EISSN: 2707-0425

East African Journal of Science, Technology and Innovation, Vol. 6 (4): September 2025

Status of brucellosis in a dairy cattle herd with history of abortion in Morogoro District, Tanzania

* [1]RAZARASOANANTENAINA, S., [1]EMMA, P. N., [2]COLETHA, M. M.

[1] Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Science, Sokoine University of Agriculture, P.O Box 3019, Morogoro, Tanzania
[2] Department of Veterinary Anatomy and Pathology, College of Veterinary Medicine and Biomedical Science, Sokoine University of Agriculture, P.O Box 3016, Morogoro, Tanzania

*** Corresponding author:** nantenainasydonie83@gmail.com

Abstract

Bovine brucellosis, caused by *Brucella* bacteria, is a zoonotic disease with major public health and economic impacts, including decreased milk production and reproductive losses such as abortion. It can spread through milk consumption, posing health risks to humans. This study aimed to investigate the presence of *Brucella* infection in a dairy herd with a history of abortions and the risk practices associated with bovine brucellosis. A cross-sectional study, conducted from January to March 2024, investigated a dairy herd of 36 cattle, with 47.22% (17/36) identified as adult cows. Milk, blood, and serum samples were collected. Cattle over six months old (n=31) were tested for brucellosis using both the Rose Bengal Plate Test (RBPT) and competitive ELISA (c-ELISA), with both tests performed on the same samples. *Brucella* DNA was detected using Real-time Polymerase Chain Reaction (qPCR) targeting the IS711 insertion sequence. Specific genes, BruAB_0168 and BMEII0466, were amplified to identify *Brucella abortus* and *Brucella melitensis*, respectively. Cohen's Kappa statistic was used to assess the level of agreement between RBPT and c-ELISA results. A focus group discussion used to assess potential risk practices associated with brucellosis focusing on abortion history, handling and disposal of aborted materials, management of aborting and sick cows, contact with other livestock and wildlife, routine health monitoring, and biosecurity practices. As the study targeted a single farm, potential risk practices were evaluated qualitatively rather than statistically. Serological testing revealed *Brucella* exposure in the herd, with 41.94% (13/31) testing positive using the RBPT and 74.19% (23/31) testing positive using c-ELISA. qPCR identified *Brucella* DNA in three out of 36 serum samples, with one sample specifically confirmed as *Brucella abortus*. These findings confirm *Brucella* infection in the herd. A comprehensive reproductive health strategy and ongoing monitoring are needed to improve herd health and reduce economic losses.

Key words: *Bovine brucellosis; Brucella DNA detection; Seroprevalence, Zoonosis.*

Cite as: Sydonie et al. (2025): Status of brucellosis in a dairy cattle herd with history of abortion in Morogoro District, Tanzania. *East African Journal of Science, Technology and Innovation 6(4).*

Received: 31/ 2 /25
Accepted: 17/07 25
Published: 30/09/2

Introduction

Brucellosis, a zoonotic infection caused by bacteria belonging to the Brucella genus, affects a diverse group of animals, ranging from domestic species like cattle, sheep, and goats, to wildlife such as bison, elk, and marine mammals. This disease poses substantial economic losses and public health concerns globally (Corbel, 2006; Godfroid *et al.,* 2014). Species of Brucella are gram-negative, facultative intracellular bacteria, categorized under the Brucellaceae family in the α-2 subdivision of Proteobacteria (Xiang *et al.,* 2006). Based on phylogenetic studies, ten Brucella species have been known to exist, including B. abortus, which affects cattle, *B. melitensis* in sheep and goats, and *B. suis* in pigs. Other species such as *B. canis*, *B. ovis*, and *B. neotomae* infect canines, ovines, and desert woodrats, respectively. Marine mammals like dolphins and seals host B. ceti and *B. pinnipedialis*, while *B. microti* and *B. inopinata* have been associated with voles and humans (de Figueiredo *et al.,* 2015; El-Sayed and Awad, 2018). Among these, *B. melitensis*, *B. abortus* and *B. suis* are the primary species linked to human disease. Human infections due to *B. canis* are rare, while no such cases have been documented with *B. ovis* or *B. neotomae* (Kılıç *et al.,* 2021). The ability of newly identified Brucella species recognized as a source of infection remains largely unexplored (El-Sayed and Awad, 2018).

