Seroprevalence and Associated Risk Factors of Bovine Brucellosis in Cattle in Tabora Region, Tanzania

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SUMMARY

Bovine brucellosis is a zoonotic disease that causes substantial economic losses in livestock production and poses a serious public health threat. Assessing its prevalence and associated risk factors is critical for designing effective control strategies in Tanzania. A retrospective study was conducted in the Tabora region using 618 archived cattle serum samples collected from eight district councils. Initial screening for brucellosis was performed using the Rose Bengal Test (RBT) followed by confirmation using Fluorescence Polarization Assay (FPA). Data on cattle characteristics including district of origin, breed, age, sex, and farming system were retrieved and analyzed. Chi-square tests and odds ratios were used to quantify strength of associations between potential risk factors and the seropositivity, with a significance level of $p<0.05$. The overall apparent seroprevalence of bovine brucellosis based on FPA was 5.1% (95% CI= 3.5 – 7.1, 31/618). The highest seroprevalence was observed in Uyui district (8.2%; 95% CI= 2.7 – 18.1) while the lowest was in Nzega DC (3.0%; CI=0.6 – 8.4). There was no significant statistical association between risk factors (age, breed, farming system and district) and seroprevalence. Agreement assessment between RBT and FPA assays yielded $\kappa = 0.907$, indicating a very good agreement. The findings confirm that *Brucella* spp. is endemic, circulating at relatively low levels among cattle herds across all districts of the Tabora region. The absence of significant associations with specific risk factors suggests that control strategies should adopt a holistic approach, addressing multiple dimensions of infection. Recommended measures include testing and culling of reactors, farmer education, vaccination, and improved livestock husbandry practices, particularly through the implementation of biosecurity measures

Keywords: Risk factors, zoonosis, Fluorescence Polarization Assay, Kappa Statistic, abortion.

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INTRODUCTION

Brucellosis is a contagious zoonotic bacterial disease caused by several *Brucella* species. In cattle, *Brucella abortus* is the primary pathogen, whereas *Brucella melitensis* affects sheep and goats, *Brucella suis* infects pigs, and *Brucella canis* is found in dogs (Ntirandekura *et al.*, 2020; Mengele *et al.*, 2023). In cattle, the disease leads to abortion, retained placenta, infertility, reduced milk production, and the birth of weak or stillborn calves. Transmission among animals occurs through direct contact with infected fetal

membranes, aborted fetuses, or vaginal secretions, as well as via infected bulls or contaminated semen during artificial insemination. Indirect transmission may result from ingestion of contaminated feed and water, contact with infected materials, or inhalation of *Brucella* contaminated aerosols (Ntirandekura *et al.*, 2020; Mengele *et al.*, 2023). In humans, infection is acquired through consumption of contaminated milk and dairy products, raw or undercooked meat, direct contact with infectious materials such as placentas, or inhalation of contaminated aerosols (Khurana *et al.*, 2021).

In humans, brucellosis presents with diverse clinical signs, including intermittent fever, malaise, anorexia, prostration, and other nonspecific symptoms. Its impacts extend beyond health, encompassing treatment costs, reduced milk production, abortions, diminished fertility, and loss of trade all of which directly affect pastoral and agropastoral communities. The disease is endemic across much of Tanzania, with documented cattle seroprevalence ranging from 0.7% to 58.1% and herd infection rates reaching up to 89.4% in Kasulu District (Swai *et al.*, 2021; Ukita *et al.*, 2021; Mengele *et al.*, 2023). Human prevalence has been reported to range between 0.6% and 58.1% (Nonga and Mwakapeje, 2016; Mengele *et al.*, 2023), whereas wildlife prevalence has been documented at 10.5% to 24% (Sambu *et al.*, 2021). Nevertheless, data on the epidemiological status of brucellosis in the Tabora region remains scarce, underscoring the need for further investigation.

Cattle production in the Tabora Region is predominantly based on the Tanzanian Short Horn Zebu (TSHZ), with Ankole, Boran, and Sahiwal breeds also present in certain areas under extensive grazing systems. The region hosts an estimated 2,841,191 cattle (National Sample Census of Agriculture, 2019/20). Herds frequently intermingle in communal grazing areas, sharing watering points and breeding bulls, practices that facilitate disease transmission. Approximately 80% of the region's land is reserved for forests and wildlife, increasing opportunities for contact between livestock and wild animals and thereby heightening the risk of cross-species disease transmission. A limited number of improved dairy crossbreeds, such as Friesian, Ayrshire, and Jersey, are raised in urban and peri-urban areas under intensive and semi-intensive feeding systems.

