Seroprevalence and factors associated with Brucella infections in cattle in Tanganyika district, Katavi Region, Tanzania

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SUMMARY

Bovine brucellosis is a chronic infectious disease of cattle, which poses serious public health and economic impacts. It leads to reproductive failures such as abortion, retained placenta, and infertility. Humans contract the infection through occupational exposure or consumption of contaminated animal products. Brucellosis is endemic in Tanzania, however, its profile in terms of magnitude and distribution is limited. Tanganyika District is one of areas in Tanzania which lacks such information for control purposes. This study, therefore, was designed to determine the magnitude of brucella infection in cattle and assess the associated factors in Tanganyika District. A total 380 cattle sera were screened for brucella antibodies by Rose Bengal Plate Test and confirmed by Fluorescence Polarization Assay. A structured questionnaire was administered to 51 herd owners to assess the potential factors associated with brucella infection. Data were analysed by descriptive statistics and logistic regression. Results showed that out of 380 cattle sera tested, 24 (6.3%, 95%CI=4.3-9.2) were positive for brucella antibodies. Bull sharing (OR=5.2, 95%CI=1.6-17.1) and open disposal of foetal membranes (OR=6.4, 95%CI=1.2-32.5) were associated with sero-positivity. The odds of brucellosis in cattle were higher in farms where farmers were engaged in managing reproduction cases (OR=20.8, 95%CI=7.3-59.5). These findings highlight the presence of bovine brucellosis in Tanganyika district and its occurrence is associated with management practices such as bull sharing, direct contact with and improper disposal of foetal membranes. This call for targeted control strategies including regular screening, public awareness campaigns, and improved animal health management.

Keywords: Zoonosis, animal diseases, Tanganyika, Brucella, serology, abortion

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INTRODUCTION

Brucellosis is a contagious zoonotic disease that affects a wide range of domestic and wild animals, as well as humans. In cattle, the disease is primarily caused by *Brucella abortus* and is characterized by reproductive disorders such as abortion, retained placenta, orchitis, and infertility (Neta *et al*., 2010; Corbel, 2006). In humans, brucellosis can cause a febrile illness known as undulant fever, with symptoms including fatigue, muscle pain, and joint discomfort (Liu *et al*., 2024). The disease is considered a significant public health concern and a constraint to livestock productivity, especially in developing countries where control measures are limited (Khurana *et al*., 2021; Mengele *et al*., 2023).

Transmission of brucellosis in cattle mainly occurs through ingestion of contaminated materials such as aborted fetuses, placental membranes, and uterine discharges. Inhalation of aerosols and direct contact with infected animals or animal products are also

important routes of transmission (Kan and Zahoor, 2018). Risk factors associated with bovine brucellosis include herd size, age, sex, reproductive disease history, management practices, and contact with other animals or wildlife (Ali *et al*., 2017; Cárdenas *et al*., 2019; Assenga *et al*., 2015).

Serological tests remain the cornerstone for the diagnosis of bovine brucellosis due to the intracellular nature of the pathogen and challenges in direct detection. Commonly used diagnostic tests include the Rose Bengal Plate Test (RBPT), Complement Fixation Test (CFT), and Enzyme-Linked Immunosorbent Assay (ELISA) (Nielsen & Gall, 2001; Ashenafi & Duguma, 2016). Despite their usefulness, they have some limitations, including cross-reactivity and low sensitivity especially in chronic cases (Nielsen *et al*., 1998; Al-Majali *et al*., 2009).

Studies in Africa and Asia have reported varying prevalence rates of bovine brucellosis and identified multiple associated risk factors. For instance, research in Ethiopia (Jergefa *et al*., 2009; Asgedom *et al*., 2016), Nigeria (Akinseye *et al*., 2016), and Tanzania (Assenga *et al*., 2015; Ntirandekura *et al*., 2021) has shown that the disease is more common in areas where close interactions between livestock, wildlife, and humans are common. Similarly, studies in Cameroon (Awah-Ndukum *et al*., 2018), Kenya (Njuguna *et al*., 2017), Zambia (Muma *et al*., 2007; Mfune *et al*., 2021), and Bangladesh (Islam *et al*., 2021) support the influence of environmental and management-related risk factors on disease occurrence.