Brucellosis often manifests as reproductive issues in livestock, including abortions, retained placenta, fetal death, genital infections, and sterility (Kubuafor *et al.,* 2000; Probert *et al.,* 2004; Corbel, 2006; Godfroid *et al.,* 2011a; Megersa *et al.,* 2011). These reproductive problems undermine the productivity and profitability of dairy farms and pose substantial health risks to humans who interact with diseased animals or consume contaminated animal products (Pappas *et al.,* 2006). The economic impact of abortion caused by Brucella infections is profound, with estimates reaching approximately US $3.4 billion annually in the animal husbandry sector in India (Singh *et al.,* 2015).

In humans, naturally occurring brucellosis is mostly derived from animal reservoirs, with only sporadic transmissions from person to person known to exist (Godfroid *et al.,* 2005). The disease spreads through direct or indirect contact with infected excretions, including urine, milk, and placental tissues (Zvizdić *et al.,* 2006; Otlu *et al.,* 2008). Despite global efforts to control and manage bovine brucellosis, the disease remains endemic in various regions, including parts of Africa (Pappas *et al.,* 2006). In Tanzania, a meta-analysis by Alonso *et al*. (2016) revealed that the proportion of bovine brucellosis overall was 8.2% [95% CI: 6.5-10.2]. In Mbeya, Sagamiko *et al*. (2018) further reported an overall seroprevalence of 9.3% at the animal level, with notable variation between indigenous (11.3% [95% CI: 9.4–13.5]) and exotic cattle (2.8% [95% CI: 1.4–5.6]), and a herd-level prevalence of 32.0%. In Morogoro Municipality, a seroprevalence of 21.3% (95% CI) according to c-ELISA was noted in smallholder dairy farms. Additionally, Weinhäupl *et al*. (2000) reported prevalence of 14.1% in Dar es Salaam, 12.3% in Lugoba, and 6.8% in the Katavi-Rukwa ecological zone (Assenga *et al.,* 2015). Within the boundaries of Mvomero district, brucellosis prevalence at the level of the farm was 52.9% [9/17 farms, 95% CI 28.5–76.1%], with the prevalence at the animal level of 7.0% [28/673 cattle, 95% CI 5.7–8.4%] (Ukita *et al.,* 2021).

While bovine brucellosis at the farm level is well-documented in Morogoro, comprehensive studies investigating Brucella spp. in dairy cattle herds experiencing abortions are limited. This case study, conducted in a single dairy cattle herd in Mvogogo village, Mikese ward, aimed to bridge this knowledge gap by applying molecular techniques to look into whether Brucella species are present as a potential causative agent of abortion.

Materials and methods

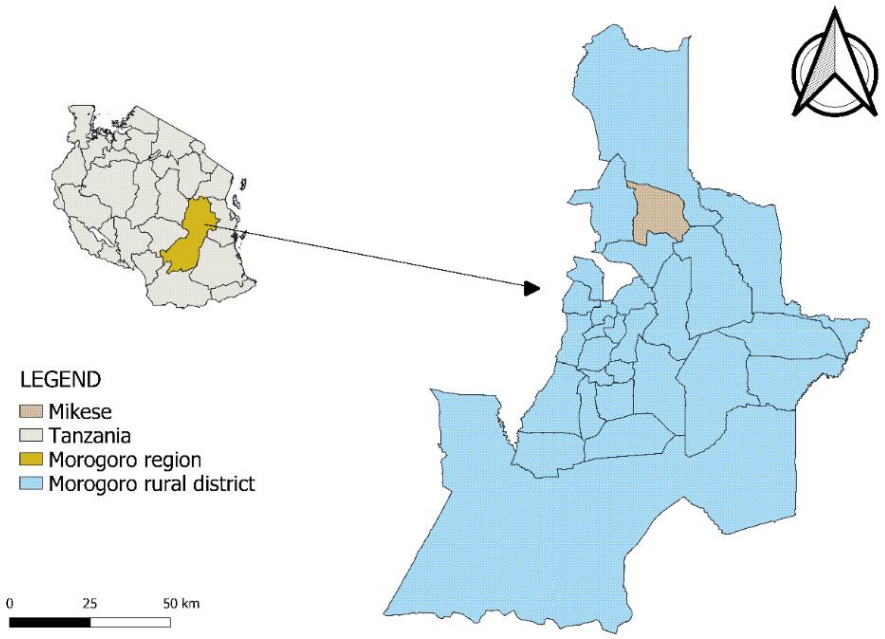
Study Area and Herd

The study was carried out on a single privately owned farm in Mvogogo village, Mikese ward, within the Morogoro District of Tanzania (Figure 1). Mvogogo village is situated at around 6°46'0" S and 37°54'0" E, with an altitude of roughly 491 meters. This area experiences a tropical weather pattern, having an average temperature around 23°C and a bimodal pattern of rainfall, where the little rains occur from November to December and the long rains from March through May. The annual rainfall typically falls between 800 and 1200 mm (Masawe, 1999). The soils are

predominantly clayey and loamy, ideal for farming, and the natural vegetation includes savanna grasses, shrubs, sporadic trees, and forest patches (Masawe, 1999). Figure 1 shows the study area boundary, showing the geographic limits of the research site within Morogoro District, Tanzania.

