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Seroprevalence of bovine brucellosis and associated risk factors among smallholder dairy cattle farmers in Hai and Meru District Councils Northern Tanzania

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Abstract

Brucellosis is an endemic zoonotic disease caused by a facultative intracellular gram-negative bacterium of the genus *Brucella*. This study was carried out to establish the current status of brucellosis and possible associated risk factors in the smallholder dairy cattle in Hai and Meru District. A cross-sectional study was conducted between January and June 2022 to investigate the current seroprevalence of bovine brucellosis and possible associated risk factors in small dairy farms in the study areas. A total of 400 cattle was sampled with blood collected from 10 villages in each district. Blood samples were analyzed for *Brucella* circulating antibodies using the Rose Bengal Plate Test and c-ELISA. A structured questionnaire was administered to 200 smallholder dairy cattle farmers to determine the potential risk factors associated with brucellosis among dairy cattle in the study areas. The overall seroprevalence of bovine brucellosis in the study area was 0.50% and 0% for the Hai and Meru districts, respectively. Analysis of knowledge and management practices of brucellosis in the study area showed that the majority of farmers 74.5%, (149/200) were knowledgeable about the name of the disease; though, the majority 87.9%, (131/149) did not know the clinical signs. Most likely, awareness and biosecurity based on the nature of the farming system (zero grazing) contributed to the low seroprevalence; thus, none of the risk factors were associated with the disease. Therefore, under the smallholder dairy farming system, a four-stage roadmap for progressive control of brucellosis in animals and humans as recommended by FAO could be implemented with farming system modifications to eradicate the disease.

Keywords: Brucellosis, dairy cattle; seroprevalence; risk factors; Tanzania

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Introduction

The smallholder dairy sector is among the growing livestock subsectors in Tanzania. This sector plays a great role in the national food security, with its importance ranging from nutrition, health, employment opportunity; manure for farm fertilization, source of energy through biogas production; and generation of household income through selling of milk surplus and milk products (URT 2015). Nevertheless, the dairy sector in Tanzania is constrained by a number of challenges, such as a seasonal change in pasture availability and quality, lack of broad-based dairy production technologies, low rate of milk processing, poor milk quality, poor milk handling facilities, long calving interval and diseases including zoonoses such as anthrax and brucellosis (Bingi & Tondel.,2015 ,Maleko et al., 2018).

Brucellosis is among the endemic-neglected zoonotic diseases of social-economic importance in many regions and countries , such as Central Asia, India, Near East countries, Mexico, South and Central America, European Mediterranean countries and African countries, including Tanzania (Corbel 2006). Globally, the prevalence of bovine brucellosis ranges between 2% and 36% (Seleem et al., 2010 ;Abu Sulayman et al., 2020; Bodenham, 2020 ; Khurana et al., 2021; Holt et al., 2021; Ntivuguruzwa et al., 2020 ; Wainaina et al., 2020; Djangwani et al., 2021 ;Mengele et al., 2023). However, the disease has been eradicated in some European countries because of successful eradication and control programmes, including active surveillance coupled with test and slaughter policy and mass vaccination of domesticated animals (Seleem, Boyle, and Sriranganathan 2010). In Tanzania, the disease is among the six

prioritized zoonotic diseases that require national attention. The endemicity of bovine brucellosis in Tanzanian dairy farming is attributed to a number of risk factors, such as inadequate surveillance of the disease, lack of vaccination of programmes , unrestricted animal replacement and movements, weak regulatory framework in the culling of brucellosis-positive reactors, poor knowledge of the disease, improper disposal of aborted fetus and retained placentas, animal interactions in grazing and watering points and breeding practices (Asakura et al., 2019 ; Sagamiko, 2019 ; Bodenham et al., 2020 ; Ntirandekura et al., 2021 ; Katandukila et al., 2021).

Brucellosis infection in smallholder dairy cattle causes not only negative social-economic impacts but also public health consequences (Akakpo 2009). Some efforts to prevent and control the spread of brucellosis in Tanzania under smallholder dairy farming system were effectively practiced early in 1980-1990 when prevalence was reduced to $\leq 2\%$ in dairy cattle. However, recent studies have showed that the seroprevalence of brucellosis in smallholder dairy cattle is re-emerging from 0%-22.1% in various hotspot areas of Tanzania following the collapse of Tuberculosis and Brucellosis control Programme in animals (Swai E, Nkya R and Kambarage, 2000; Karimuribo et al., 2007; Mellau & Wambura, 2009; Shirima et al., 2010; Swai & Schoonman, 2010 ; Shirima et al., 2016 ; Bodenham, 2020 ; Mengele et al., 2023). Despite the smallholder dairy in the northern Tanzania supplying milk to major milk processing plants; little work has been done to ascertain the current status of brucellosis in dairy cattle in this area. Therefore, this calls for a detailed investigation to establish the

disease status, spread, hot spots and possible associated risk factors in smallholder dairy cattle in the Hai and Meru District Councils representing the Kilimanjaro and Arusha regions where smallholder dairy farming is highly prominent.

Materials and Methods

Study area

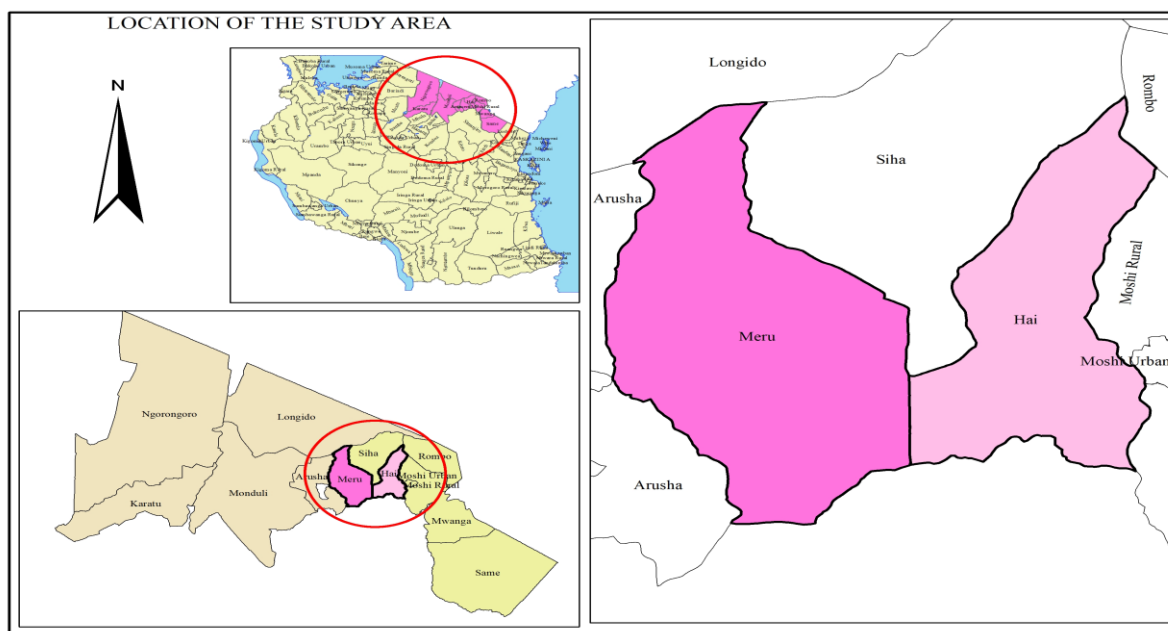


Figure 1. Map of the study area showing the boundaries of Meru and Hai Districts.

