Studies on seroprevalence and risk factors for occurrence of Bovine brucellosis in cattle in Lindi district, Tanzania

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SUMMARY

Brucellosis is a contagious bacterial zoonotic disease of public health importance worldwide. A cross-sectional study was conducted from July to November 2017 in Lindi District to estimate the seroprevalence of brucellosis in cattle, assess farmers' knowledge and to identify risk factors for *Brucella* infection in animals. Questionnaires were administered to 60 livestock keepers and blood samples collected from 300 cattle for brucellosis analysis using Rose Bengal Plate Test and competitive-enzyme linked immune-sorbent (cELISA) assay tests. Results indicated that almost half (48.3%) of the households owned small herds which were mostly (58.7%) indigenous cattle. Proportions of positive reactors to brucellosis were 6.0% and 5.2% based on RBPT and c-ELISA respectively. Adult cattle were found to be frequently affected by brucellosis than young ones, and the difference was statistically significant ($p<0.05$). Comparable frequencies of infection were found in different sex groups and among cattle from different wards and different herd sizes. Most of the farmers lacked knowledge of brucellosis. History of abortion ($p=0.00$) and improper disposal of aborted materials ($p=0.04$) were found to be significantly associated with occurrence of bovine brucellosis in cattle. This study reports for the first time on occurrence of brucellosis in Lindi District and highlights on the possible risk factors for its transmission in cattle. Control efforts need to be put in place for this and other diseases with serious public and economic impacts the public.

Key words: Cattle, brucellosis, seroprevalence

INTRODUCTION

Livestock production in Tanzania forms an integral part of agriculture; the sector which contributes immensely to the economy of the country (John *et al*., 2010). Productivity of livestock is seriously affected by animal diseases some of which are naturally transmissible between animals and humans (Nonga *et al.*, 2009, Mellau *et al.*, 2010, 2011).

Brucellosis is an economically important disease in production animals worldwide. In cattle, the disease is usually caused by *Brucella abortus* and occasionally by *B. melitensis* and *B. suis*. Brucellosis is characterized by late term abortion; infertility and reduced milk production as a result of retained placenta, endometritis, and a varying degree of sterility in the males and cows. It is amongst the neglected zoonoses largely due to lack of public awareness and yet it is one of the most important zoonotic infections, especially in pastoral and mixed crop-livestock farming systems in Africa. Though it has been eradicated in many developed countries, brucellosis remains an uncontrolled problem in regions of high endemicity such as Africa, Mediterranean, Middle East, parts of Asia and Latin America (Faye *et al*., 2005; Karimuribo *et al*., 2007).

The risk of transmission of brucellosis to humans is largely lies on the presence of the disease in the susceptible animal populations. The effects on the disease are accentuated among marginalized communities as it is associated with poverty, poor farm management practices, and high levels of illiteracy; a feature of poor communities in developing countries. Bovine brucellosis, classified as one of the neglected zoonosis of interest by the World Health Organisation, has the potential to affect both public health and people's livelihoods, as it can perpetuate the cycle of poverty.

Brucellosis is a disease of greatest importance as it reduces production and reproduction performance of cattle and is of public health significance. Lindi region is an emerging livestock keeping area as it has been receiving cattle from different parts in Tanzania but have not been screened for important diseases like brucellosis. The proposed study intends to bridge this gap by exploring the status of brucellosis, its distribution, and the associated risk factors. The gathered information will contribute to database of brucellosis in Tanzania and can be used to devise appropriate national strategies for the control of the disease. The current study was meant to determine the seroprevalence of bovine brucellosis, assess the farmer's awareness on the diseases and possible factors for its transmission in cattle in Lindi district, Lindi region, Tanzania.

