EISSN: 2707-0425

East African Journal of Science, Technology and Innovation, Vol. 3 (2): March 2022

Prevalence of *Brucella* infection and associated risk factors among children in livestock keeping communities of Morogoro, Tanzania

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Abstract

Brucellosis is among the most common bacterial zoonotic diseases worldwide. It is estimated that up to 500,000 new human cases occur each year. Brucellosis is a priority zoonosis in most East African countries. In Tanzania, the disease is one of six priority zoonoses, however, little disease burden information is available especially in children. Children are of particular interest especially in pastoral communities due to increased risk of exposure in their routine activities associated with handling of livestock and their products. This study investigated brucellosis exposure status and associated risk factors among non-febrile children in pastoral communities. A cross-sectional design was used involving a total of 361 non-febrile children of six months to 14 years old, randomly selected from households and schools. Risk practices were evaluated using focus group discussions among selected children and thematic analysis was performed. Serum samples were collected and analyzed using Rose Bengal Test (RBT) and indirect Enzyme-Linked Immunosorbent Assay (iELISA) in parallel. The overall seroprevalence of Brucella infection was 3.3% (95%CI, 2%-5%) determined by both RBT and iELISA. Risk practices observed and cited by children comprised of direct and indirect contact with animals and their products. These include: consumption of raw milk and their products, unhygienic handling of animals and their waste especially assisting cows during parturition. Girls/female respondents were more likely to test brucella positive than boys (male) respondents (2.23 OR, 95%CI 66%-754%). Brucellosis among non-febrile children in the study area was present at a relatively low prevalence. Risk practices for transmission of this zoonosis were also identified which included consumption of raw milk, contact with animals, their products and discharges including abortion materials which represent the basis for increased pathogen circulation in the area. General education on the epidemiology of brucellosis and prevention of infection spread should be provided to the community including children.

Keywords: *Brucellosis; ELISA; Infection; RBT; Risk Factors; Seroprevalence; Children*

Received: 10/11/21
Accepted: 17/03/22
Published: 31/03/22

Cite as: *Hildegalda et al., (2022)* Prevalence of Brucella infection and associated risk factors among children in livestock keeping communities of Morogoro, Tanzania. *East African Journal of Science, Technology and Innovation 3(2).*

Introduction

Brucellosis remains the most common zoonotic infection in many countries worldwide (Ducrotoy *et al.*, 2017). The disease is caused by bacteria of the genus *Brucella* which affect animals and humans (Assenga *et al.*, 2015; OIE, 2016), with estimated around 500,000 new human cases each year worldwide and approximately 10/100,000 people in endemic areas (Nyerere *et al*., 2020; WHO, 2006). The sources of infection for humans are infected domestic animals, wild animals and their products; while sources for animal infections include aborted materials, vaginal discharges, milk and semen from infected animals (Geresu *et al*., 2016). Human brucellosis occurs widely in livestock keeping communities especially in rural areas with high levels of human contact with livestock and their products (Sagamiko *et al*., 2020). Interaction between wildlife, livestock and humans favors disease transmission (Assenga *et al*., 2015). Brucellosis has become a priority zoonosis in many low- and middle-income countries (LMICs) (Rubach *et al*., 2014; Ducrotoy *et al*., 2017). In sub-Saharan Africa, the prevalence of brucellosis in humans ranges from 5-55% (Dean., 2012) in different countries, while in domestic animals it ranges between 8-46% (Smits and Cutler., 2005). In Tanzania, it is listed among six priority zoonoses that the country has to focus the control efforts as highlighted in Tanzania's One Health strategic plan (2015-2020) (URT, 2018). In the country, brucellosis has been reported in animals and humans with prevalence varying greatly in different regions. Human brucellosis in Tanzania has been reported at seroprevalences varying from 0.6% to 58.4% (Assenga *et al*., 2015; Bouley *et al.,* 2012; Carugati *et al*., 2018; John *et al*., 2010; Mirambo *et al*., 2018; Mujuni *et al*., 2018; Shirima, 2005; Swai and Schoonman, 2009). Majority of these studies report the prevalence in adults mostly with occupational exposure with little attention to children. Seroprevalences of 7% for *B. abortus* and 15% for *B. melitensis* have been reported in children in Tanzania (Chipwaza *et al*., 2015). Younger children are of particular interest because of their dependence on milk as their main source of nutrition, and if the milk they consume is unpasteurised and come from infected animals they are more likely to be exposed to the disease agent. Older children on the other hand, especially from pastoral communities, are in close contact with animals due to their roles as they most often take care of animals, like herding and milking animals, as well as cleaning animal bans which are potential ways for them to get into contact with the infectious agents in case the animals are infected. Brucellosis in children varies with clinical manifestations varying from asymptomatic infection to chronic forms (Aghaali *et al.*, 2015). Moreover, its clinical presentation is similar to other febrile illnesses such as malaria and typhoid fever (Makala et al., 2020). These factors are likely to increase the chances of misdiagnosis and mismanagement, and thus likelihood of evolution to chronic brucellosis with potentially irreversible pathological damage e.g. bone and joint complications, cardiovascular and neurological damage, blindness, just to mention a few. Most studies focus on livestock and those including humans screening have rarely considered children. Such studies mask the disease burden in such high-risk groups. The main objective of the present study was to investigate brucellosis exposure status and associated risk factors among non-febrile children in selected livestock keeping communities of Kilombero district, Morogoro, Tanzania.