Source: Created by using Quantum Geographic Information System (QGIS).

Study design, sample size and sampling procedures

A cross-sectional study was carried out between January and June 2022 involving adult dairy cattle of at least one year of age under the smallholder farming system. The sample size was calculated using Fisher's (1998) statistical procedure, with a confidence interval of 95%, a margin error of 5% and an estimated prevalence of 50% in smallholder dairy cattle.

The following formula was used for the sample size calculation:

$$N = \frac{Z^2 * P(1 - P)}{C^2} \quad (\text{eq 1})$$

where N=Sample size, P=Estimated prevalence = 0.5, Z =Level of confidence as 1.96 and c = Desired precision level = 0.05.

The research work was carried out in the Hai and Meru District Councils in northern Tanzania (Fig.1). The two districts were selected based on the high number of smallholder dairy cattle farms. Hai has a total of 49,316 dairy cattle and occupies an area of 1,011 km² (101,100 ha), while Meru has a total of 98,001 dairy cattle and an area of 1,268.2 km².

The calculated minimum sample size was 384. However, the sample size "N" used in this study was 400 smallholder dairy cattle. The study involved both purposive and multistage random sampling procedures. These sampling procedures involved 62 villages and 17 wards in the Hai District Council, while there are 90 villages and 26 wards in the Meru District Council. The study involved 10 villages and 5 wards from each District. The Wards inclusion criteria in this study was possession of at least 500 dairy cattle. First, there was a purposive selection of 5 wards with a large number of dairy cattle from each district, followed by a multistage random sampling of two villages from each ward in which 20 dairy cattle from 10 dairy cattle-keeping

households in each Village (2 dairy cattle @ household) were selected randomly for blood collection. At every stage of random sampling, run if () function in statistical software R was deployed.

Field data collection

Cattle were restrained manually and humanly to avoid harm or any causes of animal discomfort during sample collection. The exercise was performed in compliance with the Tanzania Animal Welfare Act, part V (Animal Welfare Act 2008,). Using a halter, the animal's head was fastened to an elevated position to allow visibility of the jugular vein. Then, the thumb finger was pressed at the base of the jugular groove to raise blood pressure and visualize the vein by blocking the vein. With a plain vacutainer and needle, 10 ml of blood was drawn from the jugular vein. Each animal was identified according to the identity type provided by the owner for subsequent identification. This enables proper labelling of the vacutainer tubes containing blood samples. To avoid albumin coagulation, which prevents serum formation during the centrifugation process, the blood samples were left at ambient temperature for approximately 30 minutes before centrifugation. Centrifugation was performed at the Tanzania Veterinary Laboratory Agency (TVLA) in the northern zone office, Arusha, where the vacutainer tubes were spun at 3000 rpm for 10 minutes (BHG S Segurita-Germany). After centrifugation, the tubes were removed, and the sera were decanted into 2.0 ml cryogenic vials. The sera were stored temporarily at the TVLA laboratory at -20°C soon after separation before transfer to The Nelson Mandela African Institution of Science and Technology's (NM-AIST) laboratory for analyses. Likewise, at the

NM-AST laboratory, the sera were stored at -20°C before execution of analysis.

Questionnaire survey

To investigate the possible risk factors for brucellosis in smallholder dairy cattle, a paperless questionnaire survey comprising both closed- and open-ended questions was developed and coded using the Open Data Kit [https://opendatakit.org/\(ODK\)](https://opendatakit.org/(ODK)) mobile application by the investigator. The questionnaire covered a wide range of information, such as abortion cases, recent reproductive status, history of retained placenta, knowledge about bovine brucellosis, use of vaccines, use of veterinary services, herd management practices, herd size, handling of aborting cows and aborted fetus, heifer sources, breeding methods, grazing system, milk production trends, past two calving dates, livestock movement and interaction with neighboring cattle herds as well as milk distribution channels, price and value chain (Appendix 1). Pretesting of the questionnaire was performed in smallholder dairy cattle-keeping households in Monduli District before the development of the final version. During the field visit, the questionnaire was administered to the respondents (head of household or someone knowledgeable with the herd and above 18 years old). It took approximately 25-30 minutes to complete the questionnaire successfully.

Serological analysis

Laboratory analysis was carried out at The Nelson Mandela African Institution of Science and Technology laboratory, where a Rose Bengal Plate Test (RBPT) was used as a screening test for all 400 serum samples, while the competitive enzyme-linked immunosorbent assay (cELISA) was used for confirmation of the positive reactors.

Through RBPT, all 400 serum samples were screened for Brucella antibodies using the Zoetis™ (Rev 4/2017) Brucella Rose Bengal test kit from Delpharm Biotech (Lyon Cedex 07-France). Briefly, according to the manufacturer's instructions, equal volumes (30 µL) of the test serum and antigen were mixed thoroughly on the glass plate using an applicator stick, and the plate was gently hand rocked to allow mixing. After four minutes, the plates were visually examined for agglutination in comparison with a positive control. Any degree of agglutination was considered positive, while the absence of agglutinates was considered negative. The results were recorded and saved in a Microsoft Excel spreadsheet. The white glass plate was washed with clean water and methylated spirit and then dried for approximately 5-10 minutes before reuse.

The serum sample that reacted positive to RBPT was tested by cELISA according to (COMPELISA Rev01/2020) APHA SCIENTIFIC (Animal and plant Health Agency); New Haw Addlestone, Surrey, KT15 3NB United Kingdom. The only one RBPT positive reactor and 39 serum samples that reacted negatively on RBPT were randomly selected to make a single full microtitre plate during the cELISA test.

Data storage and statistical analysis

The obtained data were saved in Microsoft Excel spreadsheet software which was also used for descriptive analysis. The rest of data statistical analysis were done by using R statistical software version 4.0.3 (2020-10-10). The prevalence of brucellosis was determined using descriptive analysis based on the RBPT and cELISA tests using the following equation.

$$p_i = \frac{x_i}{n_i} \quad (\text{eq 2})$$

where x_i is the number of animals testing positive for brucellosis in a given administrative area, and n_i is the total number of animals tested in that administrative area. Furthermore, 95% exact binomial confidence intervals (CIs) were calculated using the binom.test function from the core R (www.R-project.org) stats package. The same formula was used to compute the overall seroprevalence across the study area.