MATERIALS AND METHODS

Study design and population

This cross sectional study was conducted in Lindi rural District which is among the six districts of Lindi Region. The district is located on the southern part of Tanzania and it is bordered to the north by Kilwa District, to the south by Mtwara Region, to the west by Nachingwea District, and to the east by the Indian Ocean and Lindi Urban District. It has cattle population of 14, 900 and human population of 215,764 (National census, 2002). Only about 15% of people in Lindi district are involved in livestock keeping and subsistence farming. Cattle migrate from different parts of Tanzania to the district. The dominant pastoralists in the district are Maasai and Sukuma. The District is administratively divided into twenty-four wards. Chikonji, Chiponda, Kilolambwani Kitomanga, Kiwalala, Kiwawa, Mandwanga, Matimba, Milola, Mipingo, Mchinga, Mnara, Mtama, Mtua, Nachunyu, Nahukahuka, Namupa, Nangaru, Nyangamara, Nyangao, Nyengedi, Rutamba, Sudi and Tandangongororo. In most of these wards there are no livestock keeping activities instead they are involved in cashew nut production and fishing due to presence of Indian Ocean. The study was based on purposive selection of three wards where there are livestock keepers which include Kitomanga, Rutamba and Milola.

The population under study was considered to be heterogeneous comprising of varied gender and age groups. The inclusion criteria were; smallholder both women and men who keep dairy cattle or indigenous cattle, willingness to participate in the study and able to give information and accessibility of the place during data collection. The exclusion criteria included; unwillingness to participate in the study, unable to give information asked and living in inaccessible areas. Also those who had no time for interviews were excluded.

Sample Size determination and selection of study animals

The number of animals sampled was determined using the formula developed by Naing et al (1996); $n=1.96^2P\ (1-P)/d^2$. The expected prevalence (P) of 0.20, precision level (d) of 0.05 and confidence level of 95% resulted in a required sample size of 245 animals. Since multistage cluster sampling technique was adopted, in order to achieve the same precision, the original sample size was multiplied by the design effect (D) calculated using the formula D=1 + (b-1) roh (Otte *et al*., 1997). The average number of samples per cluster (b) was 7 and intra-cluster correlation coefficient or rate of homogeneity (roh) was set at 0.10. Number of clusters (c) sampled was determined by the formula $c=P\ (1-P)\ D/SE^2n$ and Standard error (SE) was calculated by SE=d/1.96 (Otte *et al*., 1997). Accordingly, the total number of animals bled was 300 and the number of clusters to be sampled was 56. Selection of villages and study herds was done by a simple random sampling technique. Individual animals in the herd were selected by a systematic random sampling technique. The study was conducted on both smallholder dairy herds and traditional herds comprised of indigenous cattle. In this study, a herd was regarded as a cluster, defined as a group of cattle (of both sexes) kept in one enclosure and constituting ten or more animals aging six months and above. Furthermore, herds were classified into two categories; large herds (>15 animals) and small herds (≥10≤ 15 animals) based on the classification used by the departments of Livestock and Fisheries Development in the study area. Cattle aging >2 years was representing adults and those aging ≤2 years was representing young animals adopting categorization of age groups used by Degefu *et al*. (2011). Also questionnaires were be administered to 60 randomly selected livestock keepers. In the 3 purposively selected wards i.e. Kitomanga, Rutamba and Milola, two villages were randomly selected from each ward, making 6 villages and from each village 10 questionnaire were administered to 10 households which were randomly selected in each village.

Ethical Consideration

Research permit was provided by Research, Publications and Ethics Committee of the College of Veterinary Medicine and Biomedical Sciences of Sokoine University of Agriculture (Appendix 2). Permission letters were obtained from Executive Director of Lindi District council. Verbal consent was obtained from each of the heads of study households, after explaining the purpose and importance of the study prior to commencement of interviews and sampling. Participation in the study was on voluntary basis. All the information collected from the participants and the laboratory results obtained after blood sample analysis were kept under the custody of the researcher as confidential and the study participants were treated anonymously.

Data collection

Recruitment of research assistants

Four research assistants were recruited; two livestock field officers who had a certificate in animal health were recruited to assist the researcher in data collection. These had good communication skills and experience on interaction with smallholder dairy farmers. The other two research assistants had Diploma in laboratory technology. Inclusion of local research assistants to the area helped in maximizing trust of respondents, interviewer- interviewee interaction and facilitation of laboratory work. The research assistants were briefed on the objectives of the study, data collection process, target respondents, selection criteria, approach during interview, understanding questions, elaborating why and how each question was asked, assuring the respondents confidentiality, how to record responses from interviewees, laboratory analysis procedures and data collection and recording.

