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Shirima, Gabriel

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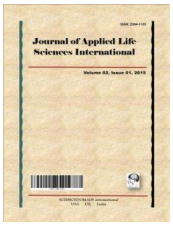

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Re-emergence of Bovine Brucellosis in Smallholder Dairy Farms in Urban Settings of Tanzania

Gabriel M. Shirima[1*], Bertilla E. Lyimo[2] and N. L. Kanuya[3]

[1]The Nelson Mandela Institution of Science and Technology, P.O.Box 447 Arusha, Tanzania.
[2]Kinondoni Municipal Council, P.O.Box 31902, Dar es Salaam, Tanzania.
[3]Sokoine University of Agriculture, P.O.Box 3015, Morogoro, Tanzania.

Authors' contributions

This work was carried out in collaboration between all authors. Author GMS prepared the manuscript and managed the literature. Author BEL designed the study and performed the laboratory and data analysis. Author NLK critically read the manuscript and layout. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Brucellosis infection was previously encountered in all livestock farming systems in Tanzania but reported to decline below 2% in smallholder dairy subsector due to the stringent calf-hood vaccination using S19 between 1979 and 1990. However, reports from the last decade indicated an increase of the infection in the smallholder dairy subsector. This prompted several researchers to conduct further studies in different urban settings to ascertain the disease and associated risk factors. This study aims to elucidate the magnitude of brucellosis in urban areas of Morogoro region and related risk factors in the advent of no control intervention in place. Presence of anti-brucella antibodies in dairy animals residing in urban areas may pose a threat to milk consumers in the cities as a significant proportion of the milk is sold informally. Therefore, generating this information will inform policy to formulate feasible intervention for controlling brucellosis in urban settings that indirectly will safeguard public health.
Study Design: This was a cross-sectional survey.
Place and Duration of Study: The study was conducted in Morogoro Municipality between May and September 2012.

**Corresponding author: E-mail: gabriel.shirima@nm-aist.ac.tz, gmshirima@gmail.com;*

Methodology: The study was to determine the prevalence of anti-brucella antibodies in smallholder dairy cattle in urban settings of Tanzania. Milk Ring Test was used as a screening technique while Competitive Enzyme-Linked Immunosorbent Assay was a confirmatory test. A questionnaire was applied to each animal owner. A total of 104 respondents were interviewed to assess possible risk factors associated with re-emergence and transmission of brucellosis among dairy cattle.
Results: 390 dairy cows from Morogoro Municipality were screened for *Brucella* circulating antibodies. Overall 35.4% (95%CI; 25.2-33.8) of milk samples tested positive based on MRT while seroprevalence was 21.3% (95% CI) based on c-ELISA. It was further revealed that abortion (p= 0.01) and herd size (0.05) were significantly associated with brucellosis seropositivity in cattle. Although 32% of herd owners vaccinate their cattle against several transboundary diseases, none vaccinated against brucellosis.
Conclusions: From this study, there is evidence that collapse of the Tuberculosis and Brucellosis Control Programme has resulted into an increase of brucellosis within the smallholder dairy farmers within urban settings. This may pose a high risk to urban farmers and milk consumers thus attracting immediate response.

Keywords: Farming systems; c-ELISA; MRT; urban brucellosis.

1. INTRODUCTION

Brucellosis is amongst the 'neglected zoonoses' [1] although in pastoral and mixed crop-livestock farming systems in Africa is a major endemic zoonosis affecting a high proportion of domestic ruminants and humans [2,3]. Though brucellosis has been eradicated in many of the developed countries, it remains a catastrophe in developing world because disease control programmes are either non-existent or inadequate due to limited resources, political commitment [4,5] and limited evidence-based intervention. Additionally, lack of information on its distribution and impact in livestock and humans may limit the evidence-based decision towards resource mobilisation for the control.