In univariate analysis, the chi-square test was used to compare two or more proportions to determine the degree of relationship and significant differences. The variables that had $P < 0.05$ at the 95% confidence interval in univariable analysis were included in multivariate analysis, where the odds ratio (OR) was utilized to investigate the relationship between brucellosis risk variables and brucellosis prognosis.

Ethical Clearance

The research project was approved by Kibong'oto Infectious Disease Hospital-The Nelson Mandela African Institution of Science and Technology and the Centre for Education Development in Health, Arusha (KIDH-NM-AIST-CEDHA)-KNCHREC) ethical committee with certificate number KNCHREC0067/04/2022 issued on 27th June 2022. Additionally, permission was granted by the respective District Executive Directors (DEDs) in response to the introduction letters from NM-AIST. The willingness of the head of households to participate in this study was sought through written consent before the execution of the study.

Results

The sociodemographic profile of the respondents

The sociodemographic profile of the respondents indicated that 72%, (144/200)

were males and 28%, (56/200) were females of which the majority of the

respondents (81%;162/200) were aged between 41 and 60 years (Table 1).

Table 1. Sociodemographic characteristics of respondents involved in the study

Variables	Category	Meru	Hai	Total (%)
Gender	male	67	77	144 (72)
	Female	33	23	56 (28)
Age	18-25	1	0	1 (0.5)
	26-40	6	8	14 (7)
	41-60	79	83	162 (81)
	>60	13	11	24 (12)
Marital status	Single	11	9	20 (10)
	Married	88	83	171 (85.5)
	Divorced	1	0	1 (0.5)
	Widowed	3	5	8 (4)

Respondents' knowledge and awareness of brucellosis

Although 74.5% (149/200) of the respondents were aware of the disease (brucellosis) majority (87.9%; 131/149) were ill of the disease clinical signs in

animals. Among the clinical signs of the disease, abortion was mentioned by 12.08% (18/149) of the respondents, while no one knew the rest of the clinical signs of brucellosis (Fig. 2).

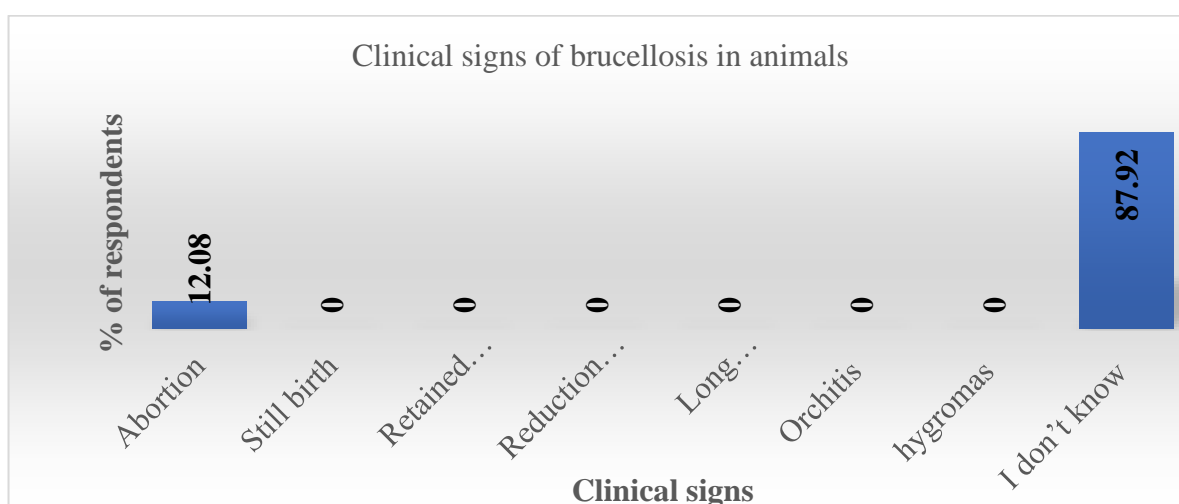


Figure 2. Awareness of the respondents on the clinical signs of cattle brucellosis in the study areas.

The results also showed that none of the respondents heard of brucellosis vaccine (S19) has never used in the study area

whereas, anthrax vaccine was well known by majority of respondents (Fig.3)

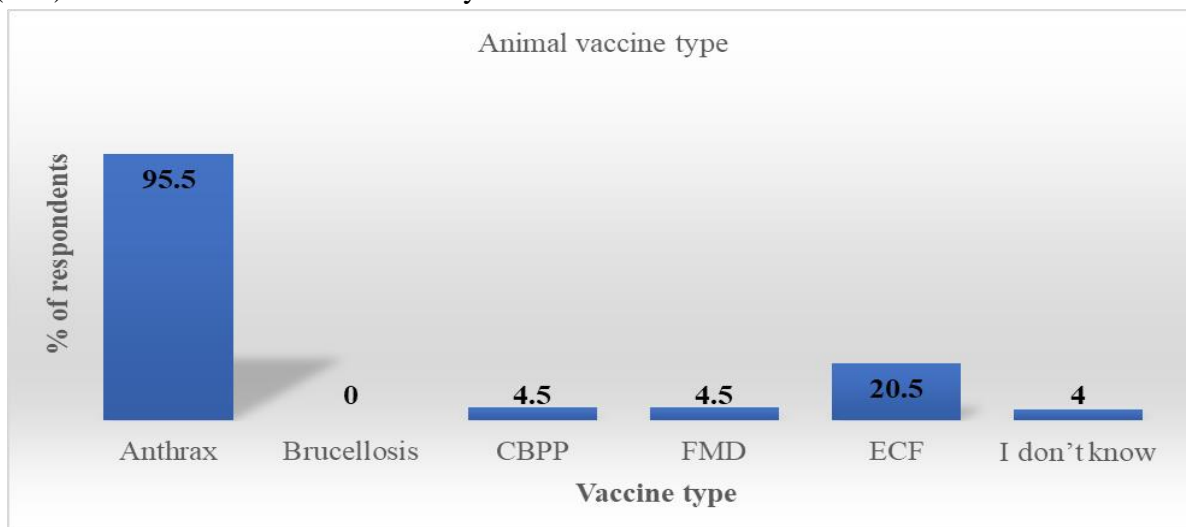


Figure 3. Respondents' awareness on commonly used vaccines in dairy cattle

Disposal methods of the aborted fetus and retained placenta.