In Tanzania, bovine brucellosis remains an underreported disease, especially in rural areas where livestock are managed under extensive systems and contact with wildlife is frequent. The Katavi region, known for its livestock-wildlife interaction have limited data on the status of bovine brucellosis. Previous studies in this ecosystem have highlighted the need for more surveillance to understand the dynamics of *Brucella* infections and guide appropriate control measures (Assenga *et al*., 2015; Mengele *et al*., 2023).

This study aimed to determine the seroprevalence and risk factors of bovine brucellosis in Tanganyika district, Katavi region. The findings will contribute to the understanding of disease distribution in this area and therefore inform the responsible parties for control and prevention.

MATERIAL AND METHODS

Study area

This study was conducted in Tanganyika District, Katavi Region, Tanzania (Figure 1). This is among three districts of Katavi region, located in the western part of Tanzania that lies between longitude 30° to 33°31’33’’ E and latitudes 5° 15' to 7° 03' S. To the North the district is bordered with Urambo district (Tabora), East with Mlele district, South with Nkasi district (Rukwa), West with Lake Tanganyika, and to the North-West with Mpanda district (Katavi region). It has the human population of 371,836 and 85% of people are crop farmers and livestock keepers (URT, 2022). Cattle population is estimated at 498,382, in which 465,778 are indigenous, and 32,604 crossbreed. Other domestic animals kept include 73,273 goats, 4,576 sheep, 2,078 pigs, 318,789 chicken, 7,425 dogs and 861 donkeys (URT, 2020). The district has two main seasons, the wet season from November to April, and the Dry season from May to October.

Study design, population and sample size estimation

This study was cross-sectional in design, involving indigenous cattle aged over 6 months of age in four wards; namely, Mishamo, Mwese, Kapalamsenga, and Sibwesa. This form of active surveillance was conducted between November 2020 and October 2021, following claims of reproductive abnormalities and losses from farmers. From each ward one village was purposely selected based on presence of complaints about reproductive disorders. In each village households with cattle herds were selected randomly. Also, selection of animals within the herds was random, except in a few instances when young animals were to be conveniently included in the sample. Animals with less than one year of age were regarded as young and those above one year were considered as adults.

A sample size was determined using Cochran’s formula (Cochran, 1977) with assumed 50% prevalence of seropositivity.

Cochran’s Sample Size Formula (for Proportions):

$$n = \frac{Z^2 \, x \, p(1-p)}{e^2}$$

whereby;

Z-score for the 95% confidence level=1.96

e is the margin of error = 0.05

p is the assumed prevalence of seropositivity= 0.5

The sample size was 384, but four samples were discarded due to deterioration. In this study, therefore, 380 samples were analysed.