Figure 1

The study area boundary, showing the geographic limits of the research site within Morogoro District, Tanzania



The farm practices livestock rearing exclusively, focusing specifically on cattle. The study involved a herd of 36 cattle, consisting of 31 females and 5 males, all of which were Ayrshire crossbreeds, a cross between Ayrshire and local breeds known for their high milk production. The herd was comprised of 17 adults older than 4 years, 9 cattle aged 1 to 4 years, 5 calves under 6 months, and 5 calves over 6 months to 12 months. Among the 17 adult cows that had already calved, 7 had experienced abortion while 10 had not. The cattle were housed in four sheds, each with distinct management practices: calves in sheds A1 and A2, replacement heifers in B1, and milking and dry cows were co-housed in B2, ensuring care specific to each group's age and production stage. The farm employed extensive management with regular pasture grazing and Artificial Insemination. Table 1 below summarizes the characteristics and reproductive status of the cattle herd.

Table 1
Demographic and reproductive characteristics of the cattle from the investigated dairy herd

Variable	Category	N
Sex	Male	5
	Female	31
Age	Calves	5
	Heifers	14
	Adults	17
Parity Level	Never Produced	14
	Once	0
	Twice	1
	Thrice	5
	More	11
	Not Applicable	5
Reproductive Status	Never produced	10
	Pregnant	4
	Lactating	13
	Dry	4
	Not Applicable	5
Abortion History	Yes	7
	No	10

Study Design and Sample Size
The study employed a cross-sectional design focusing on a single dairy farm that experienced notable abortion incidents. The entire herd, consisting of 36 cattle, was included in the sample to investigate the presence of Brucella spp.

Sample Collection
Blood Collection and Handling
Aseptic blood collection was performed from the jugular vein of each animal when 10 milliliters of blood was drawn into two types of vacutainer tubes. The initial blood collection was done using a plain vacutainer tube for serological tests. After being left to clot at room temperature throughout the entire night, the blood samples were processed the next day, with the sera carefully transferred into 1.8 ml labeled Cryovials, avoiding contact with the clots. The second tube was a Heparin-coated vacutainer tube for whole blood collection. The collected blood was thoroughly mixed with the Heparin by tilting the tube in different directions (Fonseca et al., 2023). Blood samples were labeled with the cattle ID, collection date, and sample type, then placed in a chilled box with ice packs for transport to the laboratory at the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, for analysis. Upon arrival, they were kept in storage at -20°C.

Milk Collection and Handling
Milk samples were collected from 13 lactating cows using a 50ml Falcon conical centrifuge tube. Each selected cow provided 40ml of milk, drawn from all four teats of the udder to ensure representativeness. Before milk collection, udders and teats were sanitized with a mild disinfectant to prevent contamination. Samples were collected using sterile techniques, with initial streams discarded (Mugizi *et al.,* 2015). Subsequently, the samples were labeled with the specific cattle identification number, date of collection, type of sample, and the stage of lactation and promptly chilled in a cooler with ice packs for transportation to the laboratory at the College of Veterinary Medicine and Biomedical Sciences at Sokoine University of Agriculture.

Assessment of Risk Practices
In addition to biological sample collection, a focus group discussion was conducted with farm staff to gather information on potential risk practices associated with brucellosis. The discussion focused on abortion history, handling and disposal of aborted materials, management of aborting and sick cows, contact with other livestock and wildlife, routine health monitoring, and biosecurity practices.

