Studies of brucellosis in lactating cows in Babati district, Tanzania

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SUMMARY

The present cross-sectional study was carried out to determine prevalence and risk factors for transmission of brucellosis in lactating cows in Babati district. Rose Bengal plate test (RBPT), buffered acidified plate test (BAPA), competitive enzyme-linked immunosorbent assay (c-ELISA) and polymerase chain reaction (PCR) tests were used in this study to determine the presence of antibodies against *Brucella* and *Brucella* genome. Milk and blood samples from 192 randomly selected lactating cows were collected. Furthermore, questionnaires were administered to 66 milk producers to determine the risk factors for the transmission of brucellosis in between animal populations. The RBPT and BAPA results showed 4.7% (nine cows) and 5.2% (10 cows) seroprevalence, respectively. When RBPT and BAPA positive samples were tested using c-ELISA for serologic confirmation, only eight cows (4.2%) turned out to be positive. The milk samples from eight cows that were positive for Brucella antibodies using c-ELISA were tested for the presence of Brucella DNA using PCR. Three out of the eight milk samples were positive for *Brucella abortus* indicating shedding of Brucella in milk. Analysis of risk factors for transmission of brucellosis by Fisher's exact test or Chi-square showed that livestock mixing with different herds (P=0.0097, OR=11.3333), farming system of cattle (P=0.0400, OR=3.9474), breed of cattle (P=0.0284, OR=1.9860), herd size of cattle (P=0.0030, OR=1.9537) and movement of animals through selling and purchasing (P=0.0500, OR=5.0588) were statistically associated with Brucella positivity. This study provides evidence of brucellosis in lactating cows of Babati district and shedding of *Brucella* in milk. Institution of appropriate control measures including public health education, surveillance of animals accompanied with removal of positive cases according to laws and immunisations of cattle are highly recommended.

Key words: Seroprevalence, *Brucella*, Rose Bengal plate test,

INTRODUCTION

Brucellosis is well-documented by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and also the World Organisation for Animal Health (OIE), as the most widespread bacterial zoonoses in the world posing serious public health problems and extensive economic losses (Lopes *et al.,* 2010; Neta *et al.,* 2010; Yasmin *et al.,* 2011). Brucellosis is one of the most important and well-known bacterial zoonoses in the world (Lopes *et al.,* 2010; Swai and Schoonman, 2010). The disease is additionally described as true zoonosis because all human infections are of animal origin (Kaoud *et al.,* 2010). Brucellosis is considered a re-emerging disease of special importance in East, North of the Mediterranean countries, South and Central Asia, Central and South America. Moreover, recent reports add zones as far apart because the Republic of Korea and Zimbabwe as foci representing the wide potential hazard. Brucellosis for the primary time was reported in 1859 in Malta (Lee *et al.,* 2009; Matope *et al.,* 2010).

Brucellosis is a disease that caused by Gram-negative coccobacilli, non-motile, non-spore forming, aerobic, non-toxigenic and non-fermenting bacteria of the genus *Brucella*. Brucella genus is divided into six classical species, namely *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae*, is still widely used due to historical and clinical reasons but recently identified *Brucella species* isolated from marine mammals, *B. ceti* and *B. pinnipedialis*, are now included in classification (Mariana *et al.,* 2010). The pathogenic Brucella includes *B. suis, B. melitensis, B. abortus and B. canis* which infect swine, goats, cattle, and dogs, respectively (Jelastopulu *et al.,* 2008). However, infection with any of the three species of Brucella may occur in all domestic animals. *B. canis* is also a pathogen of human but is of lesser importance. In addition, two recently identified *B. species* isolated from marine mammals, *B. ceti* and *B. pinnipedialis*, can also cause human brucellosis (Mariana *et al.,* 2010). On the other hand *B. ovis* and *B. neotomae* have never been reported to cause disease to human being (WHO, 2006). Among the four *Brucella species* known to cause disease in humans, *B. melitensis* is thought to be the most virulent and causes the most severe and acute cases of brucellosis, while *B. abortus* is reported to be the most widespread (Yingst *et al.,* 2010).

Brucellosis imposes great economic loss to the farmers since its lead to abortions in newly infected herds, retention of placenta, leading to metritis and endometritis, increased infertility, still births, reduced milk production leading to early culling and replacement of animals (Xavier *et al.,* 2009; Shafee *et al.,* 2011). Also the most serious losses are the number of humans that suffers brucellosis leading to high cost of treatment, manpower incapacitation which affects person, family, community and national economic growth (Kunda *et al*., 2010; Wankyo, 2012). Control of brucellosis in animals tremendously reduces the burden of disease in human and veterinary charges. Most of the previous studies conducted in Tanzania involved parastatal farms and few indigenous cattle herds (Karimuribo *et al.,* 2007). Limited studies about brucellosis have been carried out in Babati district, like that of Mtui-Malamsha (2001) and Shirima (2005). The studies carried out in livestock in Babati did not find or quantify risk factors associated with transmission or spillover of infection between cattle which produces milk for human consumption and other livestock as well as wild animals. There was no report that provided useful information to public and professionals about prevalence, molecular diagnosis and the risk factors of brucellosis in lactating cows in Babati district. Babati district was selected in the present study because dairy cattle business and milk production demands increasing tremendously and none livestock owners in the study area were using Brucella vaccines for the control of Brucella infections.