Materials and Methods

Study area

The study was conducted in Kilombero district, Morogoro region, South-Western Tanzania. The district is situated between 5 °58′ and 10°0′S and 35°25′ and 35°30′ E, with a population of 2,218,492 people (NBS, 2013). The district consists of Kilombero valley flood plain which is a large wetland endowed with various protected land and water resources. This district serves as a wildlife corridor between the Selous Game Reserve, Mikumi and Udzungwa National Parks, hence it is a good interface for zoonotic diseases from wildlife (Tapela, 2013). The majority of the residents of this district are subsistence farmers of maize and rice, however, emergence of livestock keepers like the Maasai, Sukuma and Barbaigs from different areas of the country progresses over time as the pastoralists search for better pastures and water for their livestock as well as better life through employment opportunities.

Study design, sampling and sampling strategy

A community-based, cross-sectional study was conducted from February 2020 to October 2021. Three out of thirty-five wards were purposively selected based on high numbers of pastoral communities. Wards with low numbers of pastoral communities and those located far were excluded. From each ward two livestock keeping villages were purposively selected depending on easy accessibility. Villages with livestock keepers but located far were excluded. Information about the purpose of the study was provided to the community by the researcher in the presence of village leaders at the meetings organized by the leaders. After the meetings, villagers/pastoralists with large numbers of livestock who agreed to participate in the study were visited and asked to sign the consent and assent forms at the household level. Within each village, five households with at least 30 heads of cattle and one primary school located in the livestock keeping community were selected, thus giving a total 30 households and six primary schools for the whole district. Households that were not willing to participate in the study, not reachable during the time of sampling and having less than 30 heads of cattle were excluded. Schools that were not within the intervened villages were excluded.