The absence of routine vaccination programs against brucellosis further exacerbates the problem. As brucellosis is a zoonotic disease transmitted through consumption of livestock products and given that the Tabora Region's 3,391,679 residents rely almost entirely on locally produced meat and milk, ensuring food safety is imperative. In pastoral and agropastoral communities, more than 85% consume raw or undercooked meat (Ngasala *et al.*, 2015). Moreover, reports indicate frequent cases of abortion during the last trimester, infertility, and retained placenta among cattle from different herds and owners (Machibywa, R.; Tabora Regional Veterinary Officer, personal communication). These observations underscore the urgent need for studies to determine the prevalence and status of brucellosis in the region.

MATERIALS AND METHODS

Study area

The study was conducted in the Tabora Region of Tanzania, covering the District Councils (DC) of Igunga, Nzega, Uyui, Sikonge, Urambo and Kaliua as well as Nzega Town Council (TC) and Tabora Municipal Council (MC) (Figure 1). Tabora lies in the central-western part of the country on the central plateau, between approximately 4°30′ S and 6°30′ S latitude and 30°20′ E to 33°50′ E longitude, at an elevation of 1,000–1,500 meters above sea level. The region experiences a subtropical climate moderated by altitude, with a rainy

season from November to April and a dry season from May to October. Average temperatures range from 22°C in July (the coldest month) to 26°C in October (the warmest month), with annual precipitation averaging 34.6 inches. Tabora hosts an estimated 2,841,191 cattle (National Sample Census of Agriculture, 2019/20) and a human population of 3,391,679 (Population and Housing Census, 2022).

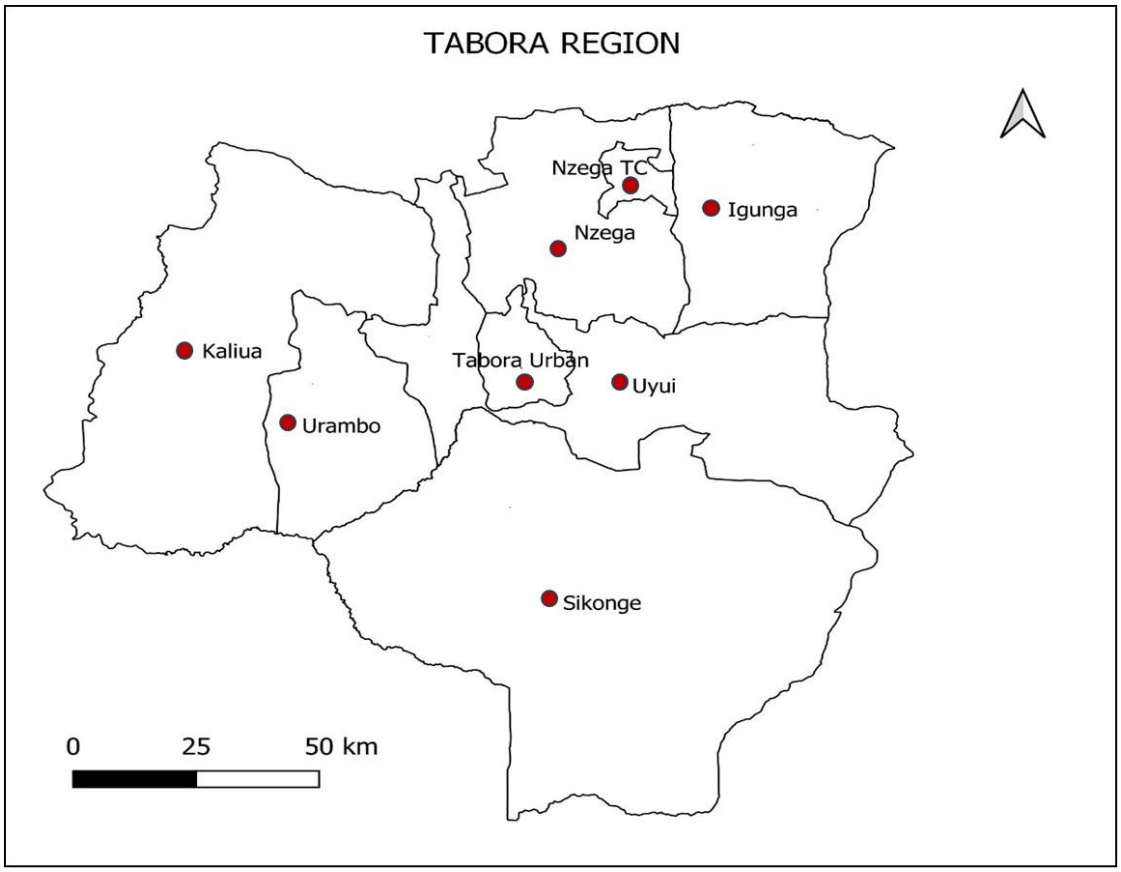

Figure 1 A map of Tabora region showing the eight (8) named study districts with red dots. Source QGIS 3.16