The study was conducted in order to determine the prevalence of brucellosis in selected lactating cows. Molecular diagnosis was used to detect *Brucella species* DNA from positive milk samples that were initially screened and confirmed using serological tests. It is well known that serological methods are not always sensitive or specific and they have repeatedly been reported to cross-react with antigens other than those from *Brucella* species (Göknur *et al.,* 2010). Due to this reason, this current study used two selected screening serologic tests and one serologic confirmatory test. The purpose of this study was to establish epidemiological data for brucellosis in cattle and determine the performance of selected serological tests.

MATERIALS AND METHODS

Study area, design and animals

This study was conducted in Babati district which is formed by two councils, namely, Babati district (BDC) and Babati Town councils (BTC). The district is at an altitude that range between 950 to 2450 meters above sea level. Rainfall in Babati district ranges between 500 and1200 mm per annum. There are two rainy seasons; the short rains from October to December and the long rains between February and May. Average temperatures range from 22ºC to 25ºC, though it can be colder in the highlands around Bashneti and warmer in the lowlands around Magugu, Mwada and Nkaiti wards. Lactating cows were sampled from both Babati district and Babati town councils (Figure 1).

The present study was cross sectional that was carried out between July 2013 and August 2014. The target study animals were lactating dairy cattle which mostly were crosses of Friesian, Ayrshire and Jersey.

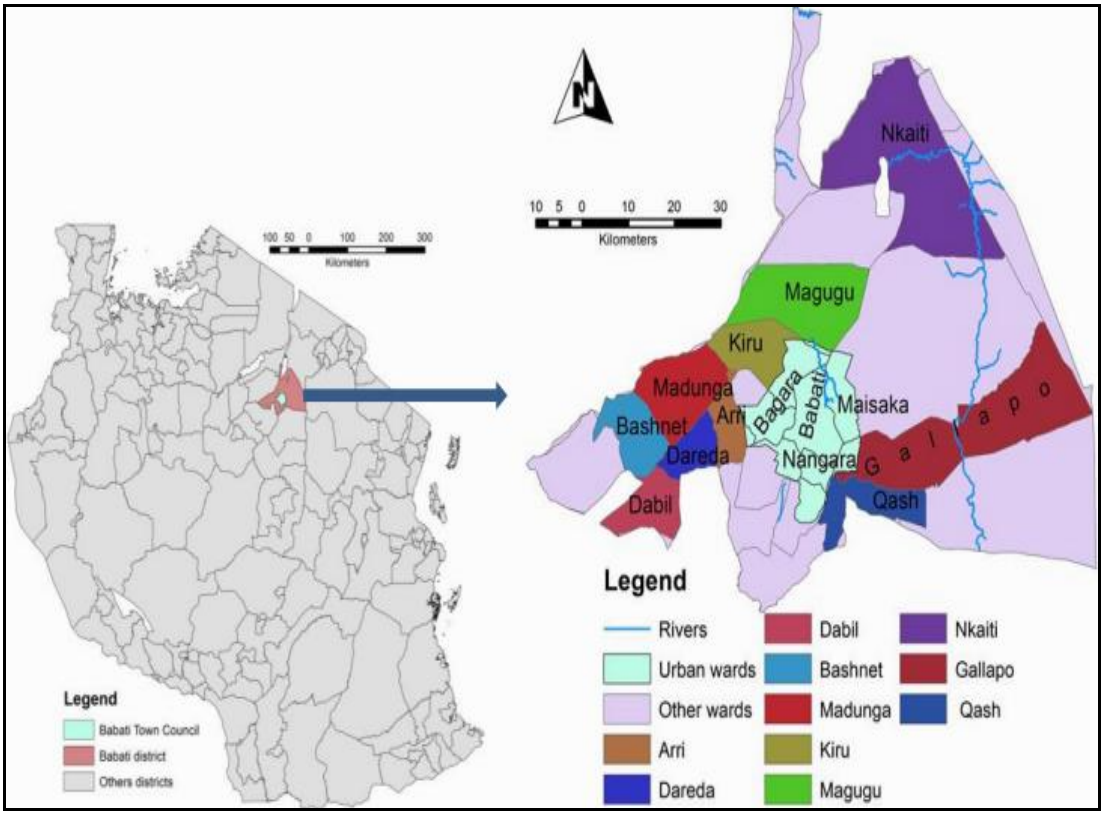

Figure 1. Map of Babati district showing sampling locations. Samples were collected from Babati district and Babati town councils. Sampling locations are indicated by names on the map.