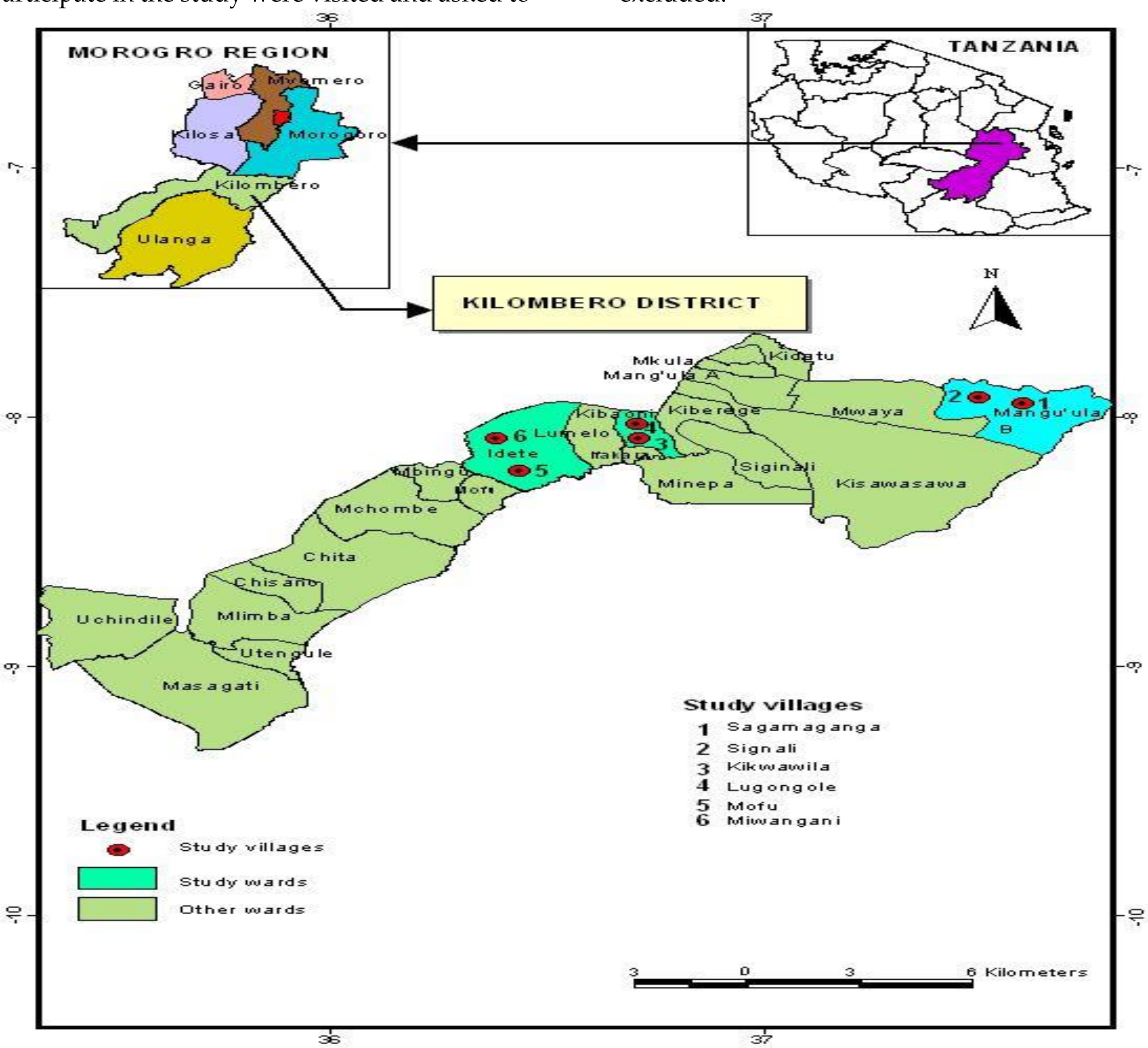

Figure 1. Map of Tanzania showing the study sites in Kilombero district within Idete, Ifakara, Signali and Sagamaganga wards.

In each household, four children within the age range between six months to 14 years were randomly selected. For households with less than four children, all were included in the study if they met the selection criteria. In the school settings, a maximum of 50 children were purposively selected from each primary school, these included children coming from livestock keeping families aging five to 14 years, and are willing to participate. At least five children from the study group, either male or female, were randomly selected to participate in the focus group discussion separately. The homes of the school children who participated in the study were visited, their siblings sampled and their interaction with livestock observed and recorded. Children from school and home clusters who refused to participate in the study were excluded. Selection of households within each village was based on the willingness to participate in the study, and accessibility by roads at the time of sample collection. Participants whose permanent residences were not within the study district were excluded. The children included were afebrile, with no history of brucellosis, and not under brucellosis treatment.

Sample size estimation

Sample size was estimated as described by Naing *et al*., (2006). An estimated/expected prevalence of 50% was used as there was not found a formal report about the prevalence of this disease in children, and that a few reports available are from studies that differ from the present study in aspects of methods, laboratory analysis and sampling design, thus giving a minimum sample size of 385 children.

Data collection

Focus group discussion

This was done after obtaining written permissions from the district education officer, heads of schools and village leaders. A focus group discussion guide was prepared to gather information on exposure risks among children. The focus group discussion method was used rather than interviews because of the language barrier, some of the children are not able to explain themselves personally. The discussion was conducted in the vicinity of primary schools located in the study villages. A total of 12 focus group discussion sessions consisting of five to six children of both sexes were conducted separately. The interviews took about 30 to 45 minutes and refreshments were supplied during the discussion as suggested by Morgan (1993). For each focus group discussion, assessment of general knowledge about brucellosis and potential risk practices for brucellosis transmission were done. General information including awareness on brucellosis, types of livestock kept, handling of livestock and their products, handling of animals during delivery/abortions and consumption of raw milk and other animal products was also collected. During the discussions, answers from children were recorded in audio recorder and in script in a written notebook; pictures were also taken.

Blood sample collection

Children were visited both at home and schools for blood sampling. From each child, approximately 5ml of blood were collected by a qualified health officer from brachial vein using a sterile disposable syringe into a plain vacutainer tube and labelled. After collection, blood samples were stored in a cool box with ice packs for cold chain before sending to the laboratory at St. Francis Referral Hospital in Ifakara for temporary storage. To obtain serum, blood samples were centrifuged at 3000 revolutions per minute (rpm) for 5min. The sera were separated from the blood clots using pipette and transferred to labeled Eppendorf tubes and stored in a -20^{o}C freezer. Before transporting to Sokoine University of Agriculture laboratory, the samples were tightly covered and packed in cool boxes with ice packs. Upon arrival the samples were stored at -20^{o}C as described elsewhere (Islam *et al*., 2018) until analysis.