Farmer interviews

Information about each herd and the animals kept was collected by means of a structured questionnaire, which was completed at all the selected herds on a single visit. The questionnaire was designed to comprise mostly closed ended (categorical) questions to ease data processing, minimize variation, and improve precision of responses. The questionnaires were pretested in the field and adjusted as required. Interviews were conducted to members of the households who were more conversant about the herds using Swahili dialect. Data on risk factors for *Brucella* seropositivity, livestock owner's knowledge, attitudes and practices regarding brucellosis was concurrently collected. Information on individual animal variables (age, sex, breed, management system, disease control programme eg. vaccination was recorded on separate sample data sheets. Other information on occurrence of reproductive events such as; history of abortion, retained placenta or other reproductive disorders was also collected. Herd personnel's knowledge and awareness of brucellosis and its transmission, disposal of placenta, aborted materials and history of raw milk consumption were also recorded. A total of 60 households were included in the study.

Blood sampling from cattle

The selected cattle for the study were properly restrained in a crash for those livestock keepers with crashes, but for those without a crash, ropes and manual restraint were used. About 5 ml of blood sample was collected from the jugular vein of each animal into plain vacutainer tube. Each tube was labeled by using codes describing the specific animal and herd. The samples were left to clot at room temperature overnight and subsequently centrifuged at 3000 rpm for 10 minutes to obtain clear serum. Thereafter about 2 ml of serum was collected into cryovials and stored at -20°C at Lindi Regional Hospital until serological analysis which was performed (a month later) at the College of Veterinary Medicine and Biomedical Sciences laboratories of Sokoine University of Agriculture.

Laboratory analysis of sera samples by Rose Bengal Plate Test

All collected sera samples were first screened using Rose Bengal Plate Test (RBPT) for *Brucella* antibodies according to the test procedure recommended by OIE (OIE, 2009). Briefly, 30 µl of RBPT antigen and 30 µl of the test serum were placed alongside on the glass plate and mixed thoroughly. After 4 minutes of rocking, any visible agglutination was considered as positive result (Omer *et al*., 2002).

Laboratory analysis of sera samples by competitive Enzyme Linked Immunosorbent assay(c-ELISA)

Rose Bengal Plate Test positive sera were then subjected to competitive enzyme linked immunosorbent assay (c-ELISA) as a confirmatory test, adopting a test procedure and interpretation of results as recommended by the manufacturer (Animal Health and Veterinary Laboratories Agency AHVLA, New Haw, Addlestone, Surrey, KT153NB, UK). Only animals positive on c-ELISA were regarded as *Brucella* seropositive. The spectrophotometer was adjusted at an absorbance of 450nm. A positive negative cut-off was calculated as 60% of the mean of the optical density (OD) of the four conjugate control wells. Any test sample that give OD equal to or below the value was regarded as positive. A herd was considered positive for the disease when at least a single animal tested positive for c-ELISA.

Data analysis

Microsoft Way, Redmond, 98052-7329, USA) and imported into Epi-Info version 7 (CDC Atlanta, USA) and MedCalc® version 13.0.2 (MedCalc software, Acacialaan 22, B-8400, Ostend, Belgium) for analysis. Frequencies were determined for proportions of positive animals and herds. Risk factor analysis adopted logistic regression in two steps i.e. univariate Multivariate analysis. The model was constructed by a forward stepwise selection of variables. The predictor variables were assessed for collinearity using Pearson's Chi-squared test. The potential confounding effect of those variables not retained in the final model was assessed using Mantel-Haenszel Chi-Squared test. The model was evaluated for goodness- of- fit using a Hosmer-Lemeshow test. The discrimination ability of final model was assessed using the receiver operating characteristics (ROC) defined as the area under the curve (AUC). The interaction term was introduced in the final model to assess effect of modification. For final analyses, a p-value of ≤ 0.05 was taken as significant.

RESULTS

Socio-demographic attributes of surveyed livestock farmers in Lindi rural District

In this study 60 farmers were reached out for interviews. Their socio-demographic attributes are summarized in Table 1.