Study design, sample size and study population

A retrospective study was conducted in the Tabora Region, Tanzania, utilizing 618 archived cattle serum samples collected during disease surveillance programs by the Tanzania Veterinary Laboratory Agency (TVLA) and the Zonal Veterinary Centre (ZVC) over a four-year period (July 2020–June 2024). Information on district of origin, breed, age, sex, and farming system was also retrieved. Records indicated that cattle farms were visited as part of active disease surveillance undertaken by TVLA. Farms were purposively selected based on accessibility and farmers' willingness to participate, with verbal consent obtained. None of the cattle sampled in Tabora had a history of brucellosis vaccination.

Serum sample data retrieved

Serum sample information retrieved included district of origin, breed, age, sex, and farming system. Cattle aged ≤12 months were classified as young, while those older than 12 months were considered adults (FAO, 1994). The study areas included two urban districts Tabora MC and Nzega TC, and six rural districts: Igunga DC, Nzega DC, Uyui DC, Sikonge DC, Urambo DC, and Kaliua DC. Breeds were classified as local or dairy

crossbreeds, sex as male or female, and farming systems as semi-intensive or extensive.

Laboratory sample analysis

All serum samples were tested for antibodies against *Brucella* spp. Initial screening was performed using the Rose Bengal Test at the TVLA Tabora Center. Subsequently, all samples were sent to the TVLA Mwanza Center for confirmation by fluorescence polarization assay (FPA).

Rose Bengal Test (RBT)

Serum samples, antigens, and both positive and negative controls were allowed to reach room temperature (25°C) after removal from refrigeration. One drop (30 µL) of test serum, positive control, and negative control was placed in separate circles on a test tile. To each, 30 µL of Rose Bengal antigen was added and mixed thoroughly. The tile was then gently rotated for 4 minutes. Agglutination was observed immediately after the rotation period under adequate lighting and results were recorded (Legesse *et al.,* 2023).

Fluorescence Polarization Assay (FPA)

All reagents (buffer, fluorescent antigen, controls, and samples) were equilibrated to room temperature (25°C) and thoroughly mixed to prevent bubble formation. The FPA assay was done as earlier described (Nielsen and Gall, 2001) using the Sentry Fluorescence Polarisation Analyser (Diachemix Sentry TM 100, single tube reader, Diachemix LLC, Wisc. USA). A fixed volume (20 µL) of serum sample, positive control, and negative control was dispensed into separate wells. An equal volume (20 µL) of fluorescent labeled *Brucella* antigen was added to each well. The mixture was gently mixed and incubated for 2 minutes at room temperature (25°C). The wells were then placed into the FPA analyzer/fluorometer for measurement. Fluorescence polarization values (millipolarization units, mP) were recorded, and sample mP values were compared against the cut-off determined by the controls, in accordance with the manufacturer's kit protocol. High mP values indicated antibody binding (positive), whereas low mP values indicated absence of antibodies (negative). Results were obtained directly from the analyzer readings and recorded accordingly (Nielsen & Gall, 2001).

Data management and analysis

Retrospective data were entered into Microsoft Excel for cleaning and verification of completeness, accuracy, and consistency. Descriptive statistics, including percentages, frequencies, medians, and means, were used to summarize the data. Statistical analysis was conducted using Epi Info version 7.2.6.0. The Chi-square test was employed to evaluate differences in seroprevalence across district of origin, breed, age, sex, and farming system. All statistical analyses were conducted at a 5% significance level.

Based on the FPA results, seroprevalence was assessed at the individual animal level. Agreement between RBPT and FPA in the diagnosis of bovine brucellosis was evaluated using Cohen's Kappa coefficient (κ). Interpretation of the Kappa value was done as follows: poor (κ = < 0.20), fair (κ = 0.21 - 0.40), Moderate (κ = 0.41 - 0.60), good (κ =0.61 - 0.80) and very good (κ = 0.81 - 1.00) (Altman, 1991).