Serological analysis

Rose Bengal test

A total of 361 serum samples were screened using RBT for detection of *Brucella* antibodies as described by Díaz et al. (2011). Briefly, 30µl of RBT antigen (Animal health and Veterinary Laboratories Agency, New Haw Addlestone Surrey, KIT15 3NB United Kingdom Lot No.325 sensitivity 14.29% specificity 97.98% (Sensitivity =TP/(TP+FN) and Specificity TN/(TN+FP), whereby TP is True positive, FN false negative, TN true negative, FP false positive, with iELISA test as the reference), (previously equilibrated at room temperature and shaken to re-suspend any bacterial sediment) and 30µl of the test serum were placed alongside on each well of the glass plate and mixed thoroughly using pipette tips. The slide was gently rocked for eight minutes and then observed for agglutination.

Agglutination and/or the appearance of a typical rim were taken as a positive result. A known positive serum (in-house control) was placed in the first well of a glass plate and mixed with equal volume of antigen in each run. Results were read by a single person well experienced in the methods.

Indirect ELISA

All 361 collected sera were subjected to IgG iELISA, by adopting a test procedure and interpretation of results as recommended by the manufacturer. (LT BIOTECH LTD, REF LT-BRU-10, Version: 003-2020-10, Lithuania, Vilnius, Sensitivity 98.94%. specificity 99.90%). According to the manufacturer instructions, analysis was considered correct, if mean value of OD for positive control was > 1 and, mean value of PP negative control was < 9. Results of the analysis for investigated samples were considered positive if PP of the samples was equal or greater than 12%, and negative if PP of the samples are < 12%.

Statistical analysis

All data were entered into Ms. Excel and then transferred to SPSS IBM Statistics 26 for analysis. Descriptive statistics, particularly frequencies and proportions, for variables such as age, sex, sampling location and education were calculated. All continuous variables were first converted to categorical variables before analysis. Sample characteristics were summarized as percentages for categorical variables. For the two serological tests (RBT and iELISA), a sample was considered positive if it reacted positively in any of the tests. This parallel interpretation was adopted to increase the detection rate of positive samples as this strategy has been suggested before in animal populations in Tanzania (Bodenham *et al.*, 2021). Chi-square test was used to compare differences in frequencies of categorical variables such as age, gender, location, tribe and education. The association between biodata/sociodemographic characteristics of the study participants such as gender, age, location and education and *Brucella* seropositivity was calculated using multivariate logistic regression. A P-value of 0.05 or less was considered statistically significant.

Diagnostic performance analysis

Kappa test was performed to evaluate the level of agreement between the two tests used (iELISA and RBT). Thus, we calculated the sensitivity, specificity, and Kappa values. The strength of agreement was interpreted as no agreement (K= 0); poor agreement (K<0.4) fair to good agreement (0.4< K >0.75); excellent agreement (K≥0.75); perfect agreement (K=1). Sensitivity of RBT was calculated using the formula; Sensitivity =TP/(TP+FN) and Specificity TN/(TN+FP), whereby TP is True positive, FN-false negative, TN-true negative, FP-false positive, as described earlier (Lukambagire *et al*., 2021).

Answers provided by children from focus group discussions were first recorded both in audio and in written form then stored accordingly in Microsoft office Word before importation into QDA Minerlite software (Provalis Research, 2997 Cedar Avenue, Montreal, QC. H3Y 1Y8 Canada) for qualitative analysis. The text from interviewed groups were then imported into the software and encoded during analysis. Codes were obtained through deductive analysis by the collection of risk practices to brucellosis transmission that were previously mentioned from other publications. Encoding was done as adapted from Onwuegbuzie *et al*., (2009). Categories that appeared by 50% of all the groups were considered as the main ideas (*themes*) and were taken as risk factors/practices for *Brucella* transmission in children (Onwuegbuzie *et al.*, 2009). However, useful information that was mentioned by less than 50% of the groups was also taken as the idea supporting the main themes (Christian and Carey, 1989).

Results

A total of 361 participants were included in the present study. These were drawn from three wards of Kilombero district. Both, males and females, school aged and those not yet at school were involved in the study (Table 1).

Table 1. Demographic information of the study participants from Kilombero district (N = 361).

Variable	Description	Frequency n (%)
Ward	Kibaoni	134 (37.1)
	Idete	90 (24.9)
	Mangula	137(38.0)
Gender	Male	188(52.1)
	Female	173 (47.9)
Education	In school	238 (65.9)
	Not in school	123 (34)

Seroprevalence of brucellosis among sampled individuals

Overall, with both serological tests we had 12 (3.3%, 95% CI 2%-6%) seropositive children. Using RBT, five children tested positive giving a prevalence of 1.7% (95% CI 1%-3%). With the iELISA, seven children were positive making a prevalence of 1.9% (95%CI 1%-3%).