Table 1. Respondent's socio-demographic characteristics and livestock keeping experience in Lindi rural (N=60)

Variable	Category	Frequency	Percent
Gender	Male	51	85.6
	Female	9	14.4
Age (years)	20-45	20	32.5
	46-65	36	60.1
	66 and above	4	7.5
Education level	Non formal	15	25.4
	Primary	32	54.0
	Secondary	7	12.0
	Graduates	0	0.0

Livestock production and health management practices among surveyed farmers in Lindi rural District

Of the visited farmers (n=60), 65.8% practice extensive, 9.2% practice intensive and 30.0% practice Semi-intensive production system. Majority of them had a 3-10 yrs experience in livestock farming. Around two thirds had an experience of 10-20 years and the rest kept animals for less than three years. Only 8.6% of the farmers had attended livestock production and/ or health related training. Only 5.8% of the herds were bred using artificial insemination, while 94.2% used natural breeding. Indigenous cattle produce between one and three litres of milk a day; whereas majority of the cross-bred dairy cattle (78.79%) produced between 5 and 10 litres a day. A small proportion of the dairy cattle (21.21%) produced between 10 and 15 litres of milk a day. All the respondents reported access to veterinary care for their animals, majority of them (86.7%) from the public veterinary services. None of the farmers vaccinated his/her animals against brucellosis. However, 31.8% of herds were vaccinated against other diseases such as Foot and Mouth Disease (FMD), Rift Valley Fever (RVF), East Coast Fever (ECF), Contagious Bovine Pleuro Pneumonia (CBPP), Anthrax and Lumpy Skin Disease (LSD). Records keeping for vaccination and other routine treatment were reported from 52.8% of the respondents. Most farmers reported other common diseases and they seem to be not aware of brucellosis as one of the most common zoonotic disease which can have a very big implication on human health.

Livestock owner's knowledge, attitudes and practices (KAPs) regarding brucellosis in the study area

Majority of the farmers in the current study (85.0%) were not aware of brucellosis. All of those who were aware of the disease acknowledged to have seen suspected case of brucellosis in animals; both in cattle and sheep. Abortion and unthrifty new born were mentioned as common signs of the disease in animals. Two of the respondents among those who were aware of the disease seemed to be aware of the zoonotic potential of the disease but couldn't figure out how human acquire infection. In this survey it was revealed that 22.2% of those who were aware of brucellosis attempted to treat infected animals and 55.5% sold infected animals at the live animal market. All the interviewed farmers left aborted cows with other animals. Unregulated animal movement was common among 100.0% of those who practices extensive farming. About 43.3% of respondents admitted to have observed abortion in their herd. The most common method of disposing off placenta, fetuses and dead calves was by burying

in the ground as reported by 53.8% of respondents who had aborting cows in their farms. Other methods of disposal were throwing to the bush (15.4%), feeding to dog (23.1%) and disposal in pit (7.7%).

Prevalence of Bovine Brucellosis in indigenous and crossbred dairy cattle in Lindi District council

A total of 300 cattle from sixty herds in three wards were sampled in this study. Out of these 61 were males and 239 were females. At individual animal level, the proportions of positive reactors to brucellosis were 6.0% and 5.2% on RBPT and c-ELISA tests, respectively. At ward level the proportions of positive animals were 5.0% for Kitomanga (n=200), 6.0% for Milola (n=50) and 6.0% for Rutamba (n=50). The positive animals were detected in 15% of the sampled herds (n=60).

Around 13.8% of the small herds (n=29) tested positive, whereas 16.13% of the large herds (n=31) tested positive. There existed no statistically significant differences in proportions of sero-positive animals between small and large herd sizes; as well as between wards. Table 1 below displays results of sero-positivity to brucellosis by different categories of sampled animals based on a confirmatory test.