Ethical clearance

Sokoine University of Agriculture (SUA) granted a research clearance through a letter with reference number SUA/DPRTC/MPVM/D/2024/0007/01.

Ethical clearance was granted by Tanzania Livestock Research Institute (TALIRI) with Ref. NO. TLRI/CC.21/078 and TVLA agreed on the use of their archived cattle serum samples.

RESULTS

Overview of demographic information of study cattle

Over a four-year period, 618 cattle were sampled and recorded at TVLA Tabora, as summarized in Table 1. The majority originated from Tabora Municipality (18%; 111/618). Most sampled cattle were of local breeds (90%; 556/618), female (90.6%; 560/618), adult (79.4%; 491/618), and predominantly managed under extensive farming systems (90%; 556/618).

Table 1 Distribution of samples collected in Tabora region by potential variables (n=618)

Parameter	Category	No. of cattle sampled	Percentage
District	Tabora MC	111	18.0
	Nzega DC	101	16.3
	Igunga	90	14.6
	Sikonge	83	13.4
	Urambo	67	10.8
	Uyui	61	9.9
	Nzega TC	58	9.4
	Kaliua	47	7.6
Breed	Local	556	90.0
	Dairy cross	62	10.0
Sex	Female	560	90.6
	Male	58	9.4
Age	Young	127	20.6
	Adult	491	79.4
Farming system	Extensive	556	90.0
	Semi-extensive	62	10.0

Seroprevalence of brucellosis in cattle across eight districts of Tabora Region

The overall seroprevalence of brucellosis in cattle, as determined by FPA, was 5.1% (95% CI: 3.6–7.0; n = 618). The highest seroprevalence was observed in Uyui district (8.2%; 95% CI= 2.7–18.1) while the lowest was in Nzega DC (3.0%; CI=0.6 – 8.4) as shown in Table 2.

Association between district, age, breed, and farming system and brucellosis seroprevalence in cattle

Table 3 shows the association between district, age, breed, and farming system and brucellosis seroprevalence in cattle. Higher seroprevalence was observed among cattle from rural districts, adults, dairy crosses, females, and those managed under semi-intensive systems. However, these differences were not statistically significant ($P > 0.05$), suggesting that location, age, breed, and farming system were not associated with an increased risk of Brucella infection (Table 3).

Table 2 Seroprevalence of brucellosis in cattle across eight districts of Tabora Region

District	Categorization	95% confidence interval (CI)	Number infected	Percent
Uyui DC n=61)	Rural	2.7 – 18.1	5	8.2
Igunga (n=90)	Rural	2.5 - 14.0	6	6.7
Urambo DC (n=67)	Rural	1.7 – 14.6	4	6.0
Nzega TC (n=58)	Urban	1.1 – 14.4	3	5.2
Tabora MC (n=111)	Urban	1.5 – 10.2	5	4.5
Kaliua (n=47)	Rural	0.5 – 14.5	2	4.3
Sikonge DC (n=83)	Rural	0.8 – 10.2	3	3.6
Nzega DC (n=101)	Rural	0.6 – 8.4	3	3.0
Total	8	3.6 – 7.0	31	5.1

Table 3 Seroprevalence of brucellosis in cattle in relation to location, age, breed and farming system

Variable	Category	No. of cattle tested	No. positive	Seroprevalence (%)	OR	95% CI	P value
District	Rural district (6)	449	23	5.1	0.9	0.5 – 2.4	0.8436
	Urban districts (2)	169	8	4.7			
Age	Adult	491	27	5.6	0.5	0.6 – 5.3	0.2309
	Young	127	4	3.1			
Breed	Dairy cross	62	4	6.6	0.7	0.5 – 4.0	0.5613
	Local	556	27	4.9			
Sex	Female	560	29	5.2	0.7	0.4 – 6.6	0.5655
	Male	58	2	3.5			
Farming system	Extensive	556	27	4.9	0.4	0.5 – 4.0	0.586
	Semi-intensive	62	4	6.5			

Note: OR = odds ratio; CI = confidence interval

Agreement between RBT and FPA tests

The Kappa statistic for assessing agreement between RBT and FPA was estimated at 0.907 (standard error = 0.038; 95% CI: 0.833–0.981), indicating very good agreement between the two tests. Overall, 37 cattle tested positive with the Rose Bengal Test, while 31 cattle tested positive with the Fluorescence Polarization Assay (Figure 2).