The results show that 7.5%, (15/200) of respondents' herd had a history of abortion while 1.5% (3/200) reported

retained placenta cases. Majority of the respondents (80%, 12/15) buried aborted foetus and retained placenta while 20%, (3/15) thrown to dogs (Fig.4)

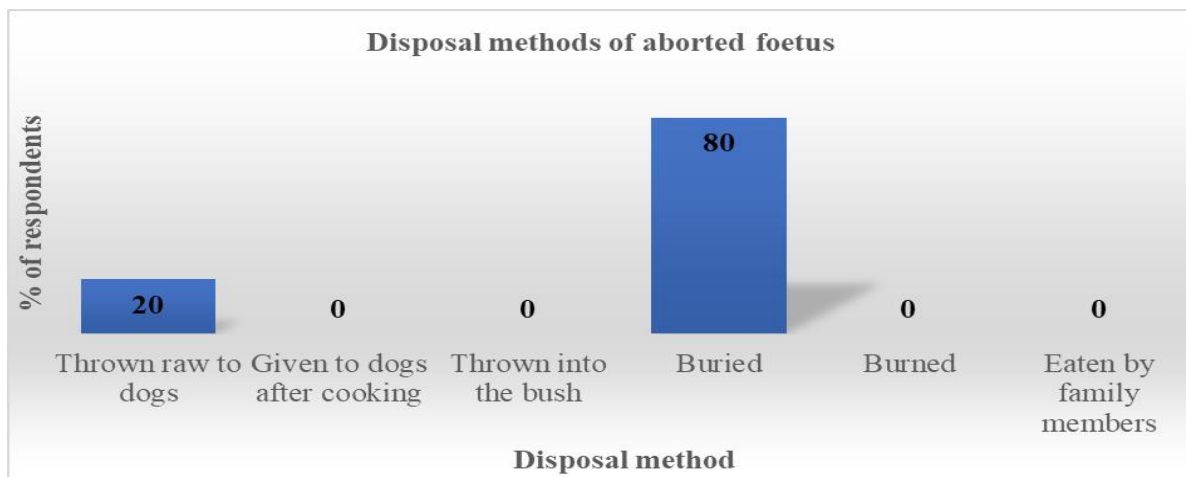


Figure 4. Disposal methods practiced by respondents on the aborted fetus and retained placenta

Animal breeding methods used in the study area.

Majority of the respondents (55%, 110/200) use artificial insemination compared to 32%, (64/200) who use bulls for breeding (Fig. 5), Of these who are using

bulls, 64%, (41/64) hire from another smallholder dairy farms. However, opinions on choice decision were based on cost (51.5%, 103/200), accessibility (34%, 68/200) and efficiency of the service type (14.5%, 29/200).

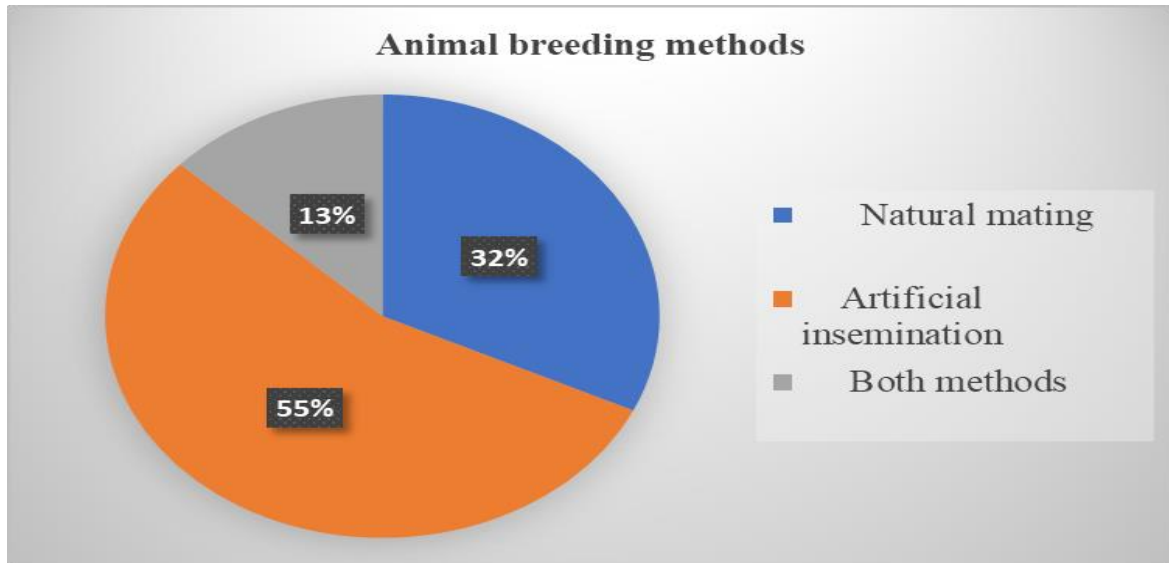


Figure 5: Dairy cattle breeding service type used in the study area.

Grazing system and animal interactions.

The majority of respondents (87.5%, 175/200) practise zero grazing system with only 1%, (2/200) practised a free-range

system (Fig. 6 Furthermore, 99%, (198/200) of the respondents reported that their cattle were neither herded nor fed together with sheep and goats.

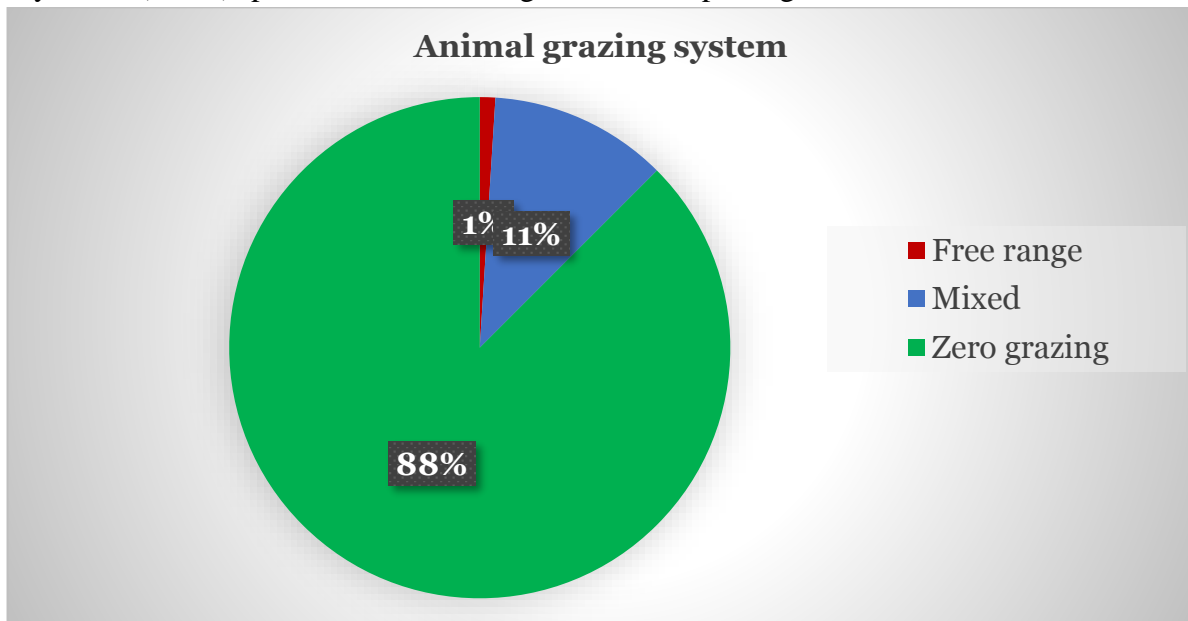


Figure 6. Smallholder dairy cattle - grazing system in the study area

Animal sources for heifer replacement.