Table 2. Prevalence of brucellosis among different categories of cattle in Lindi rural District based on c-ELISA (n=300)

Attribute	Categories	No. of animals tested	No. of positive animals (%)	Chi square (95 CI)	P-value
Sex	Male	61	2 (3.3)	0.24 (-5.9% to 7.36%)	0.63
	Female	239	14 (5.9)		
Genotype	Indigenous	201	10 (5.0)	0.008 (-4.5% to 8.1%	0.93
	Crossbred dairy	99	6 (6.0)		
Age	Young	108	1 (0.9)	5.24 (1.59% to 11.72%	0.02
	Adults	192	15 (7.8)		

Risk factors for occurrence of brucellosis in indigenous and cross-bred dairy cattle in Lindi rural District

Several factors were screened for association with occurrence of bovine brucellosis at herd level. These included cattle breed, age, sex, breeding method (natural service v/s artificial Insemination), herd size, production system (extensive, intensive, semi-intensive), history of abortion, contact with animals from other herds, contact with other animals, sharing of bulls, introduction of new animals into the herd and disposal of aborted materials. On univariate analysis; contact with pigs, contact with small ruminants, contact with wildlife, source of replacement from livestock market, improper disposal of aborted materials, use of bulls from other herds and use of dogs as guardians during grazing qualified ($p<0.10$). On multivariate analysis; history of abortion and improper disposal of aborted materials were found to be significantly associated with occurrence of bovine brucellosis in a herd.

Table 3. Results of multivariate logistic regression analysis of risk factors for occurrence of bovine brucellosis in indigenous and cross-bred dairy cattle in Lindi rural District

Risk factor	Category	OR	P-value
History of abortion in the herd	Yes	12.3	0.00
	No		
Disposal of aborted materials	Improper	2.97	0.04
	Proper		

*OR = Odds Ratio

DISCUSSION

We are reporting an animal level prevalence of brucellosis of 5.2% in Lindi rural District, which is slightly lower than those reported in previous studies in the country (Assenga *et al*., 2015, 6.8%; Chitupila *et al.*, 2015, 5.6%; Karimuribo *et al.*, 2007, 6.2%; Swai *et al.*, 2010, 7.3%). A study in Ethiopia, however, reported lower seroprevalence

levels of 3.19% (Berhe *et al.,* 2007) than what is reported in the current study. The same two studies in Ethiopia, however reported higher herd level seroprevalences of 26.1% and 42.31% respectively; contrary to 15.0% obtained in the present study. Similarly, in the country, Swai *et al.* (2010) reported a slightly higher (20%) herd level seroprevalence than what is reported in this study.

There is no controlled study that has been conducted on the relative susceptibility of female and male cattle to brucellosis. Similar to our finding, earlier studies (Berhe *et al.,* 2007; Mellau *et al.,* 2009; Desefu *et al.,* 2011) contend that there is no sex predisposition of cattle to *Brucella* infections. However, based on the reactor rates, some authors (Degefu *et al.,* 2011) propose that bulls are more resistant than sexually mature heifers and cows but less resistant than sexually immature heifers. A study by Ferede *et al.* (2011) discovered that *Brucella* infections limited to testes in male animals result into non reactors or reactors displaying low antibody titers. The same authors associated less susceptibility of male animals to *Brucella* infection with lack of erythritol (Ferede *et al.,* 2011). Seroprevalence may increase with age as a result of prolonged exposure to pathogens particularly in traditional husbandry practices where females are maintained in herds for long period of time than males (Blood and Radostitis 1990).

No statistically significant difference in proportions of positive reactors to brucellosis was observed between small and large herds. Our observation is consistent with those made by previous authors (Jergefa *et al.,* 2009; Chitupila *et al.,* 2015). Some other studies in the country (Swai *et al.,* 2010) and elsewhere (Berhe *et al.,* 2007) have reported a contrary finding to ours. The authors of these works have observed higher prevalence in large herds than in small herds. These different observations among studies may be influenced by the presence of varying exposure scenarios in the different study populations.