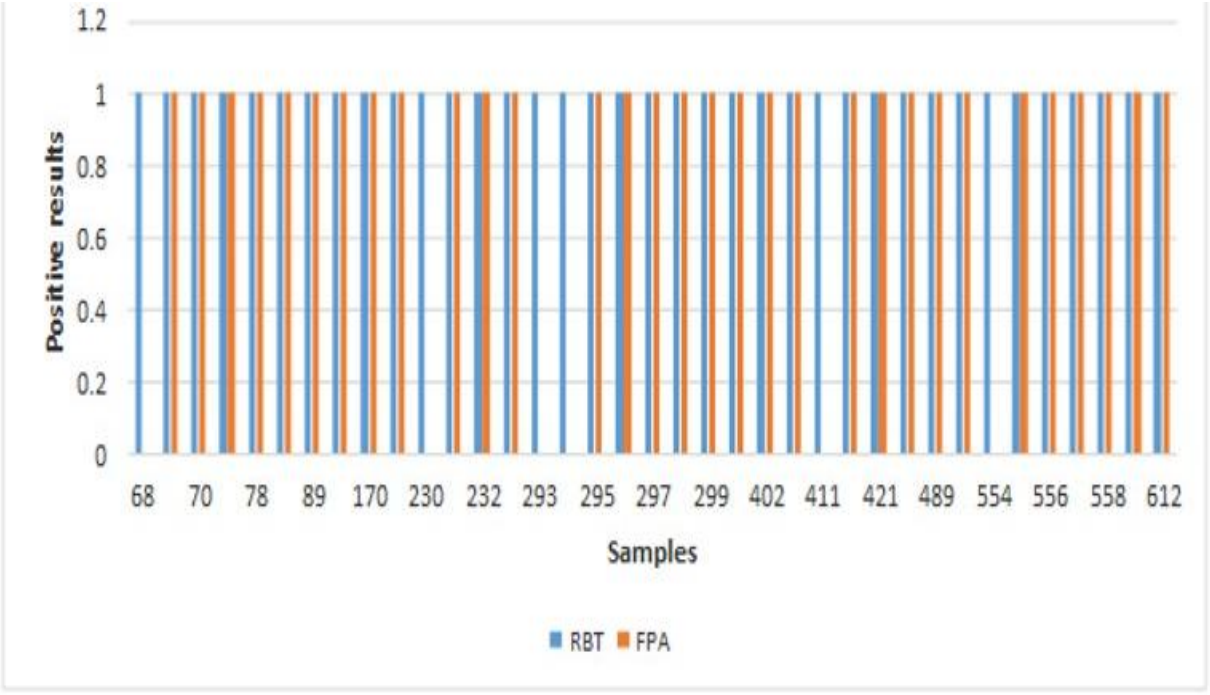

Figure 2 Shows the level of agreement between the RBT and FPA. The bar chart, shown in blue and orange, illustrates the samples that tested positive with the RBT and FPA, respectively. Of the 37 cattle that tested positive with the RBT, 31 were also confirmed positive by the FPA. Samples numbered 68, 230, 293, 294, 411, and 554 display only blue bars, indicating positivity for RBT alone.

DISCUSSION

The overall seroprevalence of bovine brucellosis in the Tabora region was 5.02%, with Nzega District Council recording the lowest prevalence (3.0%) and Uyui District Council the highest (8.2%). These findings place Tabora within the range of previously reported prevalence in Tanzania, which varies widely from 0.7% to 58.1% (Swai *et al.*, 2021; Ukita *et al*., 2021; Mengele *et al*., 2023). This moderate prevalence confirms that *Brucella* spp. is endemic and actively circulating within the cattle population of the region. Given that Tabora hosts over 2.84 million cattle, the largest population in Tanzania (National Sample Census of Agriculture, 2019/20), a 5.02% seroprevalence represents a substantial *Brucella* reservoir. This poses a persistent zoonotic threat to livestock keeping communities, particularly through the consumption of raw milk and dairy products, as well as direct contact with aborted materials (Godfroid *et al.*, 2011). Moreover, the seasonal migration of agro-pastoralists in search of pasture and water facilitates the wider transmission of brucellosis across regions. These findings underscore the urgent need to integrate animal and human health interventions within the One Health framework to mitigate the impact of brucellosis in Tabora and nationwide.