The results show that more than sixty percent of the respondents (129/200) acquired heifers from other smallholder

dairy cattle farms with the district for replacement, whereas 35% (69/200) used to upgrade female calves to become heifers from their own farm. (Fig. 7)

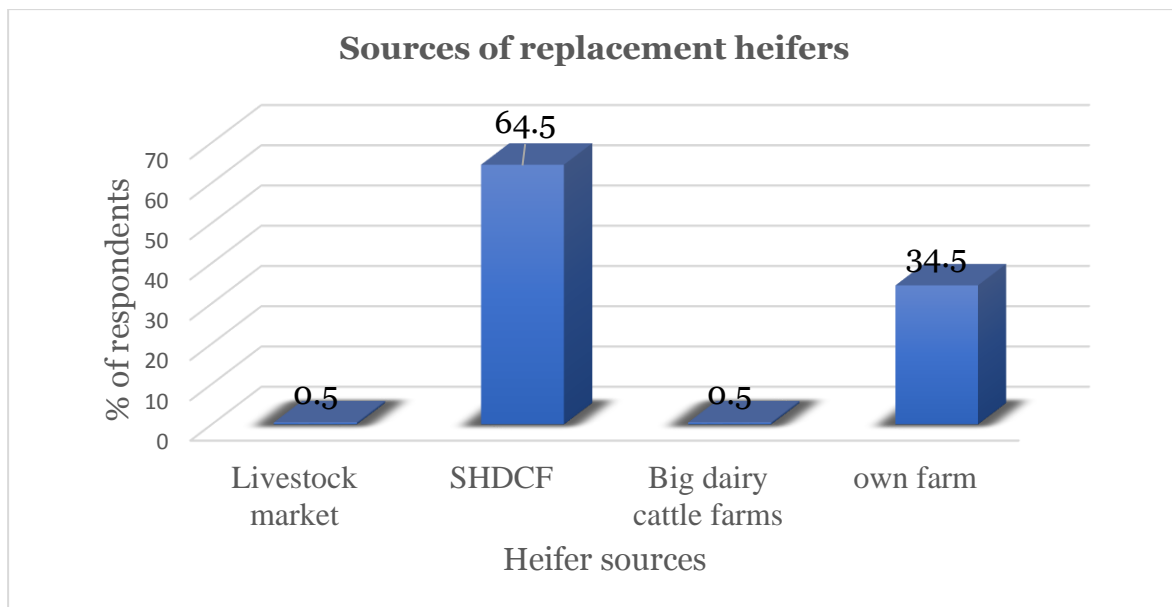


Figure 7. Source of heifers for replacement in the study area. SHDCF= Smallholder dairy cattle farmers

Management of animals that have failed to conceive.

Although majority of farmers (90.5%, 181/200) reported high conception rate by using both artificial insemination and bull

service, few farmers with animals that failed to conceive sold to other smallholder farmers as reported by 63.16%, (12/19) of the respondents (Fig. 8).

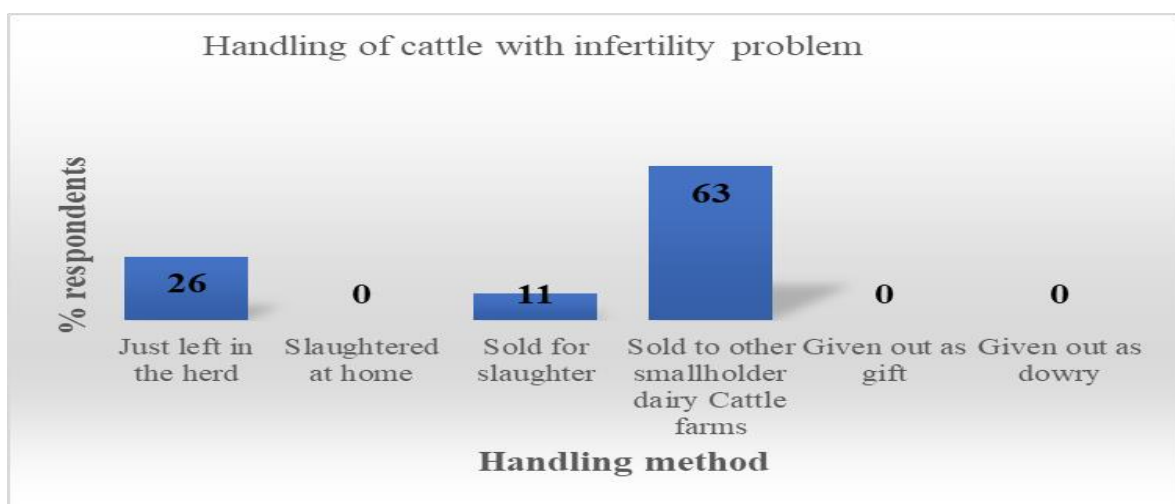


Figure 8. Management of dairy cattle that failed to conceive in the study area.

Seroprevalence of bovine brucellosis in smallholder dairy cattle in the Hai and Meru districts

A total of 400 dairy cattle from smallholder farms were tested for brucellosis using RBPT and cELISA tests.

The animal-level seroprevalence of brucellosis in Meru and Hai Districts was 0% and 0.5%, respectively, while the herd-level seroprevalence was 0% and 1% in Meru and Hai Districts, respectively. However, the difference between the two

districts was not statistically significant at $p < 0.05$ and 95% CI. The overall animal- and herd-level seroprevalence of brucellosis

in the study area was 0.25% and 0.5%, respectively. (Table 2).

Table 2. The individual and overall seroprevalence (%) of bovine brucellosis in the Hai and Meru District Councils

Results of c-ELISA across study Districts				
Study District	Animal level seroprevalence		Herd level seroprevalence	
	N (n)	Prevalence (%)	N (n)	Prevalence (%)
Meru	200 (0)	0	100(0)	0
Hai	200 (1)	0.5	100(1)	1
Overall results	400 (1)	0.25	200(1)	0.5

N = Number of animals tested n = Number of positive reactors

Association of the seroprevalence of bovine brucellosis at animal level risk factors.

Analysis of animal level risk factors and seroprevalence of bovine brucellosis among smallholder dairy cattle showed that the animal that tested positive was an adult Friesian female with neither abortion

history nor infertility problems as well as history of retained placenta. Also, the positive reactor animal had no history of retained placenta. All animal levels risk factors (Age, sex, breed, abortion history, retain placenta and failure to conceive) were statistically insignificant (p values > 0.05) under univariable analysis (Table 3).