The 12 seropositive children were from Mangula (7), Kibaoni (4) and Idete (1) wards. Out of 12 positive children, nine were at the age greater than five years. In terms of gender, eight were females and four were males. When the age was analysed as continuous variable versus log odds positivity, for every increase in one unit of age (year), the log odds of positivity increased by 0.069. However, the change was not statistically significant. Among the children who participated in the focus group discussion only two children tested positive, and from these two children one tested positive in both tests.

Comparison of serological tests used in detection of Brucella seropositivity

The Kappa score test showed that there was poor agreement between the RBT and IgG iELISA (K= 0.152). Results of the scores of the agreement between the serological tests are summarized in table 2 and 3.

Table 2. Cross tabulation of the serological tests used in detection of Brucella infection

Rose Bengal test		Positive	Negative	Total	Apparent prevalence
iELISA test	Positive	1	6	7	0.02
	Negative	4	349	353	0.98
	Total	5	355	360	
	Apparent prevalence	0.01	0.98		

Table 3. Kappa scores obtained in comparison of serological tests

Title	Value
Observed proportional agreement	0.972
Chance proportional agreement (both +)	0.0003
Chance proportional agreement (both -)	0.967
Chance proportional agreement	0.967
Observed minus chance agreement	0.005
Maximum possible agreement beyond chance level	0.032
Kappa	0.152
Kappa in %	15.294
SE(K)	0.948

K= $(P_o-P_e)/(1-Pe)$

Where: P_o observed proportion of agreement, Pe proportion expected by chance

Key to Kappa score: 1-perfect agreement; ≥0.75-excellent agreement; 0.4<K>0.75- fair to good agreement; 0-No agreement.

Relationship between biodata/sociodemographic characteristics and Brucella seropositivity

Results from multivariate logistic regression showed that there was no statistically significant association between the socio-demographic characteristics and *Brucella* seropositivity in children. Girls/female respondents were however more likely to test *Brucella* positive than boys (2.23 OR, 95%CI 60%--754%). (Table 4).

Risk practices that favor brucellosis transmission in the study population

Analysis of responses from focus group discussions obtained five themes representing risk practices for brucellosis transmission in the study area. The risk practices included i) consumption of raw milk, ii) assisting animals during parturition without personal protective equipment iii) sharing shelters with animals or sleeping close to animals, iv) improper handling of aborted materials, afterbirth and dead animals and, v) adoption and playing with small ruminants as pets (Figure 2). Other mentioned risk practices include entering animal houses bare footed and sucking of waste from noses of newborn animals soon after parturition.

Consumption of raw milk

All groups reported that they consumed raw milk at different occasions which included during herding animals because of lack of optional food while grazing.

"When I am thirsty, I milk the animal and drink milk, I just milk the animal and put the milk in the bottle and drink, I move with it". We milk the animal and drink; you just find the cow you are familiar with and get milk for drinking. You find the gentlest cow and milk".

(Responses from males, group 7 in Kikwawila village, Kilombero).

Table 4. Association between Brucella seropositivity in children and social-demographic characteristics

Variable	**Category**	**n**	***Brucella* seropositivity**		**Sig**	**Odds ratio**	**95% CI for OR**	
			No. positive	**No. negative**	**P<0.05**		**Lower**	**Upper**
Gender	Male	188	4	184				
	Female	173	8	165	0.197	2.23	0.659	7.543
Age	<5	89	3	86	0.967	1.04	0.203	5.269
	6-10	180	6	174	0.975	1.02	0.250	4.187
	11-14	92	3	89	0.999			
Ward	Kibaoni	134	4	130	0.381	0.57	0.163	1.999
	Idete	90	1	89	0.381	0.023	0.025	1.726
	Mangula	137	7	130	0.295			
Education	In school	238	8	230		0.03	0.038	2.663
	Not in school	129	4	119	0.956	0.29	0.285	3.275

Table 5. Analysis of age as continuous variable with log odds of seropositivity

iELISA	Coefficient	Standard error	z	p>[z]	95% confidence interval	
Age	.0690282	.1211108	0.57	0.569	-.16834446	.306401
Constant	-4.495127	1.112765	-4.04	0.000	-6.676106	-2.314147

Other children reported having consumed raw milk directly from the cow's udder.

"You can drink directly from the teat, but the cow will strike you by its leg, you have to look for the gentle one whom you are familiar with, for example the one you milk everyday".
Males, group 11 in Signali village, Kilombero.

Also, some children use their palms to collect and drink milk flowing from the cow's udder because they lack containers for collecting the milk.

"I was with my friend; he was milking while I catch up the flowing milk with my hands and drink". *(Response from male, group 11 in Signali village, Kilombero).*

The habit of drinking raw milk was mostly mentioned by the male groups (100%) compared to female groups (90%). Kilombero district consists of Kilombero flood plains, the area that is used for grazing animals. During dry seasons children move with animals to the plains seeking for pasture, a kind of transhumance, thus they are required to stay far from their homes for a long period. It is apparent that, during this period the readily available source of food for these children is raw milk from the livestock. Another circumstance that makes children in this area drink raw milk is following snakebite.