The current study did not detect significant variation in seroprevalence across the eight districts of Tabora. Although apparent prevalence ranged from 3.0% in Nzega DC to 8.2% in Uyui DC, these differences were not statistically significant. This uniformity suggests that the risk of *Brucella* exposure is not confined to localized hotspots but represents a region-wide challenge. In other words, across all districts of Tabora, the factors influencing transmission and the likelihood of exposure appear broadly similar. This pattern points to widespread, common risk factors operating across district

boundaries. The predominance of extensive grazing systems in Tabora characterized by herd mixing at water points and grazing areas, as well as seasonal cattle migration in search of pasture likely facilitates efficient transmission of *Brucella* throughout the region (Musallam *et al.*, 2015). Furthermore, the practice of illegally grazing cattle in wildlife protected areas may expose livestock to wildlife reservoirs such as buffalo (Sambu *et al.*, 2021). The absence of significant clustering underscores the need for control measures to be implemented at a regional scale, rather than targeting only selected districts, to effectively reduce the burden of brucellosis in Tabora.

No significant difference in seroprevalence was observed between crossbred dairy cattle (n = 62) and local breeds (6.5%). This finding is inconclusive but biologically plausible, as dairy crossbreeds such as Friesian and Ayrshire are often more susceptible to infectious diseases due to limited genetic adaptation compared to local breeds (Ntirandekura *et al.*, 2020). However, the very small sample size of crossbred animals (n = 62) likely limited the statistical power to detect a true difference, representing a classic case of type II error. Thus, while this result does not exclude a potential breed effect, it highlights the need for larger, more targeted studies to properly investigate this relationship.

The higher prevalence observed in adult cattle can be explained by the cumulative exposure over time and the activation of infection during pregnancy (Glynn and Lynn, 2008; Lyimo *et al.*, 2024). The lack of statistical significance (p = 0.278) may, however, be influenced by the relatively small sample size of young animals included in this study, which limited the power to detect a true difference.

The higher prevalence observed in female cattle (5.2%) is consistent with the pathophysiology of brucellosis, which exhibits tropism for reproductive organs and is frequently associated with abortion and placental retention in females (Khurana *et al.*, 2021). The very low number of positive males (n = 2) limited the robustness of statistical comparisons, resulting in a wide confidence interval for the odds ratio (0.4–6.6) and a non-significant p-value (0.552).

The study found no significant difference in *Brucella* seroprevalence between cattle raised under extensive and semi-extensive systems (p = 0.586). The slightly higher prevalence observed in semi-extensive systems was unexpected, as more intensive management is generally associated with improved biosecurity. This counterintuitive result may be explained by the small sample size from semi-extensive farms (n = 62) or by confounding factors such as higher animal density or the introduction of infection through purchased feed or animals. Additionally, the common practice of farmers sharing breeding bulls across households or neighboring farms when cows are in heat may contribute to transmission. These findings warrant further investigation with a larger sample size and more detailed management data.

Regarding serological test comparison, very good agreement was observed between RBT and FPA ($\kappa = 0.907$) (Altman, 1991). This indicates a strong correspondence between high RBT scores and positive FPA results, suggesting that sera with high RBT scores may not require confirmation with additional tests such as competitive ELISA or complement fixation test (CFT). Our findings are consistent with those of Paulin et al. (2012) and Montagnaro et al. (2007), who reported a Kappa coefficient of 0.8. However, Muma et al. (2009) reported lower agreement, and Falzon et al. (2019) observed only moderate intra-test agreement ($\kappa = 0.41$). Such variations may reflect differences in test repeatability across laboratories, equipment, and personnel.

Study limitations

The most significant limitation of this study is the reduced statistical power resulting from the low number of positive cases (n = 31) and small subgroup sample sizes, which greatly increases the risk of Type II errors and likely explains the absence of statistically significant associations. The cross-sectional design, based on archived serum samples, further limits causal inference and may underestimate true prevalence due to potential antibody degradation over time. In addition, the analysis was constrained by the absence of herd-level data critical for understanding brucellosis epidemiology alongside incomplete risk factor information (e.g., abortion history, animal movements) and potential selection bias introduced by the non-random, purposive sampling method. Collectively, these limitations restrict the generalizability of the findings to the broader cattle population of the Tabora region.

Conclusions and recommendations

This study demonstrates that bovine brucellosis is prevalent and widely distributed across the Tabora region. This reflects a generalized risk environment and underscores the continuing need for robust control measures. Given the uniform distribution, control programs such as vaccination should be implemented at the regional level rather than targeting specific hotspots. Molecular studies are needed to identify the circulating *Brucella* species and strains, such as *B. abortus* and *B. melitensis*.

CONFLICT OF INTEREST

Authors have no conflict of interest regarding the issues presented in this article.

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