A significantly small proportion of young animals turned out to be positive for brucellosis in the present study. This observation underscores findings reported by previous authors (Berhe *et al*., 2007; Mellau *et al*., 2009; Swai *et al.,* 2010; Degefu *et al*., 2011; Assenga *et al*., 2015). It is argued that age is one of the intrinsic factors which influence the susceptibility to *Brucella* infection such that young and sexually immature animals are more resistant to primary infection and often clear infection although latent infections do occur (Degefu *et al*., 2011). Similarly, growth and multiplication of *Brucella* organisms are stimulated by sex hormone and erythritol which tend to increase in concentration with age and sexual maturity (Walker, 1999; Dinka and Chala, 2009; Ferede *et al*., 2011). Authors of a study conducted in the country associated higher sero-prevalence of brucellosis in adult animals with under nutritional, stress and lower immunity that develops following acute infection (Swai *et al.,* 2010).

Lack of knowledge on brucellosis was a feature in majority of the livestock keepers in the study area. This compromises disease prevention and control efforts. This jeopardizes the health of these farmers as lack of knowledge and awareness about the disease implies that they don't take required precautions when handling infectious products. Moreover, with these results it is obvious that no precaution is taken to prevent spread of the disease to other herds within or outside the study area. With the perception that brucellosis in animals can be cured and the practice of farmers disposing suspect animals into the food chain or selling them to others is likely to result into propagation of the disease. he use of antimicrobials against *Brucella* infections in animals causes L-transformation on the cell wall in a way creating carrier. Regarding disposal of infected animals, Holt *et al*. (2011) points out that, selling infected animals may increase transmission of brucellosis between households in a village, between villages and even to larger geographical areas. In its Regulation, 2007, The Animal Disease Act, 2003 indicates that animals should be tested for brucellosis, reactors separated from the healthy animals and destroyed. Isolation of anmals suspected of having brucellosis is not practiced by livestock keepers in the study area. Holt *et al*. (2011) asserts that failure to separate animals that abort increases the risk of susceptible animals acquiring brucellosis through contact with aborted materials.

In this study, history of abortion in the herd was revealed to have a positive association with the serological prevalence of bovine brucellosis in the study area. This could easily be linked by the possibility of most of the abortion occurring as a result of infection with *Brucella* organisms. Contamination of environment with aborted materials, lochia, urine and uterine discharges from *Brucella* positive cows have been documented to be major sources of infection to other incontact susceptible animals (Blood and Radostitis 1990).

Some few studies have reported on risk factors for bovine brucellosis in the country. A study by Chitupila *et al.* (2015) revealed that improper

disposal of aborted materials is among the factors. This observation has surfaced again in this study whereby improper disposal of aborted materials has been linked with the likelihood of occurrence of brucellosis. Susceptible animals can be infected via direct contact with aborted materials or by products of parturition. Similarly, improperly disposed aborted materials contaminate the environment with *Brucella* organisms and become major sources of infection to susceptible animals (Blood and Radostitis 1990). Such contamination of environment can lead into contamination of pasture and hence cattle become infected when grazing. Once embedded in protein like aborted materials and /or uterine exudates *Brucella* organisms can survive in dry conditions for 42 days to 75 days as they are protected from the effects of direct sun light (Blood and Radostitis, 1990). Some other authors report that under ambient temperature and relative humidity, *Brucella* organisms can survive in aborted fetuses in sheds and in liquid manure for up to 8 months (Blood and Radostitis, 1990). The organisms can also survive in the grass for up to 100 days depending on the season as suggested by Blood and Radostitis, (1990). In some cases dogs can acquire *Brucella* infection through access to infected aborted materials from cattle; and infected dogs shed the organisms into environment via urine, vaginal secretions, aborted materials or feces (Blood and Radostitis, 1990).

The present study provides evidence of presence of bovine brucellosis in an emerging cattle keeping area in Southern Tanzania. It also points out to associated risk factors with occurrence of the disease, all of which have been mentioned earlier. With the prevalent production system, poor bio-security and congregation of animals at grazing and watering points, it is likely that the disease can spread within and between herds. It is therefore advocated to institute control measure for the disease focusing more on preventing exposure of susceptible animals to the pathogen. Public education on the epidemiology and health impacts of the disease will help to prevent further spread of the disease and protect human health within and outside the study area. General principles of hygiene and mass vaccination of susceptible population to achieve herd immunity will also help to prevent the disease.

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