"If someone is bitten by a snake the victim is fed with raw milk to neutralize the poison. "It is believed that drinking a lot of raw milk after snake bite neutralizes the venom in the victim's blood and then other traditional medicines are used. If for example you are bitten by a snake when herding cattle, the first aid is raw milk. So, it helps you to return the animals safely home.
(Response from male, group 7 in Kikwawila village, Kilombero).

During focus group discussions, the children were shy, looking down and looking at each other and giggling. This made an impression that they were not comfortable responding to the questions raised. However, they appeared to know that drinking raw milk, especially direct from the udder is a bad habit and is forbidden by their parents.

i. Assisting animals during parturition without protective gears

Males are directly involved in assisting animals during parturition, however all groups mentioned being involved in assisting animals during parturition especially in cases of dystocia. This includes pulling legs of the newborn, mouth to mouth respiration administered to the newborn calf or clean calf with bare hands or using a piece of cloth and water.

"You enter your hand inside the womb, touch the leg of the newborn and pull it slowly"
(Response from male, group 1 in Mofu village, Kilombero).

"If you find that the newborn is not able to breath, you blow the air through the nose and wipe all the waste off the body" (Response from male, group 5 in Lugongole village, Kilombero).

Most of the practices of assisting parturitions were done in poor and unhygienic conditions which have a potential to predispose these children to most zoonotic infections including brucellosis;

"I have seen a goat during parturition, I helped by pulling the leg of the newborn until it came out, after that I washed my hands, and if it happens that there is no water around, I clean my hands using grasses and later with water when available". Also *"I assisted the lamb, when I saw her unable to move here and there, I pulled the baby slowly until it came out and after that I blew air through the nose and removed the layers away from the eyes and nose. If it happens that still the newborn cannot breathe, we take them to our elders"*
(Response from male, group 9 in Sagamaganga village, Kilombero).

Some of the children participated in unhygienic assisting animals' parturition together with their parents;

"I have pulled the newborn of the cow during parturition, my mother was also assisting the cow, and after the newborn was out, we put her beside her mother. I saw my mother blow air into the nose and mouth until it became alive. Then we left the calf beside the mother".
(Response from female, group 10 in Sagamaganga village, Kilombero).

It is therefore certain that when the children are on their own, they could practice this without being aware of the risk of infection.

"I have assisted a goat during parturition; no one was around to help so I pulled the legs of the newborn until the baby came out. You don't tie him first, the animal will lie itself down".
(Response female, group 10 in Sagamaganga village, Kilombero).

iii) Residing in the same shelter with animals

About 75% of all groups reported to keep the animals inside the houses where they also reside. This is due to threats from predators, such as lions, bad weather conditions and to protect young animals from being harmed by the older animals within the herds.

"But the young animals are sleeping inside, our mother told us to take them inside, we sleep in the same room with them" (Responses from female, group 2, Mofu village).

The other reason for residing in the same house with animals was due to traditions and customs

"The Maasai houses have no more than two rooms so animals and people are sleeping in the same house.

This is different from Sukuma, whereby they have a kraal for cattle, for calves and the one for sheep and goats" (Responses from male, group 9, Sagamaganga village).

iv) Improper handling of abortions, afterbirths and dead animals

Children are responsible for handling of birth materials such as placenta. About 75% of all the groups mentioned that they are involved in the disposal of birth materials. However, the handling and disposal of afterbirth is done without wearing any protective gears.

"After the newborn is out, the afterbirth membrane is thrown away, it can be thrown away by any person, I have also done" (Response from male, group 7, Kikwawila village).

They may throw the membranes away in the bushes, chopping it and giving it to dogs, or burying the afterbirth into the ground. This practice is performed either on pasture during grazing of animals or when animals are at home. Most often these activities are done by male children; this was reported by 80% of male's groups.

"If it happens during the herding activities that the newborn is dead you have to carry the newborn first to be seen by parents at home. After they see it, they will tell you to dispose into the bush or give it to dogs" (Response from male, group 11, Signali village).

v) Adopting and handling sick/infected animals as pets

Both male and female children do play with animals, this includes running and carrying them. Almost half of the groups mentioned adopting small ruminants as pets.

"*Yes, we play with small ruminants, but they are running faster than us*".
(Response from *male, group 11 Signali village).*

Some of the groups (30%) reported that younger children are the ones playing with small ruminants while older children only take care of them.

"No, I do not play with the small ruminants; our young ones are the ones playing with them". (Response from male, group 9 Sagamaganga village).