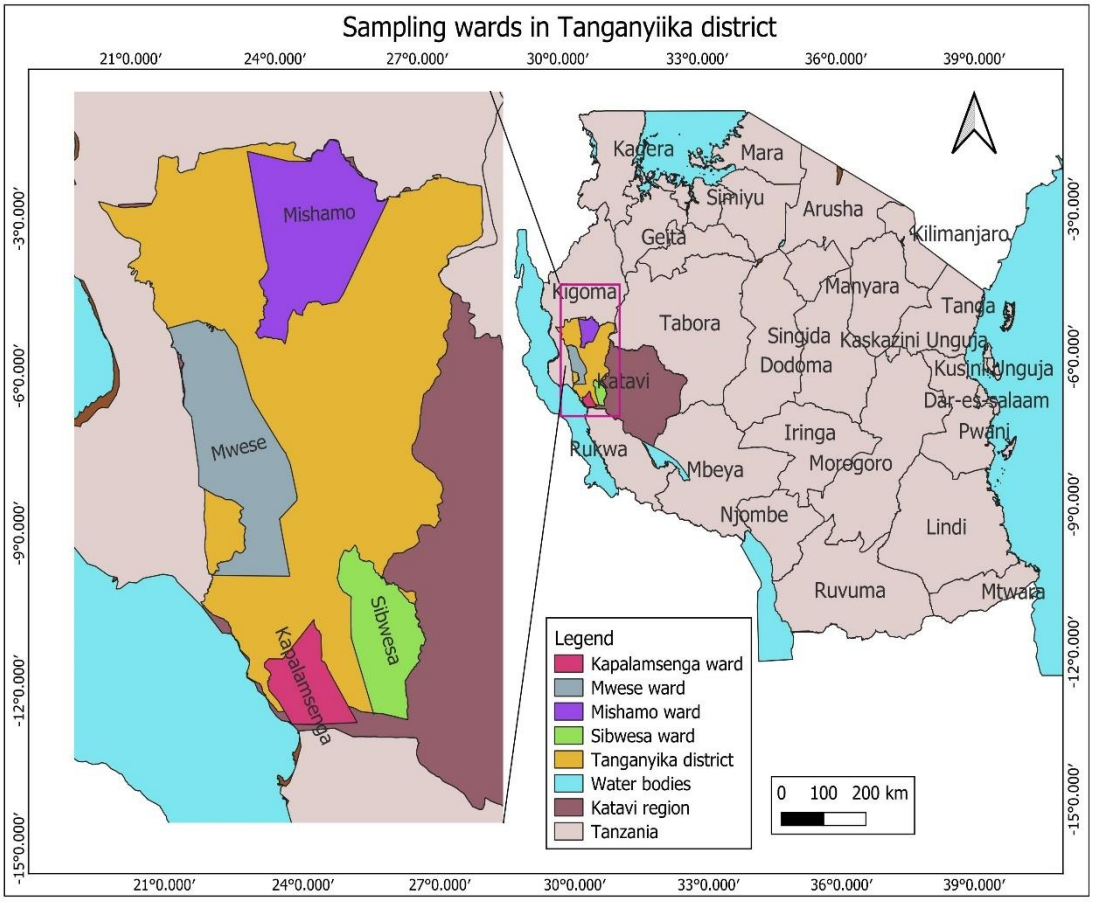

Figure 1: Study area: The map shows Tanganyika districts on the western part of Tanzania and the four wards where samples were collected: Mishamo, Mwese, Sibwesa and Kapalamsenga (The map was drawn Using QGIS 3.38 using public shape files).

Sampling

Five millilitres of blood samples were collected from the jugular vein using Plain vacutainer tubes. Blood was left to stand at room temperature for six hours and serum was separated by centrifugation. Sera were temporally stored at -20°C at Sumbawanga centre and then transported in ice packed cool box to TVLA Dar es salaam and stored at -80°C until testing.

Laboratory testing for brucellosis

Two diagnostic tests, the Rose Bengal Plate Test (RBPT) for initial screening and Fluorescence Polarization Assay (FPA) for confirmation, were used to detect brucellosis antibodies at the TVLA laboratory in Dar es Salaam.

Rose Bengal Plate Agglutination test

Rose Bengal Test was carried out using OIE, (2009) guidelines by using Standardized Brucella Antigen manufactured by Alpha Scientific Pty Ltd. Briefly, equal volumes of 30 µl from both serum sample and *Brucella* antigen were mixed together on a glass slide and manually agitated for 4 minutes. The reaction was compared to positive and negative controls. The presence of pink clumps was considered as tentative positive, while negative samples maintained a smooth, homogeneous pink suspension.