Estimation of sample size

Each cattle keeping household was regarded as a cluster. The number of clusters (C), required for this study was calculated using the formula $C=\frac{P(1-P)D}{(SE^2)n}$, where D= 1+ρ (n-1), SE= $\frac{L}{Z_\alpha}$, P = estimate value for proportions, D = design effect, SE = standard error; n = average cluster size =3, ρ = intra-cluster correlation coefficient = 0.15 (Otte and Gumm, 1997). The average herd size was three animals per herd (BDC and BTC annual reports, 2012), the expected prevalence of brucellosis in dairy cattle was assumed to be 12.2% (Swai *et al.,* 2005). The statistical confidence level was decided to be 95%, and desired absolute precision was 5%. Accordingly, using the formula and the values above, the sample size required was determined to be 66 households (cluster), and 192 lactating cows.

Animal selection and data collection

This study included 66 households owning 192 lactating cows. Based on cattle concentration, the households were sampled from BDC and BTC. The list of heads of the households with at least three lactating cows was obtained from BDC and BTC's Livestock and Fisheries Departments and they were used as sampling frame. The household was a sampling unit and the lactating cows for study were selected randomly. Age of animals was classified as young, middle age and adult lactating cow with less than three years, three to five years and above five years, respectively. There was no history of vaccination against brucellosis in cattle of Babati district.

Sample collection and handling

Before milk and blood sampling was undertaken, the head of selected household was interviewed with the questionnaires which focused on general livestock husbandry, epidemiology, ecological factors and assessment of knowledge and awareness of transmission of brucellosis. Thereafter, the selected lactating cows were restrained using ropes, crush and bull ring depending on what restrain technique was favourable on specific herd situation found. Approximately 10 ml of whole blood was collected from the jugular veins by venipuncture into plain vacutainer tubes (Griner Bio-One GmbH Kremsmunster, Austria). In addition, from the same animal, 10 ml of milk was collected from washed and dried udder into Falcon tubes. Blood samples were stored in cool box and later on transferred to

Babati District Veterinary Office where they were allowed to clot in a slant position on a table and serum was harvested into Eppendorf tubes after 24 hours. Serum and milk samples were kept in ice box during transfer to laboratories at Sokoine University of Agriculture. Both sera and milk samples were stored at -20°C until used for serological and molecular screening of brucellosis.

Laboratory analysis of samples

Serological detection of *Brucella* antibodies

Serum samples from cattle were tested for antibodies against Brucella. In the present study, screening of Brucella antibodies was done using RBPT and BAPA while c-ELISA was used as confirmatory test, according to protocols for *Brucella species* detection described by OIE, 2009. Laboratory analysis of samples for all tests was carried out at the Faculty of Veterinary Medicine (FVM), SUA. A village was considered positive if there was at least one animal in herd in that village responding positive to c-ELISA confirmatory test while a herd was considered positive if at least one animal was detected to have Brucella antibodies in that herd.

Sera were tested for antibody against *Brucella species* using rapid Rose Bengal plate test (RBPT) using the Rose Bengal stained antigen (Central Veterinary Laboratory, UK). Briefly, 30 µl Rose Bengal antigen (Weybridge standard**)** was placed on glass-plate and followed by mixed with an equal volume of test serum. Afterwards, the antigen and test serum were thoroughly mixed using stirring stick and the slide was gently rocked for four minutes at room temperature. The reactions were then examined for agglutination by naked eyes. The sample was considered positive if serum in the glass plate agglutinated and the test was repeated for samples with weak agglutination.

Sera were tested for antibody against *Brucella spp.* using Buffered acidified plate test (BAPA). Briefly, 80 µl of serum followed by 30 µl of antigen were placed onto a clear glass plate and mixed with a stirrer to cover a circle with approximately 27 mm. Positive and negative control serum were also separately included during the testing. Afterwards, the plate was rotated four times, covered and the antigen-antibody mixture incubated for four minutes at room temperature. Then, the plate was again rotated for four times, followed by incubation of the antigen-antibody mixture for four minutes. The reactions were then examined and scored for agglutination by naked eyes. The sample was considered positive if serum in the glass plate was agglutinated and test was repeated for samples with weak agglutination.

Competitive enzyme-linked immunosorbent assay

The confirmation of the presence of *Brucella* antibodies was performed using c-ELISA following a protocol described by Veterinary Laboratory Agency (VLA), Surrey, United Kingdom. Briefly, the conjugate solution was prepared and diluted to working strength and 100 µl added onto each well of 96 well ELISA plate. Afterwards, 20 µl of each test serum sample was added per well. After addition of the conjugate, plate was vigorously shaken to allow mixing of the serum and conjugate solution. The plate was covered with the lid and incubated at room temperature (21°C ± 6°C) for 30 minutes on rotary shaker, at 160 revolutions per minutes to allow the interaction between antibodies and the antigen coated on the plate. After incubation, the contents of the plate were discarded and the plate rinsed five times with washing solution and thoroughly dried by tapping on absorbent paper towel. The ELISA reader was switched on and the unit was allowed to stabilize for ten minutes. Before the unit was used, the substrate and chromogenic solution was prepared by dissolving one tablet of urea and hydrogen peroxide in 12 ml of distilled water. Afterwards, 100 µl of substrate and chromogenic was added to each well. The plate was left at room temperature for minimum of 10 minutes and maximum of 15 minutes. The reaction was stopped by addition of 100 µl of acetate buffer and condensation on the bottom of plate was removed by absorbent paper towel. Reading of the plate was made at 450 nm by using Thermo Labsystems Multiskan R.C. The lack of colour development indicated that the sample tested was positive. 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 gave OD equal to or below the value was regarded as positive.