Laboratory Analysis

Serological Assays

a. Rose Bengal Plate Test

Serological testing for Brucella was initially performed using the Rose Bengal Plate Test (RBPT), a quick agglutination test outlined by Díaz *et al*. (2011). A 30 µl drop of serum and controls was pipetted onto each section of the test plate, adjacent to a 30 µl of RBPT antigen. Each serum and antigen drop was mixed with a fresh toothpick, and the plate was manually rocked in circular motions for about 4 minutes. Afterward, the results were read in good lighting, with visible clumping indicating positive results (Corbel, 2006).

b. Competitive Enzyme-Linked Immunosorbent Assay (c-ELISA)

The serum samples were examined for Immunoglobulin G antibodies using SVANOVIR* Brucella-Ab C-ELISA kits (Article numbers SV-104893 and SV-104894). Following the manufacturer's instructions, 45 µl of Sample Dilution Buffer was introduced into every well for the serum samples, controls, and conjugate controls. Positive, weak positive, and negative serum controls were added to designated wells in duplicates, along with 5 µl of sample dilution buffer for the conjugate control wells. Then, 5 µl of the test samples were incorporated in duplicates. A 50 µl monoclonal antibody (mAb) solution was added to all wells, followed by thoroughly mixing for 5 minutes and incubating for 30 minutes at ambient temperature (18–25°C). The plates were rinsed four times with PBS-Tween Buffer, and then 100 µl of Conjugate Solution was introduced to each well. Following this, the plates underwent incubation for an additional 30 minutes under ambient conditions. After the second washing, 100 µl of Substrate Solution was introduced into every well and incubated for 10 minutes. The reaction was then ceased by introducing 50 µl of stop solution into each individual well, and the optical density (OD) readings at 450 nm were obtained using a microplate photometer. For validation, the Conjugate Control (Cc) OD should be 0.75 – 2.0, the Positive Control PI should be 80 – 100, the Weak Positive Control PI should be 30 – 70, and the Negative Control PI should be less than 30. A PI < 30% was considered negative, while a PI ⩾ 30% was considered positive.

Genomic DNA Extraction

The HighPrep™ Blood and Tissue DNA Kit (BioNordika, Stockholm, Sweden) was used to extract genomic DNA (gDNA) from milk samples. Initially, 1000 µl of the milk sample was added to bead tubes, with controls prepared using nuclease-free water (negative control) and Zymo Community Microbial Standard (positive control). After adding 300 µl of lysis buffer (AS Buffer) and vortexing, the tubes were processed using a bead beater and centrifuged to separate the supernatant. To the supernatant, an aliquot of 20 µl of Proteinase K solution was infused and subjected to incubation at 65°C. MAG-S1 beads were then introduced, along with 430 µl of ethanol. After thorough mixing and incubation at ambient temperature, the samples were placed on a magnetic plate to separate the beads. The beads were rinsed twice with 70% ethanol, permitted to dry in the open air, and subsequently reconstituted in 100 µl of MB elution buffer. DNA was eluted by incubation at 65°C, then detached from the beads and preserved at -20°C for further use.

Genomic DNA extraction derived from whole blood and serum samples was executed utilizing the QIAamp® Blood Mini Kit (Qiagen, Hilden, Germany). The process started with the addition of 20 µl of QIAGEN protease to a 1.5 ml microcentrifuge tube, then adding 200 µl of sample and 200 µl of Buffer AL. After giving this mixture a vigorous vortex, it underwent incubation for ten minutes at 56°C. After a brief centrifugation to eliminate droplets, 200 µl of ethanol (96-100%) was infused. Subsequent to vortexing, the mixture was centrifuged once more. It was then transferred to a QIAamp Mini spin column in a 2 ml collection tube and centrifuged at 6000 x g (8000 rpm) for 1 minute. The column was rinsed with 500 µl of Buffer AW1 and centrifuged again, then washed with 500 µl of Buffer AW2 and centrifuged at maximum speed (20,000 x g; 14,000 rpm) for 3 minutes. The column was centrifuged once more to remove any residual Buffer AW2 before being moved to a fresh 1.5 ml microcentrifuge tube. Finally, 200 µl of Buffer AE was added, and the column was kept at room temperature for one minute, after which DNA was eluted via centrifugation at 6000 x g (8000 rpm) for one minute, resulting in high-quality DNA suitable for further procedures like PCR.

Identification of Brucella with Real-time PCR Assay

To determine the genus Brucella, the extracted genomic DNA was analyzed by targeting the IS711 insertion sequence gene via the use of gene-specific primers listed in Table 2 and a Real-time PCR technique. For distinguishing Brucella abortus and *Brucella melitensis*, additional genotyping was conducted with primer/probe sets described in Table 2, targeting the BruAB_0168 genes for Brucella abortus and the BMEII0466 gene for *Brucella melitensis* (Kumar *et al.*, 2018).

Table 2

Primer and Probe Sequences for Real-Time PCR Detection of Brucella Genus, Brucella abortus, and Brucella melitensis.