Although livestock brucellosis encountered in all farming systems in Tanzania, smallholder dairy was considered to have the least prevalence (0-4.4%) based on the management practices and control measures instituted during the introduction of dairy animals in early 1980's [6]. During this period, active surveillance and calf-hood vaccination were instituted in imported dairy breeds kept in parastatal farms, and few smallholder farms though collapsed 1990's (Kambarage personal communication 2015). Through this strategy coupled with brucellosis awareness, it was thought would maintain the disease at a low level in the subsector. Nevertheless, subsequent studies revealed brucellosis was on the increase [3,7,8] in small holder dairy subsector being as high as in pastoral and agropastoral communities. This stimulates more studies in the subsector to establish the source, distribution and magnitude of brucellosis [9,10] to justify further consideration for control and raise community awareness. The current study aims at elucidating the magnitude of brucellosis in urban areas of Morogoro Municipality and associated risk factors following the collapse of Tuberculosis and Brucellosis control programme. Following livestock intensification in urban and peri-urban settings with little disease management strategies prompted this study to inform the community and other key stakeholders on the distribution and magnitude of brucellosis in smallholder dairy cattle for appropriate interventions.

2. MATERIALS AND METHODS

2.1 Study Area

The present study was carried out in Morogoro Municipality as one of the urban centres in Tanzania. The Municipality occupies an area of about 260 km² which is divided into 19 wards [11] with a human and livestock (dairy cattle) population of 228,863 and 6,982 respectively, [12]. The major economic activities include diverse business activities (35%), livestock and subsistence farming (33%), office works (16%), elementary occupation (11%) and industrial production (5%) [12].

2.2 Smallholder Dairy Production System

In this study, a smallholder dairy farm is defined as a dairy unit keeping one to ten dairy cows. The subsector keeps different type of breeds

such as crosses of Ayrshire, Friesian and Tanzania Short Horn Zebu. Majority of smallholder dairy farms fed mainly on native grasses supplemented with a varying amount of homemade concentrate mixture of cereal grains, i.e. maize bran and cotton seed cake or sunflower seed cake.

2.3 Study Design

It was a cross-sectional study carried out from May to September 2012. 13 wards that were keeping dairy cattle were purposively selected from the list of 19 wards in Morogoro municipality, then two streets with cattle herds in each ward were selected and then four households randomly selected in each street. This made a total of 104 livestock keeping households. However, selection of households was also considering those with two or more dairy cows.

The livestock sample size was estimated to provide 80% power with 95% confidence. Based on the 50% prevalence of brucellosis and 0.05 error margin the sample size was calculated as described by Martin et al. [13], to obtain the total number of animals to be screened from the Municipality.

$$n = \frac{z^2 \times P(1-P)}{d^2}$$

Where:

n = the required sample size
P = estimated prevalence = 0.5
z = level of confidence as 1.96; d = Desired precision level = 0.05

A total of **384** animals were obtained for brucellosis screening.

2.4 Data collection

Each household interviewed through the personal administration of a structured questionnaire to any person above eighteen years. Herd-level information was collected on a single visit. Important herd level data collected includes herd size, history of abortion, grazing pattern, vaccination regime and breeding methods. A total of 104 households were interviewed in this study after obtained written permission from Municipal Authorities and verbal consent from herd owners.

2.4.1 Collection and handling of milk samples

Milk samples were collected from all lactating cows in the herds that interviewed. The udder was washed and dried with a clean towel, and approximately 10 mls of milk was hygienically collected into a sterile bottle (Universal bottles) according to OIE guidelines [14]. The first stream of milk was discarded. Samples were screened using Milk Ring Test (MRT) within six hours of the collection as described by Shafee et al. [15]. Briefly, milk samples were tested for antibodies against *B. abortus* by (MRT). Each milk sample was thoroughly mixed to disperse the cream and 1ml of whole milk dispensed into each test tube, and one drop (0.03 ml or 30 µl) of MRT antigen was dispensed into each test tube and shaken gently to ensure that the antigen and milk were thoroughly mixed. Test tubes were incubated for one hour at 37ºC. A strongly positive reaction was indicated by the formation of a dark blue ring above a white milk column. The test was considered negative if the colour of the underlying milk exceeds that of the cream layer.