Molecular diagnosis

DNA extraction from milk samples

The collected milk was thawed and used for DNA extraction using ZR Genomic DNA™ Tissue MiniPrep kit (Zymo Research, USA). To 3 ml of milk samples, 200 µl of water, 200 µl of 2x digestion buffer and 20 µl proteinase K were added and incubated overnight at 55^{o}C. After overnight incubation 500 µl lysis buffer was added and mixed thoroughly by vortexing. Then samples were centrifuged at 10 000 *g* for one minute to pellet precipitated proteins. Thereafter, supernatant was transferred to a Zymo-Spin™ IIC column and DNA allowed to bind to the column by centrifugation at 10 000 *g*. DNA bound to the column was washed using buffers to remove PCR inhibitors. DNA was eluted into a micro-centrifuge tube. The eluted DNA was stored at -20^{o}C until PCR.

Amplification of *Brucella* species DNA by PCR

Detection of the presence of *Brucella* spp. genome in milk samples was done using PCR. The components of the PCR mix and the primers used for the detection of different *Brucella* spp. are shown in Table 1 and 2, respectively. PCR was performed using a DNA polymerase from Bioneer, Korea. The amplification conditions consisted of an initial denaturation at 95°C for 10 minutes followed by 40 cycles of denaturation (30 seconds at 95°C), annealing (30 seconds at 55°C) and extension (90 seconds at 72°C), and a final extension at 72°C for 10 minutes on a thermal cycler (TaKaRa, Japan). After PCR, 5 µl of the PCR products was mixed with a 6x loading dye. The PCR products were then electrophoresed in one per cent agarose gel in buffer containing Gel Red (Biotium, USA) and a marker of one kilo base pairs ladder was used. Electrophoresis was performed at 80 Volts for 45 minutes. Finally the results were read and image captured using a gel documentation system (Gel doc EZ Imager, BioRed, USA).

Table 2. Preparation of PCR mix for the detection of *Brucella* in milk samples

Component	Volume for one reaction (µl)
PCR premix containing DNA polymerase and dNTPs	0.5
Forward primer (10 µM)	0.5
Reverse primer (10 µM)	0.5
Nuclease free water	13.5
DNA template	5

Table 3. Primers used for the detection of *Brucella* in milk samples

Primer	Sequence 5'→3'	Target *Brucella* spp.	PCR product size (bp)	Reference
IS711	TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT-TGC-CAG	Forward primer for both *B. melitensis* and *B. abortus*		Bricker and Halling, 1994
abortus	GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC	Reverse primer for *B. abortus* biovars 1, 2 and 4	495	Aggour *et al*., 2013
melitensis	AAA-TCG-CGT-CCT-TGC-TGG-TCT-GA	Reverse primer for *B. melitensis* biovars 1, 2, and 3	730	Aggour *et al*., 2013

Livestock owner's cross-sectional questionnaire survey

A pre-designed structured questionnaire with both close and open ended questions was used to collect information on herd level risk factors we believed to be associated with *Brucella* prevalence. Pre-testing of the questionnaire was carried out in one of the study areas to ten dairy owners to detect any lack of clarity of questions was noted and later revised and few changes were made before final version was developed. A questionnaire form comprising of variables such as herd size, source of their cattle, mixing of cattle with cattle from other herds, purpose of his/her dairy farm, milk and meat consumption habit and presence of brucellosis patient in their family. In addition, data on individual animal such as sex and age were recorded. Questions related to general livestock husbandry, livestock and wild animals feeding pattern, contacts between wild and domestic animals and livestock movement. The revised questionnaire was then administered to households where animal blood and milk samples were taken. The interviews were conducted by the author alone after harvesting blood and milk from livestock and one member of

family was involved. The selected respondent was the one knowledgeable about the herd, usually the head of the house. When the head of house was absent, other members of the household like the wife, child, parents/parents in law of head of house or other specified member with knowledge of herd under investigation were interviewed. The interview took about 30-40 minutes.

Data analysis

Data was entered in Microsoft Excel 2010 and analysed by Epi Info (Epi Info™ 7.1.3, Atlanta, USA). Individual animal level prevalence was defined as the number of positive reactors per 100 animals tested. Herd level prevalence was computed as the number of herds with at least one-reactor cattle divided by the total number of herds tested. Descriptive statistics such as frequencies and percentages were calculated. A confidence limit of less than 5% ($P< 0.05$) was used to indicate a significant level. Chi-square test and Fisher's exact were used to compare the statistical significance in prevalence of brucellosis in livestock using Epi Info statistical software. Logistic regression analysis was used to assess strength of association of different factors to the occurrence of brucellosis in cattle and its potential risks. A multivariate logistic regression model of risk factors was fitted by backward stepwise selection of variables (McDonald, 2009). The variables were retained in the model based on likelihood test ratio p-value ($p<0.25$ for the first model and $p<0.05$ for the final model). The goodness of fit of the model was tested by Hosmer and Lemeshow test (MedCalc version 13.1.1). Furthermore, the agreement of the tests RBPT, BAPA and c-ELISA used in the diagnosis of bovine brucellosis were analysed using kappa statistic (κ).