Fluorescence Polarization Assay

The samples positive for RBPT were tested with *Brucella* FPA for confirmation. The test was conducted as per manufacturer's instructions (Ellie LLC) by Fluorescence polarization Instrument (Sentry ®200, Ellie LLC). Sample diluent was prepared by mixing one part of 25X sample diluent with 24 parts of distilled water, heated up to 37°C and then brought to room temperature for use. Then 20 µl of samples and controls were pipetted into tubes (negative controls in triplicate). One millitre of the sample diluent were pipetted into all tubes mixed. and then incubated for 3-30 minutes at room temperature. Blank readings of all samples and controls were obtained. Then 10µl of Tracer was added into all tubes containing samples and controls, mixed

and then incubated for 3-5 minutes at room temperature. Then millipolarization (mP) readings of all samples and controls were obtained and recorded. Results were interpreted based on the following:

ΔmP= (Sample mP -Average Negative Control mP)

ΔmP <20= Negative

ΔmP >20-59= Positive

Collection of metadata and risk associated information

A semi-structured questionnaire was administered to livestock keepers whose animals were sampled. Biodata such as age (<1 year as young, ≥ 1 year as adult), sex, and herd management practices, and brucellosis awareness were collected. The completed data sheets were archived for analysis.

Data analysis

Data were processed using Microsoft Excel and Epi Info™ 7.2 for descriptive and inferential statistical analyses. The chi-square test was used to examine associations, and multivariable logistic regression identified risk factors for brucellosis. In logistic regression, the binary dependent outcome (sero-positive or sero-negative) was fitted against the predictors, namely; sex, age, season, management of brucellosis cases, bull sharing, brucellosis awareness, fetal material disposal method, vaccination status, cattle movement, feeding system, and animal housing. A univariate analysis was run and predictors with arbitrary p-value ≤ 0.2 qualified for multivariable logistic regression. A backward elimination method was used to build the final model at 0.05 significance level. Interaction terms between variables in the final model were tested.

RESULTS

Characteristics of sampled animals

The distribution of cattle samples shown in Table 1 revealed majority of cattle samples were collected from Kapalamsenga ward, followed by Mishamo, Mwese and Sibwesa. Also, the majority of the samples were from adult cattle. In terms of sex, there were more female cattle in the sample, than males.

Table 1: Sampling distribution based on wards, age and sex of animals

Variable	Frequency	%
Ward		
Sibwesa	11	2.9
Mishamo	25	6.6
Kapalamsenga	331	87.1
Mwese	13	3.4
Age		
Adult	315	82.9
Young	65	17.1
Sex		
Female	342	90.0
Male	38	10.0

Seropositivity of brucella antibodies in cattle

Out of 380 sera from cattle, 32 (8.42%) were positive on RBPT, and of these 32 RBPT positive sera, 24 were confirmed brucella positive by FPA. Therefore, the sero-prevalence of brucella antibodies in cattle in Tanganyika district was 6.3% (95% CI: 4.3 – 9.2). Twenty out of 24 positive samples (83.3%) were from females, while 23 (95.8%) were adult cattle.

Brucella seropositivity did not differ significantly among the sampling wards/ location (p=0.609), sex (p=0.282), age of animals (p=0.095) and seasons (P=0.414) (Table 2). However, although seasonality was not detected, monthly variation (Figure 2) of brucella infection seropositivity varied significantly (p=0.029, χ^2 =4.7). December recorded the highest percentage of positive cases (17.9%). In contrast, January, February, April, July and August had the lowest prevalence ranging from 2.4% to 3.3%.

Table 2: Sero-prevalence of brucellosis in cattle by season, age, sex and location (n=380)

Variable	Category	Frequency	Positive	% Positive	p value
Season	Rainy	191	14	7.3	0.414
	Dry	189	10	5.3	
Age	Adult	315	23	7.3	0.095
	Young	65	1	1.5	
Sex	Female	342	20	5.8	0.282
	Male	38	4	11	
Location	Kapalamsenga	332	20	6.0	0.609
	Mishamo	25	2	8.0	
	Mwese	12	1	8.3	
	Sibwesa	11	1	9.0	

