transmission could be high due to increase in source of infection to humans.

Of the bovine species adult cows had a 14.3% prevalence of brucellosis than heifers (7.54%), does (11.9%) or ewes (10%) in this study. The high prevalence observed in cows however, could be attributed to the predilection site of the pathogen and presence of higher concentration of erythritol in the uterus which favours rapid multiplication of the pathogens in adult cows as compared to heifers or bulls (Alton, 1985). Due to this fact the infection persists most commonly in sexually matured animals (Adams, 1998). The other observation could be the ability of some young animals including heifers to clear the infection or the fact that infection may remain latent until adulthood when the animal is sexually mature (Bishop *et al.,* 1994; Radostits *et al.,* 2000).

A little variation in prevalence has been observed between the two serological tests. The differences could be attributed probably by false positives in the case of RBPT due to presence of residual antibodies and cross reaction with other pathogens such as *Escherichia coli* O: 057, *Salmonella* O: 30, *Yersinia enterocolitica* O: 9 LPS and *Vibrio cholerae* O: 1 which is common (Arthur *et al*., 1998).

Prevalence of brucellosis in goats and sheep has also been observed to be higher than expected. In pastoral communities goats are very important due to their hardship nature and disease resistance and thus very important as far as brucellosis is concerned. The commonest cause of brucellosis in goats is *Brucella melitensis* which causes a severe disease in human as well (Young, 1983). The equally high seroprevalence of brucellosis observed in goats could be probably due to tendency of infected does to carry the infection for months or to their lifetime. *Brucella melitensis* is the most infectious species of the *Brucella* genus and this may have contributed to the high prevalence observed among humans in this study. These findings will be a challenge to public health officials in the area because pastoralists prefer cattle milk followed by goat milk as their daily meal compared to sheep milk. Furthermore, goats are slaughtered frequently than either cattle or sheep posing a more risky hazard to pastoralists at NCA.

Among the three villages, Misigiyo village has shown the highest seroprevalence of brucellosis as compared to the other two villages. Although preventive methods have an influence on the transmission of brucellosis, a considerable numbers of wild animals in this area which share the same grazing areas and watering points with domestic ruminants may not be ignored. More focused studies are necessary in future to prove our hypothesis which has based on a limited size of samples and tests.

Despite few blood samples obtained, brucellosis seroprevalence in sheep, as reflected by both tests, indicated that the

higher prevalence was recorded among sheep in Misigiyo followed by Endulen in which there is a low level of interaction between wild ungulates and domestic animals. The small sample size for sheep was attributed by the farming system practiced in this area whereby sheep are transferred to the lowland areas and only few have been left behind for immediate uses mainly for meat. Further studies are recommended in order to get the true prevalence of brucellosis in sheep. More studies should be done to classify the species responsible for infection in sheep in NCA because sheep can be infected with *B. ovis*, *B. mellitensis* and *B. abortus*. *Brucella ovis* causes a mild disease and it does not cross react with other *Brucella* organisms like *B. bovis* or *B. mellitensis* (Kusiluka and Kambarage, 1996).

In conclusion brucellosis prevalence in domestic ruminants at NCA may have an association with brucellosis in wildlife in the same area and the increase in human brucellosis cases so observed in a hospital in Ngorongoro may also have an association with animal brucellosis. More research studies on zoonoses are necessary in this important world heritage site.

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