2.4.2 Collection and handling of blood samples

Blood samples were collected from all lactating dairy cows. Approximately 10 mls of whole blood was drawn from the jugular vein using plain vacutainer tubes (Becton Dickson UK). Each tube was labelled using codes (number) describing the specific animal and herd. The test tubes were kept overnight at room temperature to allow clotting. The tubes were centrifuged at 3000 rpm for 10 minutes to obtain clear serum and decanted into Eppendorf tubes in duplicates and stored at -20ºC until tested by using c-ELISA at Sokoine University laboratory. The c-ELISA was performed and interpreted as described by Veterinary Laboratory Agency-VLA (Weybridge) protocol [16]. Briefly, a 96-well polystyrene microtitre plate pre-coated with *B. melitensis* LPS antigen was used. Using a single channel micropipette 20 µl (1 of each test serum has added to polystyrene microtitre wells in duplicate except wells in column 11 and 12. Twenty microlitres of the positive control antisera from VLA was dispensed into the first six wells of column 11 and 12 and 20 µl (1 of the negative control antisera from VLA was dispensed into the last six wells. One hundred microlitres of the conjugate buffer were added to all wells and incubated at room temperature for 30 minutes. Plates were rinsed five times and dried

thoroughly followed by the substrate and stop solution, and the plate read within 10 minutes.

Each plate was measured by both visual observation (any colour change) and ELISA reader at 450 nm. The plate results were considered invalid if any of the following applied:

(i) The binding ratio (BR) was less than 10.
(ii) The optic density (OD) of the mean of the six negative ODs was less than 0.70. The optimal mean negative OD is 1.0.
(iii) The OD of the mean of the six positive wells was greater than 0.10.
(iv) The mean OD of the four conjugate control wells was less than 0.70.

The cut-off value for c-ELISA positivity was based on the conjugate control where the cut- off was taken as 60% of the mean of the OD of the four conjugate control wells. Any test sample giving an OD equal to, or below this value, was considered positive. All results were expressed as a percentage of the conjugate control and referred to as percentage positive values (pp values).

2.5 Statistical Analysis

Data from the questionnaires and laboratory results were stored in a computer, using Microsoft Excel spreadsheet program 2007. Descriptive statistics for the animal and herd level explanatory variables was done using Epi-Info version 7.1.1.14 and statistical significance was determined at 95% CI at critical probability (P<0.05).

3. RESULTS

A total of 390 dairy cows from 104 households were screened for brucellosis circulating antibodies using Milk Ring Test antigen and c-ELISA as a confirmatory test. The proportions of positive reactors to MRT were 35.4% (n=138) of the milk samples and 21.3% (n=83) to c-ELISA for serum samples (Table 1). Out of 104 households screened, 35% (n=36) were seropositive. Of the MRT positive samples, 37% (n=51) were negative to c-ELISA. The seropositivity at herd level ranged from 0-66.7% with Kingolwira, and Mbuyuni wards had the highest herd seropositivity and least at Mafiga ward.

History of abortion at herd level (P=0.01) and herd size (P=0.05) were significantly associated with Brucella seropositivity (Table 2). There was no significant statistical difference (P= 0.16) between herd owners who practice natural mating (80%) and those practice artificial insemination (20%) to Brucella seropositivity. Also, grazing patterns (free range, semi-intensive and zero grazing) did not show to be associated with Brucella seropositivity in the area (P = 0.08).

It was revealed that up to 78.9% of respondents interviewed had never heard of brucellosis disease in cattle and 66.4% had no idea on how the disease is transmitted.

Regarding vaccination programme it was revealed that 32% of the herds conducted vaccinations against Foot and Mouth Disease (FMD), Rift Valley Fever (RVF), East Coast

Table 1. Prevalence of brucellosis based on MRT and c-ELISA in 10 wards of Morogoro Municipality

Wards	Herds screened	C-ELISA Pos. herds [%]	Serum collected	C-ELISA Pos [%]	Milk collected	MRT Pos. [%]
Mlimani	9	3[33.3]	52	9[17]	52	13[25]
Mafiga	8	0[0]	13	0[0]	13	1[7.6]
Boma	11	3[27.3]	22	2[9]	22	6[27.3]
Bigwa	15	3[20]	21	7[33]	21	9[42.9]
Mbuyuni	8	5[62.5]	10	4[40]	10	5[50]
Magadu	5	2[40]	76	15[19.7]	76	27[35.5]
Kingolwira	9	6[66.7]	39	10[25.6]	39	19[48.7]
Kichangani	15	6[40]	74	23[31]	74	34[45.9]
Mazimbu	12	3[25]	37	5[13.5]	37	11[29.7]
Kihonda	12	5[41.7]	46	8[17]	46	13[28]
Total	**104**	**36[35]**	**390**	**83(21.3)**	**390**	**138[35.4]**

Fever (ECF), Contagious Bovine PleuroPneumonia (CBPP), Anthrax, and Lumpy Skin Disease (LSD). None of the herd was vaccinated against brucellosis.