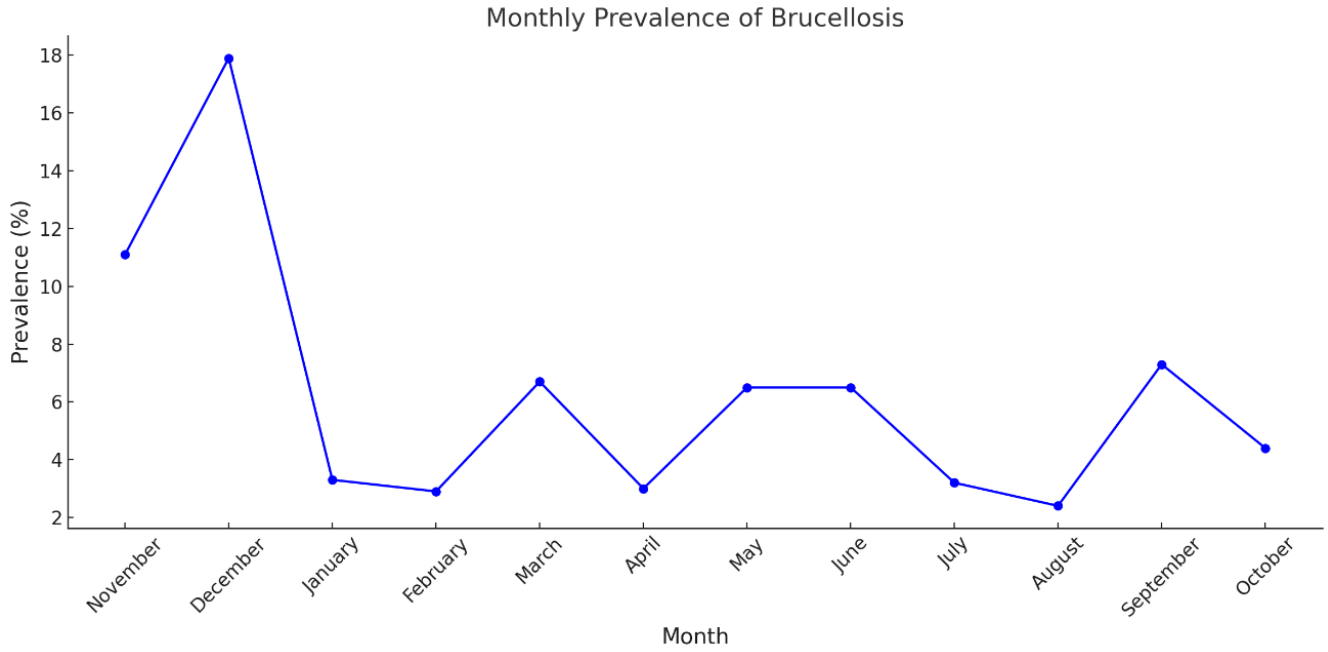

Figure 2: Monthly trend of brucellosis sero-prevalence in cattle in Tanganyika district

Risk factors for brucella infection seropositivity

Chi square test results in Table 3 shows that seropositivity for brucella infection was associated with farms that shared bulls (12%, 95%CI=7.5-17.9) compared to farms that did not share bulls (1.9%, 95%CI=0.5-4.7) ($p<0.0001$), farms that foetal membranes were disposed openly (8.1%, 95%CI=5.2-12.03) than burying (1.8%, 95% CI=0.2-6.5). Seropositivity was also high in farms where farmers were engaged in managing reproduction procedures (32%, 95%CI=18.6-47.6) which reflect farmers role on horizontal transmission of infection compared to when farmers were not engaged (3%, 95%CI=1.6-5.4). Similarly, farmers that had low awareness about brucellosis, their animals experienced higher

prevalence (7.9%, 95%CI=5.0-11.5) than those who were aware (1.9%, 95%CI=0.2-6.8).