Discussion

The present study highlighted the prevalence of brucellosis and risk factors that could possibly facilitate the transmission of the disease among children in the study area. Findings from this study showed a relatively low seroprevalence of brucellosis in the sampled children. The low seroprevalence of infection in the present study could be accounted by the fact that the study subjects were afebrile at the time of sample collection which could suggest that majority were not exposed to the infection.

Other studies conducted in Tanzania have shown higher seroprevalence of brucellosis in humans i.e. 7.7% (John *et al*., 2010), 10.9% (Makala *et al.*, 2020), and 7% (Chipwaza *et al.,* 2015). These studies used ELISA kit that detects both IgG and IgM isotype of antibodies, while in the present study we used the kit designed to detect IgG antibodies. During the early stage of *Brucella* infection, IgM are dominant while in the chronic stage IgG predominate. This suggests that the present study might have missed individuals who were in the early stage of infection.

Our findings are also different from those reported in Kenya and Uganda and Jordan with relatively higher seroprevalences of 38.9%, 11%, and 11.6%, respectively (Akoko *et al*., 2021; Al-Majali and Shorman, 2009; Miller *et al*., 2016). However, findings from the present study are similar to what was reported in Iran with seroprevalence of 4.3% and in Chad indicating the same level of seropositivity and exposure risks as expected in endemic regions (Aghaali *et al*., 2015; Schelling *et al*., 2003). The differences in prevalence between the present and previous studies cited in this article could be attributed to differences in sampling strategies and analytical test KITs used. For instance, studies by Chipwaza *et al*. (2015) and Akoko *et al*., (2021) were done among febrile patients which is likely to increase chances of detecting *Brucella* infected individuals especially in their early stages. The use of commercial IgG/IgM lateral flow assay can detect acute and chronic infection (Miller *et al.*, 2016); these tests could give different results from what could be obtained by other techniques that detect either of the immunoglobulins.

There was poor agreement between the serological tests used in the study. RBT had few positive results compared to iELISA. This can be due to low sensitivity of RBT particularly in chronic cases (Geresu and Kassa, 2016)). It is well documented that RBT has relatively low specificity especially in endemic areas due to cross reactivity with other bacteria such as *Yersinia enterocolitica O:* 9, *Escherichia coli* O157: H7, *Salmonella* species and *Pasteurella* species (Nielsen, 2002; Nielsen *et al*., 2004; Islam *et al*., 2018). ELISA (especially the one detecting both IgM and IgG) is more sensitive in acute and chronic cases of brucellosis and it offers a significant diagnostic advantage in the diagnosis of brucellosis in endemic areas (Geresu and Kassa, 2016).

This study used parallel interpretation of the results so as to increase the chances of obtaining the positive individuals as participants were afebrile and they were recruited from their households. The effect of parallel interpretation could be over or under estimation of results attributed to the qualities of the tests used.

The positive serological result from the present study indicates that an individual has been in contact with the *Brucella* bacteria in the past or has an active infection which could not be differentiated in the tests used but provide useful information about circulation of the bacteria in the population. Prevalence of the disease in humans indirectly also reflects its occurrence in livestock reservoirs. It has been documented that in areas where brucellosis exists in sheep and goats, the disease incidences are also high in humans (WHO, 1997). Similarly, prevalence of brucellosis in humans has also been associated with the presence of it in cattle (John *et al*., 2010) and wild animals (Assenga *et al*., 2015). Therefore, attention has to be given to animals as they are the source of human infection and could continue shedding the bacteria in the environment.

From this study, children less than five years were less affected with brucellosis and those above six to ten years were more likely to be *Brucella* seropositive. This finding is similar to that reported by Tanir *et al*. (2009) where the lowest frequency of *Brucella* infection was among under-fives, presumably because exposure to unpasteurized milk is low at that age as they are breastfed. Therefore, children above five years are more affected with brucellosis, this has been reported elsewhere that children above five years constitute the majority of childhood brucellosis cases (Al-Majali and Shorman, 2009; Gül *et al*., 2014), due to higher probability of tendering animals and consumption of unpasteurized milk and milk products (Bodenham *et al.,* 2020). When the age was analysed as continuous variable versus log odds positivity, for every increase in one unit of age (year), the log odds of positivity increased by 0.069. However, the change was not statistically significant.

Our data showed that being female increases the likelihood of being *Brucella* seropositive, however this association was not statistically significant. Similar findings have been reported previously in the country where by females had a relatively higher infection rate than males (James, 2013). This is in contrast with studies reported by Schelling *et al.,* (2003) and Sagamiko *et al*., (2020) who reported brucellosis seroprevalence being higher in males than females. Differences in prevalence rates between the sexes may be explained by different roles, behavioral attitudes towards livestock handling, preparation of food from animals as well as tradition and customs which predispose them to different levels of infection. In livestock keeping communities, females do most of the work associated with harvesting of animal products (such as milking), preparation of food from raw meat, and cleaning of livestock houses. On the other hand, boys are involved in herding activities, assisting animals during parturition/abortion which may predispose both groups to infection.