Table 2. Some risk factors associated with the occurrence of brucellosis at household level in Morogoro Municipality

Variables (Factors/Practices)	Percent	P value
Raw milk consumption		
Yes	72.2	0.65
No	27.8	
Breed		
Ayrshire	19.3	
Friesian	12	0.16
Cross breed	68.7	
Calf-bucket feeding		
Yes		
No	59.6	
Calf sucking 0.32		
Yes	59.6	
No	40.4	
Breeding methods		
Natural services		
Yes	92.4	
No	34.7	
Artificial insemination	19.8	
Yes	80.2	
No		
Herd size		
1-15 cows	63.9	
16- 30 cows	14.5	0.05
>30 cows	21.7	
Grazing system		
Free-range system	45.8	
Mixed system	18.1	0.08
Zero grazing system	36.1	
History of abortion		
Yes	34.9	0.01
No	65.1	

4. DISCUSSION

The results of the current study indicated anti-Brucella seroprevalence of 21.3% in Morogoro Municipality under smallholder dairy management system. The higher seroprevalence noted in this study is contrary to other previous studies under small holder dairy in different zones of Tanzania [8] with the prevalence ranged between 0-4.4% [6]. Previous studies reported relatively low seropositivity in the small holder dairy due to control activities implemented by Brucellosis and Tuberculosis Control Programme in the country between 1980 and late 1990. The collapse of this programme in late 1990's might have attributed to the increase of Brucella seropositivity in some regions and Municipalities of Tanzania [8] including Morogoro. Calf-hood vaccination using S19 and screening at farm level ceased after the collapse of the programme (Kambarage, Personal communication, 2015). This may have attributed to brucellosis re-emergence under smallholder dairy systems.

The seropositivity at ward level was variable ranging from 0-66.7%. This variation could be explained by the fact that these animals are strictly zero grazed, [housed and feed with grasses through cut and carry practices] thus within herd factors may contribute to such variations. Farm management practices such as the introduction of infected animals and handling of calving's/abortions and placentas may have contributed to herd variations as reported elsewhere [6].

C-ELISA seropositivity was associated with a history of abortion and herd size and was in agreement with several other studies [9,17,18,6,19]. Despite of this association, (abortion) a range of other disease conditions prevalent in the area including tick-borne diseases, Rift Valley Fever and Trypanosomosis may also cause abortion in cattle [19].

Although 32% of herd owners vaccinated their cattle against several other diseases, none was done for brucellosis. From the questionnaire survey, it was also revealed that majority (78.9%) of respondents had never heard of brucellosis. This complicates for any interventional approaches as its impact on livestock production was not realized by farmers. The nature of the disease does not exhibit conspicuous clinical symptoms except abortions that sometimes occurred once, and subsequent gestations just deliver normally and thus complicates any intervention strategy.

Therefore, in areas where prevention and control programmes are not in place, annual screening may be advocated with continuous education to farmers to safeguard their animals and protect public health. Also, there is a need to resume the Brucellosis and Tuberculosis control programme that was collapsed to rescue the current situation in Morogoro Municipality and elsewhere.

5. CONCLUSION

Therefore, encountering high prevalence of brucellosis in smallholder dairy cattle in urban

settings indicated that the disease is re-emerging in the subsector thus calls for reviving the programme. Test and slaughter coupled with a screening of incoming animals and calf-hood vaccination may reduce the disease prevalence in the smallholder dairy sector as reported in other studies [20]. Although humans were not sampled along with this study, it may not exclude from being exposed, and hence awareness creation may be required during the control programme.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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