Table3: Factors associated with seropositivity of brucella infection in cattle in Tanganyika District

Variable		Frequency	Seropositive	% Seropositive	95%CI	p-value
Involvement in managing brucellosis cases	Involved	44	14	**32**	18.61-47.58	P<0.0001
	Not involved	336	10	3	1.62-5.39	
Bull sharing	Shared	167	20	**12**	7.47-17.89	P<0.0001
	Not shared	213	4	1.9	0.51-4.74	
Awareness of Brucellosis	Aware	103	2	1.9	0.24-6.84	0.032
	Not aware	277	22	**7.9**	5.04-11.78	
Disposal	Burying	109	2	1.8	0.22-6.47	0.022
	Open disposal	271	22	**8.1**	5.16-12.03	

Based on multivariate logistic regression (Figure 3), the risk factors for brucellosis were bull sharing (OR=5.2, 95%CI=1.6-17.1, p=0.007), open disposal of foetal membranes (OR=6.4, 95%CI=1.2-32.5, p=0.03) and engagement in managing reproduction cases (OR=20.8, 95%CI=7.3-59.5, p <0.0001).

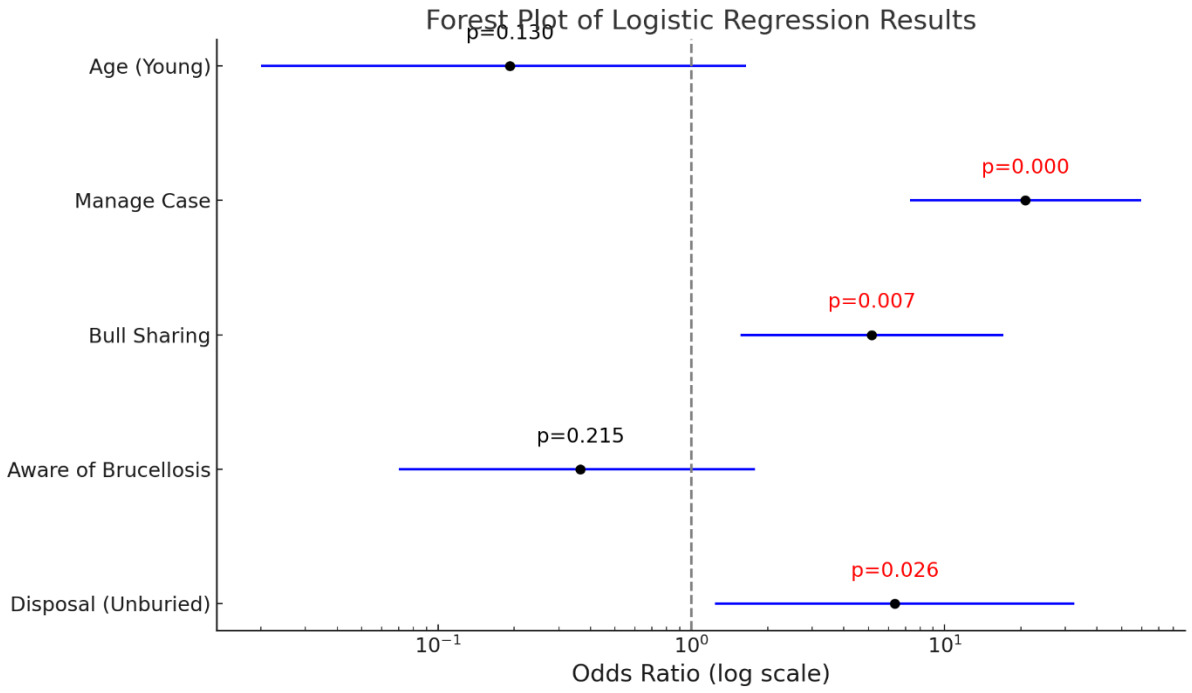

Figure 3: Multivariate logistic regression results showing risk factors for brucellosis in cattle in Tanganyika district