Table 3. Association of the seroprevalence of bovine brucellosis at animal level risk factors

Risk factors	Category	N (n)	Prevalence (%)	Univariable analysis	
				χ^2	p value
Age	Young	22(0)	0	1.51e-28	1
	Adult	378(1)	0.25		
Sex	Male	23(0)	0	9.95e-32	1
	Female	377(1)	0.25		
Breed	Jersey	33(0)	0	0.30369	0.8591
	Ayrshire	61(0)	0		
	Friesian	306(1)	0.25		
History of abortion	Yes	29(0)	0	1.72e-29	1
	No	371(1)	0.25		

Failure to conceive	Yes	30(0)	0		
	No	370(1)	0.25	1.86e-27	1
History of RP	Yes	7(0)	0	1.26e-26	1
	No	393(1)	0.25		

N =Number of animals tested; n = Number of positive reactors; RP=Retained placenta

Detailed information of the seropositive cattle

Further information revealed that the seropositive cattle was a Friesian adult female kept under zero grazing system. The herd had 4 dairy animals with no small ruminants. Natural mating and artificial insemination were interchangeably used to service the animals in the herd. The

knowledge and practises related to bovine brucellosis and husbandry with the herd owner did not differ from the rest of farmers. All herd level variables (grazing system, type of service, source of heifers, contacts and vaccination) were not associated with the brucellosis seropositivity (p values >0.05 (Table 4).

Table 4. Association of the seroprevalence of bovine brucellosis at herd level risk factors

Risk factor	Category	N (n)	Prevalence (%)	Univariable analysis	
				χ^2	p value
Grazing system	Free range	4(0)	0	0.14322	0.9309
	mixed	46(0)	0		
	zero grazing	350(1)	0.25		
Type of service	AI	224(0)	0	2.155	0.3404
	Bull	126(0)	0		
	Both	50(1)	0.25		
Heifer source	livestock markets	2(0)	0	0.01861	0.8915
	smallholder dairy cattle farms	258(0)	0		
	Big dairy cattle farms	2(0)	0		
	own farm	138(1)	0.25		
Frequent contacts with other herds	Yes	2(0)	0	8.24e-23	1
	No	398(1)	0.25		
Disease name	Yes	299(1)	0.25	0	1
	No	101(0)	0		
Brucellosis diagnosis	Yes	11(0)	0	2.56e-26	1
	No	389(1)	0.25		
Vaccinated against brucellosis	Yes	0 (0)	0	8.38e-27	1
	No	400(1)	0.25		
Access to veterinary services	Yes	(0)	0	1.26e-26	1
	No	44(1)	0.25		

Livestock sharing house at night	Yes	1(0)	0	8.38e-	1
	No	399(1)	0.25	27	
N =Number of animals tested; n = Number of positive reactors					

Discussion

Brucellosis is among the six prioritized zoonotic disease in Tanzania that has social economic effects. This study was carried to establish the current status of brucellosis and possible associated risk factors in the smallholder dairy cattle in Hai and Meru District.

Seroprevalence of bovine brucellosis among smallholder dairy cattle.

The findings from this study, indicate that the sampled cattle in Meru district did not show antibodies against brucella infection, however, one dairy cattle in Hai district indicated to have been exposed to brucella infection. This indicates that the two districts are not among the brucellosis hotspots as reported elsewhere (Mengele et al., 2023; Shirima et al., 2018). Similarly, in this study, it was found that dairy farmers are not using the S19 vaccine against brucellosis and this indicates that the infected animal was due to natural exposure of the pathogen. The low seroprevalence of bovine brucellosis in the study area lies within the range of brucellosis studies in dairy cattle carried out in several parts of Tanzania (Mengele et al., 2023; Mdegela et al., 2004; Karimuribo et al., 2007; Alexander, 2017; Mhozya, 2017). Similarly, the findings of this study are in agreement with other studies conducted across the World that indicate a low seroprevalence in smallholder dairy cattle (Kothowa et al., 2021, (Hesterberg et al. 2008), (Getahun 2021), (Nguna et al. 2019), (Hassan et al. 2014). The results of this study are contrary to the findings of other studies where the seroprevalence was very high (Swai & Schoonman, 2010 ; Mengele

et al., 2023). The difference in seroprevalence in various studies in Tanzania can be caused by variations in study design, farming systems, management practices and other biosecurity measures taken by farmers. The very low seroprevalence obtained in this study might be attributed by a number of factors such as zero grazing system, animal replacement practices, disposal method of the aborted foetus and breeding methods.

In this study, it was found that zero grazing system was the dominant grazing system practiced by most of the smallholder dairy cattle farmers. Through zero grazing system, animals are fed on fodder using cut and carry practices. Therefore, there is less interactions of animals between herds thus acts as one of the key biosecurity control options against disease transmission including brucellosis. This observation agrees with the findings of other studies (Karimuribo, 2007 ; Swai & Schoonman, 2010) that zero grazing system minimize the level of infection since animals from different herds do not interact to each other. This is in contrary to other farming systems such as pastoral and agro-pastoral systems where herds with multispecies interact frequently in grazing and watering points thus perpetuates disease transmission (Assenga et al., 2015; Shirima, 2005). Furthermore, the zero grazing system practised in the study area separates small ruminants from being herded together with the dairy cattle thus minimize the cross-infection risk as well (Shirima, 2005 ; Rubegwa, 2015 ;Oromia et al., 2022 ; Mengele et al., 2023).

Based on the fact that both districts practised dairy farming for decades, animal replacement becomes feasible from within minimising introduction of animals from outside herds/districts. another reason for the low seroprevalence observed in this study. It was found that most animals for replacement were originated from either within the herds or between the herds in the study area. The mode of animal acquisition in the study area does not favour the introduction of animals that might be infected from other areas. This observation concurs with the findings from other studies (Shirima, 2005 ; Karimuribo , 2007 ; Alexander, 2017). However, the findings of this study are not similar to that of Rubegwa et al., (2015) and Abera et al., (2019) who reported higher bovine seroprevalence in homebred animals than brought animals.

In this study, it was found that most common breeding method used was artificial insemination instead of sharing bulls. This type of breeding method could contribute to the observed low seroprevalence of brucellosis in the sampled cattle. The disease's free semen used in artificial insemination limits possible transmission of *Brucella* pathogens from the infected bulls to cows. This findings from the study areas is also supported by others elsewhere (Corbel, 2006, Shirima, 2005) and Mfunne, 2015) who reported that there is high prevalence of *Brucella* in areas with low rate of using artificial insemination breeding method. Although proportion of dairy cattle farmers used bulls for mating; they are sourced from within the district and when screened were negative to both RBPT and cELISA. The use of bulls is not uncommon in pastoral farming systems thus may attribute to the level of infection reported compared to dairy cattle farming (Mellau and Wambura,

2009 ; Swai and Schoonman, 2010 ; Nguna et al., 2019).