RESULTS

Seroprevalence of Brucella in lactating cows of Babati district

A total of 192 of lactating cows that produce milk for public consumption were involved in this study. All animals were tested for the presence of antibodies against *Brucella spp* using RBPT, BAPA and c-ELISA. The number of animals that tested positive is indicated in Table 3. Out of 192 animals, nine animals were positive for Brucella antibodies after screening using RBPT and 10 animals were positive for *Brucella* antibodies after screening using BAPA. The agreement between the RBPT and BAPA to detect brucellosis was good (κ = 0.94). When the 10 positive animals were tested using c-ELISA, eight of these animals were confirmed to be positive (Table 3). The agreement between the c-ELISA and BAPA to detect brucellosis was good (κ = 0.88). Out of the eight animals confirmed to be positive for Brucella antibodies, all of them were found within animals originating from traditional cattle farmers and large scale dairy farmers and no positive animal originated from small and medium scale dairy farmers. There was no statistical significance difference between the prevalence of Brucella and the breed of animals, location of cattle, farming system, herd size or age.

Molecular detection of Brucella species DNA by PCR

Eight milk samples from animals that were confirmed to be positive by c-ELISA were screened for the presence and type of *Brucella* spp. using PCR. Primers that specifically amplify *B. abortus* and *B. melitensis* were used in the PCR (Table 2). When PCR was performed using primers that specifically amplify the IS711 gene of *B. abortus*, three out of eight cows were positive, producing an expected PCR product of 495 bp (Figure 2). No PCR product was observed when PCR was performed using primers that specifically amplify the IS711 gene of *B. melitensis*.

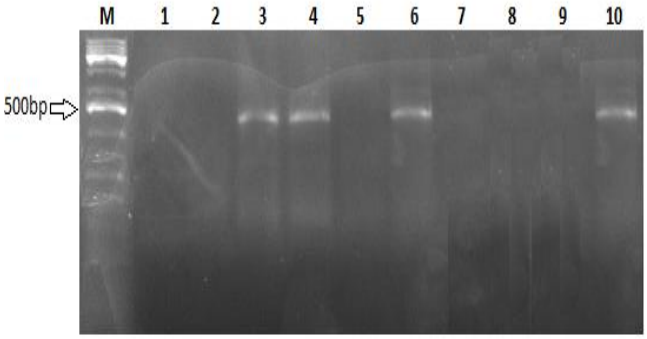

Figure 2: Amplification of *B. abortus* IS711 gene using PCR. A 1% agarose gel electrophoresis of *B. abortus* IS711 gene amplicon of 495 bp from total DNA of cow milk. Lane M; 1kb DNA ladder; Lane; 3, 4 and 6 are positive milk samples; Lane; 1, 2, 5, 7 and 8 are negative milk samples; Lane; 9 negative control containing water; Lane 10; positive control containing DNA of *B. abortus.*

Table 4. Seroprevalence of brucellosis in lactating cows of Babati district

Parameter	Category	Number of cows screened	Number (%) of positive cows by different serological tests		
			RBPT	BAPA	c-ELISA
Production system	Dairy	142	6 (4.2)	7 (4.9)	5 (3.5)
	Traditional	50	3 (6.0)	3 (6.0)	3 (6.0)
Cattle breed	Friesian	94	2 (2.1)	3 (3.2)	2 (2.1)
	Ayrshire	48	4 (8.3)	4 (8.3)	3 (6.3)
	TSHZ	50	3 (6.0)	3 (6.0)	3 (6.0)
Age	≥ 3 years	28	0 (0.0)	0 (0.0)	0 (0.0)
	≥ 5 years	152	8 (5.3)	9 (5.9)	7 (4.6)
	≤ 6 years	12	1 (8.3)	1 (8.3)	1 (8.3)
Herd size	Small (1-5 animals)	104	0 (0.0)	0 (0.0)	0 (0.0)
	Medium (6-10 animals)	24	0 (0.0)	0 (0.0)	0 (0.0)
	Large (≥ 10 animals)	64	9 (14.0)	10 (15.6)	8 (12.5)
Location	BDC villages tested	24	5 (20.8)	5 (20.8)	5 (20.8)
	BTC villages tested	12	1 (8.3)	1 (8.3)	1 (8.3)

Risk factors associated with transmission of brucellosis between lactating cows and other animals in Babati district

Total number of 66 respondents was administered with questionnaire for assessing risk factors of transmission of brucellosis. Majority (68%) were adult men above the age of 25, among 66 respondents, 53 (80.3%) were dairy cattle owners, 13 (19.7%) traditional cattle farmers, in whom 59.1% had knowledge on brucellosis. The results related to awareness about brucellosis (Table 4).