DISCUSSION

The study found 6.3% seropositive cattle to brucella infections in Tanganyika cattle herds, suggesting the economic and public health threat of the disease in the study area. Our results are comparable to other studies from different regions in Tanzania. For example, Asenga *et al*. (2015) found a prevalence of 6.8% in Katavi, which was among the earlier studies that focused on the disease's distribution in the country. With regard to more recent studies, Sagamiko *et al*. (2018) observed a prevalence of 11.3% in Mbeya, Tanzania, which shows an increase from previous findings. This might reflect changes in animal management practices or an improvement in diagnostic techniques that have allowed for the detection of more cases. In Kagera, Tanzania, Ntirandekura *et al*. (2021) found a prevalence of 5.9%, indicating that brucellosis remains endemic across various Tanzanian regions, albeit with varying prevalence levels depending on the area.

The results also align with studies conducted in other countries such as Ethiopia (Negash and Dubie 2021) and in Pakistan (Arif *et al*., 2019) where a prevalence of 11.9% and 8.7% respectively were reported, suggesting that brucellosis is widespread in both African and South Asian cattle populations. This may further suggest common factors like livestock management and environmental conditions that may influence the prevalence rates (Dadar and Godfroid, 2021; Lyimo *et al*., 2024). These differences in prevalence in different studies may probably be due to several factors such as sample sizes, sampling techniques, different diagnostic tests and interpretations.

The present study highlights the association between involvement of farmers in managing reproductive disorders and the prevalence of brucellosis. This finding is in agreement with previous reports (Arif *et al*., 2017 and Al-Majali *et al*., 2009) in which poor case management such as poor disposal of aborted fetus, placenta and other uterine materials, and poor veterinary services were associated with high prevalence of brucellosis. High prevalence of the disease during managing brucella cases might be associated with failure to proper dispose infected tissues, aborted fetuses, or contaminated bedding that can lead to environmental contamination and spread of brucellosis to other animals in the herd or to nearby farms (Robi *et al*., 2021).

Sharing bulls between herds was found to be a risk factor for disease transmission, having five times more likelihood of contracting the infection. This finding was similar to the finding by Cárdenas *et al*., (2019) and Muma *et al*., (2007) who mentioned that sharing of bulls was associated with seropositivity of the disease in the farm, hence a risk factor. Lack of awareness about the disease is a risk factor for disease transmission as we found that animals owned by informed farmers have significantly lower odds of contracting the disease. This finding is similar to the one by Islam *et al*. (2021) who also reported that awareness and knowledge on Brucellosis was related with positive cases. This means that farmers were not taking necessary precautions when handling brucella infected animals, their products or by-products and by so doing, they propagate the disease to uninfected animals.

Similarly, hygienic disposal of reproductive wastes was a preventive measure to brucella infections in cattle. Prevalence was lower in farms where wastes are buried as compared to when the wastes were exposed, either through giving them to dogs, throwing to the bushes or surrounding farms. In addition, the results indicate that safe disposal practices, such as burying wastes, were associated with a significantly lower risk of contracting brucellosis. This finding is similar to the one by Vinueza *et al*. (2023) who reported that incineration/burial of aborted materials were a protective factor compared to when these materials are not incinerated/buried, or left in the pastures or given to farm dogs. Njuguna *et al*. (2017) documented that placenta of an infected animal and products of abortion contains large number of brucella organisms, and transmission occurs by oral ingestion or following exposure to fetal tissues, vagina discharges, aborted fetus and placenta containing brucella organisms. Tendency of cattle to lick carcasses and vaginal discharge, and mechanical transmission role played by dogs on aborted material across the ground increase further the spread of disease.

It is concluded that brucella infections are circulating in cattle herds in Tanganyika district, Katavi region and poor management practices related to bull sharing, poor disposal of foetal materials and farmer's involvement in managing dystocia cases are strongly associated with seropositivity among the sampled cattle

LIMITATION OF THE STUDY

The study was limited to only one region. The results may not reflect the wider status of brucella infection. Also, archived samples may have contributed to low positivity.

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