Reports of abortion incidences and retain placentas in the current study may be clear evidence of low reproductive diseases including brucellosis. From this study, it was found that abortion and retained placenta were not common cases to happen in dairy cattle. However, cases of aborted foetus and retained placenta were disposed of properly by burying them onto the ground. This further prevents disease perpetuation as reported earlier that feeding raw to dogs amplify spread of the disease (Shirima 2005; Sijapenda et al., 2017 ; Ntirandekura et al., 2018 ; Ismail et al., 2019 Ntivuguruzwa et al., 2020 ; Mengele et al., 2023).

Animal and herd level factors associated with brucellosis in smallholder dairy cattle

Both animal level (sex, age, breed type, history of retained placenta and abortion) and herd level (farmer's awareness of the disease name, clinical signs of brucellosis in cattle, proper disposal methods of the aborted fetus, management of infertile animals, grazing system, breeding method, animal replacement, animal housing system, animal vaccination) risk factors were not significantly associated with the seropositivity due to a very low seroprevalence of the sampled cattle in this study. Since this happened in the absence of any formal prevention and control interventions it calls for a systematic surveillance and monitoring to maintain the status. Farms brucellosis certification coupled with continues education and awareness may be a strategic approach.

Conclusion and recommendations

In this study, it was found that the sampled cattle had a very low

seroprevalence. Likely, brucellosis prevalence in smallholder dairy cattle in Hai and Meru districts is relatively low. Zero grazing system coupled with in-house breeding and animal replacement from within the study areas may have provided biosecurity measures for brucellosis spread thus, need to be intensified to limit introduction of infections in the study areas.

Based on the findings from this study; it is recommended that; the surveillance monitoring approach may shift to bulk milk sampling to detect exposed herds in the event of low seroprevalence. Also, Knowledge about brucellosis is highly recommended as it can guide control efforts and improve information about local risk factors as well as the extent of dairy farmers' understanding of the disease to foster creation of better extension campaigns on the brucellosis. Importantly, brucellosis certification scheme initiated after this study be strengthened and monitored to ensure a disease-free area.

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References

- Abu Sulayman, Sulaiman Mohammed, Roop Singh Bora, Jamal S.M. Sabir, and Mohamed Morsi M. Ahmed. 2020. "Brucellosis: Current Status of the Disease and Future Perspectives." *Postępy Mikrobiologii - Advancements of Microbiology* 59 (4): 337–44. <https://doi.org/10.21307/pm-2020.59.4.25>.
- Akakpo, Ayayi Justin. 2009. "The Impact of Brucellosis on the Economy and Public Health in Africa," 85–98.
- Alexander, Maengo Respich. 2017. "Epidemiology of Brucella Infection in Cattle in Urban and Peri-Urban Areas of Sumbawanga Municipality, Tanzania. Msc.Thesis. Sokoine University of Agriculture (SUA)." "Animal Welfare Act 2008." 2008.
- Asakura, Shingo, George Makingi, Kunda John, Rudovick Kazwala, and Kohei Makita. 2019. "Seroprevalence and Risk Factors for Human Brucellosis in Agro-Pastoral Areas in Tanzania" *21* (1): 1–10.
- Bingi, Susan, and Fabien Tondel. 2015. "Recent Developments in the Dairy Sector in Eastern Africa Towards a Regional Policy Framework for Value Chain Development," no. 78.
- Bodenham, Rebecca F. 2020. "Brucellosis in Northern Tanzania : Investigating the Epidemiology of Human Infection and Evaluating Diagnostic Test Performance in Animal Hosts. University of Glasgow."
- Bodenham, Rebecca F, Abdulhamid S Lukambagire, Roland T Ashford, Joram J Buza, Matthew P Rubach, Philoteus Sakasaka, Gabriel M Shirima, and Emanuel S Swai. 2020. "Prevalence and Speciation of Brucellosis in Febrile Patients from a Pastoralist Community of Tanzania." *Scientific Reports*, 1–11. <https://doi.org/10.1038/s41598-020-62849-4>.
- Corbel, MJ M.J. 2006. "Brucellosis in Humans and Animals." WHO

- Library Catalogue in Publication Data, 1–88.
- Djangwani, Juvenal, George Ooko Abong', Lucy Gicuku Njue, and Dasel W.M. Kaindi. 2021. "Brucellosis: Prevalence with Reference to East African Community Countries – A Rapid Review." *Veterinary Medicine and Science* 7 (3): 851–67.
<https://doi.org/10.1002/vms3.425>.
- Getahun, Temesgen Kassa. 2021. "Seroprevalence of Bovine Brucellosis and Its Public Health Significance in Central High Land of Ethiopia."
- Hassan, Abdullah Al, M Bashir Uddin, Rafiqul Islam, Ho-seong Cho, and Mukter Hossain. 2014. "Serological Prevalence of Brucellosis of Cattle in Selected Dairy Farms in Bangladesh" 54: 239–43.
- Hesterberg, U. W., R. Bagnall, B. Bosch, K. Perrett, R. Horner, and B. Gummow. 2008. "A Serological Survey of Leptospirosis in Cattle of Rural Communities in the Province of KwaZulu-Natal, South Africa." *Journal of the South African Veterinary Association* 80 (1): 45–49.
<https://doi.org/10.4102/jsava.v80i1.168>.
- Holt, Hannah R., Jasbir Singh Bedi, Paviter Kaur, Punam Mangtani, Narinder Singh Sharma, Jatinder Paul Singh Gill, Yogeshwar Singh, et al. 2021. "Epidemiology of Brucellosis in Cattle and Dairy Farmers of Rural Ludhiana, Punjab." *PLoS Neglected Tropical Diseases* 15 (3): 1–17.
<https://doi.org/10.1371/journal.pntd.0009102>.
- Ismail, A.A.R.A., T.E.I.K.E. Angara, and A.M. Ibrahim. 2019. "Research Article Knowledge, Attitudes and Practices Associated with Brucellosis in Small-Holder Dairy Farms in Suburbs of Khartoum State, Sudan." *EC Veterinary Science* 4.4 4 (July): 241–50.
- Karimuribo E D, Ngowi H A, Swai E S and Kambarage D M. 2007. "Prevalence of Brucellosis in Crossbred and Indigenous Cattle in Tanzania. Livestock Research for Rural Development. Volume 19, Article."
- Katandukila, Jestina V, Yerima J Chuhila, and Fred D Chibwana. 2021. "Serological Analyses of Human Brucellosis in Ngara and Kibondo Districts ," 47 (3): 1225–35.
- Khurana, Sandip Kumar, Anju Sehrawat, Ruchi Tiwari, Minakshi Prasad, Baldev Gulati, Muhammad Zubair Shabbir, Rajesh Chhabra, et al. 2021. "Bovine Brucellosis—a Comprehensive Review." *Veterinary Quarterly* 41 (1): 61–88.
<https://doi.org/10.1080/01652176.2020.1868616>.
- Kothowa, J.P., Mfune, R.L., Godfroid, J., Hang'Ombe, B.M., Simuunza, M. & Muma, J.B. 2021. "Documenting the Absence of Bovine Brucellosis in Dairy Cattle Herds in the Southern Region of Malawi and the Associated Knowledge , Attitudes and Practices of Farmers," 1–7.
- Maleko, David. 2018. "Smallholder Dairy Cattle Feeding Technologies and Practices in Tanzania: Failures , Successes , Challenges and Prospects for Sustainability.Nternational Journal of Agricultural Sustainability <https://doi.org/10.1080/14735903.2018.1440474> Downloaded From ."