Risk factors of transmission of brucellosis basing on statistical analysis

Analysis for risks factors of brucellosis transmission in the study area reported by household respondents through questionnaire revealed some variables which are potential risk factors that were considered to be associated with brucellosis were based on individual cattle and herd level. At herd level factors where farming system, herd size, mixing of cattle from different herds (livestock contact), sale or purchase of animals from and to unknown farm and cattle breed to be associated with increased risk of brucellosis transmission. These variables were subjected to univariate logistic regression analysis (Table 4). Univariate analysis indicated that herd size (OR=1.9537, P=0.0030), mixing of cattle (OR=32.5000, P=0.0027), selling and buying of cattle from and to unknown farms (OR=5.0588, P=0.0500), farming system (OR=3.9474, P=0.0400) and cattle breed (OR=1.9860, P=0.0284) were significantly associated with risk for having brucellosis, hence were subjected to multivariate analysis.

Table 5. Risk factors of transmission of brucellosis in cattle

Variable	Category	Frequency	Percentage
Number of cattle	1-5	55	83.3
	6-10	6	9.1
	11-above	5	7.6
Sale or purchase of animals	Yes	21	31.8
	No	45	68.2
Livestock mixing	Yes	13	19.7
	No	53	80.3
Grazing in communal pasture	Yes	14	21.2
	No	52	78.8
Livestock-wild animals contacts	Yes	9	13.6
	No	57	86.4
Brucellosis knowledge	Yes	39	59.1
	No	27	40.9
Farming system	Dairy	53	80.3
	Traditional	13	19.7
Type of cattle kept	Indigenous	10	15.2
	Exotic	41	62.1
	Both	15	22.7

Table 6. Univariate analysis of risk factors of transmission of brucellosis

Term	Odds ratio	95%CI	P-value
Cattle type	0.7998	0.2022-3.163	0.7502
Communal pasture	2.0000	0.3268-12.238	0.4533
Communal water	1.1333	0.1186-10.833	0.9135
Farming systems	3.9474	1.0645-14.638	0.0400*
Herd size	1.9537	1.2546-3.042	0.0030*
Brucellosis knowledge	3.8235	0.4210-34.727	0.2335
Livestock contacts (mixing)	11.3333	1.8016-71.294	0.0097*
Livestock wildlife contact	3.7857	0.5828-24.591	0.1632
Livestock-wild common water	3.7857	0.5828-24.591	0.1632
Placenta into bush	3.2500	0.5094-20.737	0.2126
Sale or purchase animals	5.0588	0.8466-30.231	0.0500
Veterinary services	0.7143	0.1331-3.835	0.6948
Cattle breed	1.9860	1.0752-3.669	0.0284*

Note: * statistically significant

The multivariate analysis indicated no statistical significant association between cattle breed (OR= 1.9516, P=0.1533), farming systems (OR=0.5724, P=0.5633), herd size (OR=1.7773, P=0.0729), mixing of cattle (OR=1.8513, P=0.7190), selling and buying of cattle (OR=0.6213, P=0.7530) with having brucellosis. The study showed mixing of cattle from different herds (OR=1.8513), cattle breeds (OR=1.9516), and herd size (OR=1.7773) had higher chances of contracting brucellosis compared to those who do not mix their cattle and have small herd size (Table 5).

Table 7. Multivariate analysis of risk factors of brucellosis transmission

Terms	Odds ratio	95% CI	P value
Cattle breed	1.9516	0.7795-4.886	0.1533
Farming system	0.5724	0.0863-3.796	0.5633
Herd size	1.7773	0.9479-3.333	0.0729
Livestock mixing	1.8513	0.0646-53.045	0.7190
Sale or purchase	0.6213	0.0321-12.044	0.7530

Hosmer and Lemeshow test showed the model fit the data (P=0.6018)

DISCUSSION

Seroprevalence of brucellosis in lactating cows

The present cross-sectional study was carried out to determine the prevalence and the risk factors associated with transmission of the brucellosis in lactating cows in Babati district. The tests used in the study were RBPT, BAPA, c-ELISA and PCR. The selected serologic screening tests were RBPT and BAPA and had good agreement for detecting brucellosis when compared by kappa statistics (κ = 0.94), (Table 3). The RBPT is capable of detecting infected animals earlier due to its ability to detect presence of IgG1, which is produced early after exposure. False positive reactors are normally due to residual antibodies from vaccination history of the herd, colostral antibodies in calves, cross-reaction with certain bacteria and laboratory errors. The positive predictive value of this test is low and a positive result is required to be confirmed by some other more specific test like ELISA, SAT or CFT (OIE, 2009; Megersa *et al.,* 2011). Due to the fact that, RBPT can give up false-negative reactions mostly due to prozoning (OIE, 2009; Göknur *et al.*, 2010), BAPA was used as second screening test in this study. The agreement between the screening tests and confirmatory test (c-ELISA) was good in detecting brucellosis when compared by kappa statistics (κ = 0.88), (Table 3). The c-ELISA was chosen to be used in this study due to its several diagnostic merits which include high sensitivity and specificity, ability to differentiate vaccinated animals from naturally infected ones, or those infected with cross reacting organisms and its use in areas where disease prevalence is low (OIE, 2009). The results from this study consequently are reliable and indicated that brucellosis is prevalent in Babati district.