Consumption of raw milk and milk products was identified as the leading risk practice in the present study. Similar findings have been reported in other pastoral and agropastoral communities in Tanzania (John *et al*., 2010; Swai and Schoonman, 2009). Likewise, it was reported in Uganda that consumption of locally made dairy products from unpasteurized milk increases the risk of being seropositive (Nguna *et al.*, 2019).

Consumption of raw milk was associated with some traditional engraved cultural beliefs. From the group discussion, it was noted that most people believe that raw milk is more nutritious than pasteurized/boiled milk. This is similar to findings obtained from the survey by Mullin *et al*. (2014). Other reasons for consumption of raw milk include snake bite and /or overdosing with medications which make people to consume raw milk as a first aid intervention. This is similar to the findings by Muturi *et al*., (2018) who found that, even though most of the pastoralists around the world know about the risk associated with consumption of raw milk, the majority of them still consume raw milk for traditional and for cultural reasons.

The present study revealed that children drink raw milk directly from animals during herding activities when they are hungry. A similar explanation was given by Tumwine *et al*., (2015) in Uganda. From these observations, it is important to provide appropriate education about zoonotic infections to children and their parents as family education has been associated with a lower rate of brucellosis (Azza El *et al*., 2007).

Assisting animals during parturition, and improper handling and disposal of birthing and abortion materials from animals were also the risk practices observed in the present study. This finding corroborate the findings of other authors who found strong association between brucellosis and assisting aborting livestock (Jansen *et al*., 2019; John *et al*., 2010; Muturi *et al*., 2018). The association between assisting animals during delivery and increased risk of *Brucella* infection is due to the fact that the predilection site for *Brucella* species are reproductive organs particularly the placenta and aborted fetuses, due to the presence of growth factor erythritol (Doni *et al*., 2017; WHO, 2006).

It was apparent from this study that residing with animals is a common practice whereby people share the same shelters with their animals especially newborn animals. Cultural reasons, climatic conditions such as rainfall, predators such as lions are some of the reasons for sharing shelters with animals especially at night. Like other airborne diseases such as tuberculosis, brucellosis can also be acquired through aerosol inhalation (Bosilkovski *et al*., 2015; John *et al*., 2010). This is supported by Osoro *et al*., (2015) in Kenya who documented aerosol exposure to brucellosis among livestock keepers. Further, excretions from infected animals through urine and feces may contain zoonotic infectious agents that can contaminate the surroundings and can be accidentally inhaled (Genene, 2009; Kledmanee and Liabsuetrakul, 2019; Makala *et al.*, 2020; Young, 1983).

Conclusions

Brucellosis is prevalent in Kilombero district in Morogoro region in non-febrile children with relatively low prevalence. Risk practices were identified and could constitute a possible basis for emergence of full-blown brucellosis in the future. Provision of general education concerning brucellosis and how to prevent infection spread should be provided to the community to bring awareness on the social, and economic impact of the disease. Any control strategy should include children as they are infected and exposed to different risk practices.

Recommendations

It is recommended that more studies be conducted targeting clinically sick individuals so that relevant samples can be collected to determine circulating *Brucella* species in the area.

Acknowledgement

A word of thanks is extended to all the laboratory personnel from health facilities in Ifakara, St. Francis referral hospital, and Sokoine University of Agriculture (SUA); who assisted in sampling the study subjects and analysis of samples. Thanks to all households and schools that participated in the study. This project was supported by the framework of the DELTAS Africa Initiative [Afrique One-ASPIRE /DEL-15-008]. Afrique One-ASPIRE is funded by a consortium of donors including the African Academy of Sciences (AAS), Alliance for Accelerating Excellence in Science in Africa

(AESA), the New Partnership for Africa's Development Planning and Coordinating (NEPAD) Agency, the Wellcome Trust [107753/A/15/Z] and the UK government. The World Academy of Sciences (TWAS)-UNESCO. Grant ref. No. 19-033 RG/BIO/AF/AC_I – FR3240310150 provided funding for purchase of serological test kits. The funders had no role in the design of the study, data collection, analysis, interpretation, writing or decision on the choice for the journal.

Ethics approval

This study was approved by Medical Research Coordinating Committee of Tanzania's National Institute for Medical Research (NIMR/HQ/R.8a/Vol.1X/3102). Written informed consent were obtained from heads of the households. At school children provided their verbal assent to participate in the study. Permission letter to collect data in the schools was obtained from the District Education Officer.

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