- <https://doi.org/10.1080/14735903.2018.1440474>.
- Mellau, Lesakit, and Phil Wambura. 2009. "Seroprevalence of Brucellosis in Domestic Ruminants in Livestock-Wildlife Interface : A Case Study of Ngorongoro Conservation Area , Arusha , Tanzania Seroprevalence of Brucellosis in Domestic Ruminants in Livestock- Wildlife Interface : A Case Study of N," no. December. <https://doi.org/10.4314/tvj.v26i1.49234>.
- Mengele, Isaac Joseph, Gabriel Mkilema Shirima, Shedrack Festo Bwatota, Shabani Kiyabo Motto, Barend Mark, De Clare Bronsvort, Daniel Mushumbusi Komwihangilo, et al. 2023. "Veterinary Sciences The Status and Risk Factors of Brucellosis in Smallholder Dairy Cattle in Selected Regions of Tanzania."
- Mfunne, Ruth L. 2015. "Epidemiological Study of Bovine Brucellosis in Smallholder Dairy Cattle in Lushoto and Rungwe Districts, Tanzania. Msc.Thesis.Sokoine University of Agriculture (SUA)."
- Mhozya, Makoye. 2017. "Assessment of Brucella Abortus and Antimicrobial Residues in Raw Cattle Milk in Bukombe District, Tanzania.Msc.Thesis. Sokoine University of Agriculture (SUA)."
- Nguna, Joyce, Michel Dione, Micheal Apamaku, Samuel Majalija, Denis Rwabita Mugizi, Terence Odoch, Charles Drago, et al. 2019. "Seropositivity in Cattle , Goats and Humans in Iganga District , Uganda" 8688: 1–10. <https://doi.org/10.11604/pamj.2019.33.99.16960>.
- Ntirandekura, Jean Bosco, Lucas E. Matemba, Helena A. Ngowi, Sharadhuli I. Kimera, and Esron D. Karimuribo. 2018. "Knowledge, Perceptions and Practices Regarding Brucellosis in Pastoral Communities of Kagera Region in Tanzania." *Journal of Advanced Veterinary and Animal Research* 5 (3): 343–53. <https://doi.org/10.5455/javar.2018.e285>.
- Ntirandekura, Jean Bosco, Lucas Eliaimringi Matemba, Sharadhuli Iddi Kimera, John Bwayla Muma, and Esron Daniel Karimuribo. 2021. "Brucellosis and Its Associated Risk Factors to Humans and Domestic Ruminants in Kagera Ecosystem , Tanzania" 21 (2).
- Ntivuguruzwa, Jean Bosco, Francis Babaman Kolo, Richard Simba Gashururu, Lydia Umurerwa, Charles Byaruhanga, and Henriette van Heerden. 2020. "Seroprevalence and Associated Risk Factors of Bovine Brucellosis at the Wildlife-Livestock-Human Interface in Rwanda." *Microorganisms* 8 (10): 1–15. <https://doi.org/10.3390/microorganisms8101553>.
- Oromia, South Western, Monenus Etefa, Tadele Kabeta, Desalegn Merga, and Motuma Debelo. 2022. "Cross-Sectional Study of Seroprevalence and Associated Risk Factors of Bovine Brucellosis in Selected Districts of Jimma Zone ,," 2022.
- Gabriel Shirima. 2012. "Prevalence of Brucellosis in the Human , Livestock and Wildlife Interface Areas of Serengeti" 420 (Nbs): 2–5.

- Rubegwa, Bachana Ammeckson. 2015. "A Dissertation Submitted to the University of Zambia in Partial Fulfillment of the Requirements of the Degree of Master of Science in One Health Analytical Epidemiology (Msc OHAE)," no. October.
- Sagamiko, Frederick D. 2019. "Seroprevalence of Human Brucellosis and Associated Risk Factors among High Risk Occupations in Mbeya Region of Tanzania," no. January 2016.
- Seleem, Mohamed N., Stephen M. Boyle, and Nammalwar Sriranganathan. 2010. "Brucellosis: A Re-Emerging Zoonosis." *Veterinary Microbiology* 140 (3–4): 392–98. <https://doi.org/10.1016/j.vetmic.2009.06.021>.
- Shirima, G M, J Fitzpatrick, J S Kunda, G S Mfinanga, R R Kazwala, D M Kambarage, S Cleaveland, Pentlands Science, and Park Midlothian. n.d. "Short Communication. The Role of Livestock Keeping in Human Brucellosis Trends in Livestock Keeping Communities in Tanzania," 1–6.
- Shirima, Gabriel Mkilema (2005). 2005. "The Epidemiology of Brucellosis in Animals and Humans in Arusha and Manyara Regions of Tanzania," no. 3: 321–25.
- Sijapenda, J E, E V G Komba, and H E Nonga. 2017. "Studies on Seroprevalence and Risk Factors for Occurrence of Bovine Brucellosis in Cattle in Lindi District, Tanzania." *Tanzania Veterinary Journal* 35 (Special Issue): 82–89. <http://www.ajol.info/index.php/tvj>.
- Swai E, Nkya R and Kambarage, D.M. 2000. "The Prevalence of Bovine Brucellosis in Small Scale Dairy Cattle Herds 43-50.Pdf."
- Swai, Emanuel Senyael, and Luuk Schoonman. 2010. "The Use of Rose Bengal Plate Test to Assess Cattle Exposure to Brucella Infection in Traditional and Smallholder Dairy Production Systems of Tanga Region of Tanzania" 2010. <https://doi.org/10.4061/2010/837950>.
- United Republic of Tanzania. 2015. "Tanzania Livestock Modernization Initiative." Ministry of Livestock and Fisheries Development, 41.
- Wainaina, Martin, Gabriel O. Aboge, Isaac Omwenga, Catherine Ngaywa, Nicholas Ngwili, Henry Kiara, George Wamwere-Njoroge, and Bernard Bett. 2020. "Detection of Brucella Spp. in Raw Milk from Various Livestock Species Raised under Pastoral Production Systems in Isiolo and Marsabit Counties, Northern Kenya." *Tropical Animal Health and Production* 52 (6): 3537–44. <https://doi.org/10.1007/s11250-020-02389-1>.