The overall seroprevalence of brucellosis in lactating cows in Babati district is 4.2% (8 of 192). The seroprevalence of brucellosis according to cattle type are as follows; 3.5% (5 of 142) for dairy cattle, 6.0% (3 of 50) for traditional cattle (Table 3). These levels of seroprevalence observed in this study are in close agreement with previous studies in which the seroprevalence of brucellosis was found to be high in traditional cattle and low in dairy cattle. Other documented prevalence of brucellosis include, 4% in dairy animals and 15% in traditional cattle in northern zone of Tanzania (Swai *et al.,* 2010), 14.3% in traditional cattle in Mikumi-Selous ecosystem (Temba, 2012), 1.5% for smallholder dairy cattle and 17.9% for indigenous cattle in Iringa and Tanga (Karimuribo *et al.*, 2007), 4.9% in traditional cattle in Arusha and Manyara (Shirima *et al.,* 2005), and 3.2% in dairy cattle in Arusha (Minja, 2002). However, it is lower than the seroprevalence reported by Mtui-Malamsha (2001) and Swai *et al.* (2005) who reported 12.2% in dairy cattle in Manyara and Kilimanjaro, respectively.The difference in seroprevalence reported by Mtui-Malamsha (2001) and Swai *et al.* (2005) and the current study could be due to the difference in animal population, sample size and differences in livestock management practices.

The low seroprevalence in the smallholder dairy animals is likely due to stall feeding that minimises contacts between herds and other animals (Karimuribo *et al.*, 2007). Management practices such as breeding bulls, artificial insemination and intensive husbandry system, which involve confinement, are among elements that have an effect on the number of seropositive animals in an area or herd (Minja, 2002). The cut and carry feeding system of animals that is practiced by many dairy smallholders help to control brucellosis, however, can serve as a potential risk factor when fodder is collected from areas used by indigenous traditional cattle which encroach the peri-urban and urban settings especially during the dry season (Karimuribo *et al.*, 2007). The observed lower sero-prevalence in dairy cattle can also be explained by the altitude of farmers to consider their dairy cattle as enterprises and tend to control brucellosis or take some precautions when purchasing their replacement stock (Chimana *et al.,* 2011; Wankyo, 2012).

Traditional cattle farmers use free-range management system in which they share communal pasture and water points which leads into mixing of cattle and this has shown in this study as an important risk factor (P = 0.0097) for exposure to *Brucella species*, also Matope *et al.,* (2010) and Chimana *et al.,* (2011) found the same. Also traditional cattle farmers frequently purchase cattle from other herds or common livestock markets (*Minadani*) where screening of these cattle for brucellosis is not carried out due to limited availability of veterinary services, this further increases chance of contact with infected herds (Chimana *et al.,* 2011).

Results from this current cross-sectional study revealed, uncontrolled movement, purchasing of livestock (P = 0.0500) from unknown disease status farms and intermixing of pastoralists, agro-pastoralists and smallholder dairy cattle from different regions upcountry migrated to the study

area recently could perhaps account for no statistical significance difference in seroprevalence observed (Chimana *et al.,* 2011; Temba, 2012). In this study found large herd size to be a risk factor (P = 0.0030) due to farmers with large livestock herds with no enough owned pasture and water sources, are forced to use free-grazing farming system to find pasture and water where mixing of different herds occurs and leads to health animals contracting brucellosis (Kohei *et al.,* 2011). Type of management system was a potential risk factor (P=0.0284) for seroprevalence of bovine brucellosis rather than breed despite being potential risk factor. Since all seropositive were from the group of cattle kept in the free-range and semi-extensive management system and none from intensive management system (Table 3). This finding is in agreement with the report of (Karimuribo *et al.*, 2007; Matope *et al.,* 2010) with regard to cattle management systems.

In *Brucella* infection, prevalence increases with age, probably because of greater exposure to infection, time female animals spent in herds is longer than male for breeding reason (Omer *et al.,* 2000; Mohammed *et al.,* 2010). Female animals usually have high brucellosis prevalence due to the presence of the *eri* gene which is essential for erythritol in allantoic fluid which stimulate the growth and multiplication of Brucella organisms and tend to increase in concentration with age (Mellau *et al.,* 2009; Aggour *et al*., 2013). Older animals have higher seroprevalence rate than young animals (Table 3), the effect of age on Brucella infection is related to sexual maturity of animals. Being sexually mature female is a known risk factor to Brucella infection (Minja, 2002; Temba, 2012).

Furthermore, the results of this current cross-sectional study indicate that among 36 villages sampled, six out of 36 (16.7%) had Brucella seropositive in cattle while 30 (83.3%) had none (Table 3). Consequently, it can be inferred from the results that brucellosis is localized among villages in Babati district. The results additionally indicates that out of 66 herds sampled six (9.1%) had Brucella seropositive while 60 (90.9%) had none of the animals reacting to any of the tests. The results therefore indicate that, although brucellosis is localised among villages also it is less distributed among herds. The nature of distribution of disease in villages poses danger of further spread among herds and individual animals because most of pastoral, agro-pastorist and few dairy herds use communal grazing grounds and watering points especially during dry season as reported by Karimuribo *et al.* (2007). It is common to see overcrowding of animals at water points, especially during the dry season, and probably infected aborted animals, recently calved infected animals and infected animals with retained placenta may grossly contaminate the water source resulting in the disease (Matope *et al.,* 2010). Grazing in communal land contribute to build up of Brucella organism in the environment due to uterine discharges, urine and lochia from infected animals which have been found to be major sources of infection to other animals (Shafee *et al.,* 2011). *Brucella* organism can survive in grass for varying period of time with infectivity up to 100 days depending on season and this poses risk of infection to other animals during grazing. The rate of dissemination of brucellosis in the herds and among villages will largely depend on management system practised, animal population density and patterns of movements (Kunda *et al.,* 2010). It is likely that the routine animal's movement in search of water and pasture during dry season and intermixing of herds from different households and from different villages may exacerbate the rate of spread of disease into non infected areas (Karimuribo *et al.,* 2007).

Poor aborted materials disposal systems as seen in this study because of lack of community knowledge about the zoonotic implications of the disease. Collapse of the animal health services in Tanzania as result of the privatisation of veterinary services may also contribute to the perpetuation of the disease in the study areas (Karimuribo *et al.,* 2007; Mellau *et al.,* 2009). As it is well known that during abortion, large numbers of Brucellae are released which may, in turn, cause the infection to other animals and humans (Turatbek *et al.,* 2006; Kunda *et al.,* 2010). Tarangire and Manyara National Parks wild animals have been interacting with livestock and humans from the villages bordering the parks for several decades could perhaps suggest cross transmission of infection at interface where animals share grazing pastures and water especially during dry seasons. The higher prevalence in domestic ruminants is the coexistence of livestock and wild animals which facilitates survival and translocation of the disease causing agent (Mellau *et al.,* 2009).

Several studies have shown that buffaloes and wildebeest were most affected among African wild animals (Shirima, 2005). Seroprevalence reported in wildlife are 67% in buffalo Tarangire National Park (Anderson, 1988), 28% in buffalo in Ngorongoro-Serengeti ecosystem (Shirima, 2005), 24% and 17% in buffalo and wildebeest, respectively in Serengeti ecosystem (Fyumagwa *et al.,* 2009). Therefore, the presence of brucellosis in both domestic and wildlife animals as well humans emphasizes the need for collaboration between livestock owners,

livestock experts and wildlife experts. The importance of wildlife brucellosis is based on the difficulties in eradication, disease dynamic between wild animals, livestock and human being, and conflicts between farmers and wildlife experts (Shirima *et al.*, 2005; Mellau *et al.*, 2009).

Molecular detection of Brucella species DNA in cattle milk by PCR

Applications for PCR strategies vary from the identification of the illness to characterization of field isolates for epidemiologic functions together with classification studies (Poester *et al.*, 2010; Vivek *et al.*, 2014). Based on these facts, PCR amplification targeting the species-specific genetic element IS711 in the Brucella chromosome was performed to determine and confirm the presence of Brucella DNA in milk samples (Poester *et al.*, 2010). Only positive samples tested by serologic methods were used to determine and confirm the presence of Brucella DNA in milk samples. The results from this study indicated that only three milk samples (out of eight) had *Brucella abortus* but not *B. melitensis*. As is well known, *B. abortus* can be shed in the milk of infected animals intermittingly, in cattle and other species (Capparelli *et al.*, 2009). So it possible that the other five milk samples was taken when animals were not shedding bacteria into milk. The difference also can be due to long termpersistence of anti-Brucella antibody without presence of the disease agent in milk. Furthermore, it can be due to relatively low detection limit of PCR, because it is possible that some milk samples contained bacteria less than the detection limit and hence failed to be found as positive (Göknur *et al.*, 2010; Kechagia *et al.*, 2011). The PCR has shown in this study that it is a technique that enables for speedy and correct identification of brucellosis (Baddour, 2012). Another advantage of PCR technique is that detection can be done without necessarily culturing the bacteria that are infective to humans (Göknur *et al.*, 2010).

From this study, there is a proof that brucellosis is present within the population of milk producing cattle in Babati district. Routine screening of animals or surveillance for brucellosis is incredibly necessary in brucellosis control. It might facilitate to notice positive cases as early as doable thus on scale back the chance of cross contamination to different animals at intervals the herds or flocks and take correct measures on time. More attention should be paid towards implementing a proper control program for brucellosis and more efforts should be directed towards improving the animal health biosecurity program. Build-up immunity of animal population against brucellosis is possible approach to all livestock which can suffer from brucellosis. Mandatory vaccination of cattle with Brucella vaccine like S19 and RB51 which are present in the markets and applied into heifers of 3-8 months of age in dairy and traditional cattle. In addition, controlling brucellosis in small ruminants is done mainly by Rev-1 vaccination and will indirectly reduce the prevalence of this disease in other animal species especially cattle. Control progress should be monitored serologically and evaluated epidemiologically.

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