S/N	Genotype		Primer Sequences (5′-3′)	Target	Primer size (bp)
1	*Brucella* species	F	GCTTGAAGCTTGCGGACAGT	IS711	20
		R	GGCCTACCGCTGCGAAT		17
		Probe	FAM-AAGCCAACACCCGGCCATTATGGT		24
2	*B. melitensis*	F	TCGCATCGGCAGTTTCAA	BMEII0466	18
		R	CCAGCTTTTGGCCTTTTC		18
		Probe	FAM-CCTCGGCATGGCCCGCAA		18
3	*B. abortus*	F	GCACACTCACCTTCCACAACAA	BruAB_0168	22
		R	CCCCGTTCTGCACCAGACT		19
		Probe	FAM-TGGAACGACCTTTGCAGGCGAGATC		25

The PCR master mix was composed of 10 µl of PrimeTime Gene Expression Master Mix (2X), 1 µl of Primer/Probe assay, 2 µl of DNA template, and 7 µl of nuclease-free water to achieve a final volume of 20 µl. The PCR conditions involved an initial denaturation at 95°C for 3 minutes, followed by 40 cycles of 15 seconds at 95°C for denaturation, 1 minute of annealing, and 1 minute of extension at 60°C. Each run included two positive and two negative controls. Samples were considered positive if their cycle threshold (CT) value was ⩽35. The Real-time PCR was conducted using a 7500 Real-Time PCR System from Thermo Fisher Scientific (Waltham, Massachusetts, United States).

Data Analysis

Cohen's Kappa statistic was used to assess the level of agreement between RBPT and c-ELISA results. Data from the focus group discussion were analyzed qualitatively to identify potential risk practices associated with brucellosis. As the study was limited to a single farm, no statistical analysis was performed; instead, patterns and practices were described narratively.

Results

Seroprevalence of Brucella spp.

Among the 31 cattle over six months old, 41.94% (13/31) tested positive for Brucella spp. using the Rose Bengal Plate Test (RBPT), while 74.19% (23/31) were positive by competitive ELISA (c-ELISA). These results are summarized in Table 3. Notably, all cattle that were RBPT positive were also c-ELISA positive. The level of agreement between RBPT and c-ELISA was assessed using Cohen's Kappa statistic, yielding a value of 0.40, which indicates moderate agreement.

Table 3
Seroprevalence as determined by RBPT and c-ELISA

Test	Result	Number of Samples (n=31)	Percentage (%)
RBPT	Positive	13	41.94
	Negative	18	58.06
c-ELISA	Positive	23	74.19
	Negative	8	25.81
RBPT & c-ELISA	Positive	13	41.94
	Negative	8	25.8

Identification of Brucella with Real-time PCR
Out of the 13 milk samples, 36 whole blood samples, and 36 serum samples tested, only three serum samples showed amplification with Cycle threshold (CT) values considered positive according to the protocol (⩽35), indicating the presence of Brucella DNA. Among the three amplified samples, one was specifically identified as Brucella abortus. The other two samples did not show amplification with primers specific for *Brucella abortus* or *Brucella melitensis*, resulting in a bovine brucellosis prevalence of 8.30% on the farm. Table 4 illustrates the summary of Real-time PCR results for detecting Brucella species.

Table 4
Summary of Real-time PCR Results for Brucella spp. DNA Detection in Dairy Cattle Herd

Sample Type	Samples Tested (n)	Positive Samples (n) (CT value Range)	Negative Samples (n)
Serum	36	3 (CT: 33.8 – 35)	33
Blood	36	0	36
Milk	13	0	13

Risk Practices
Primary risk practices for brucellosis included exposure to other livestock and wildlife, inadequate isolation of aborting and sick animals, unsafe handling practices, absence of routine health monitoring, and insufficient biosecurity measures.

Discussion

This study investigated several key aspects of brucellosis in the dairy cattle herd under investigation. Specifically, it examined the seroprevalence of brucellosis within the herd and the presence of Brucella DNA in samples from the cattle.

Brucella Seroprevalence
The seroprevalence result from c-ELISA (74.19%) indicated a high exposure rate to Brucella in cattle older than six months, suggesting that a substantial proportion of the herd had been exposed to the pathogen at some point. The Competitive ELISA demonstrated high sensitivity (100%) and specificity (99.7%) in detecting antibodies associated with natural Brucella infections. Its ability to differentiate infected cattle from those vaccinated with Brucella abortus strain 19 emphasizes its effectiveness and reliability as a diagnostic tool for accurately identifying ongoing natural infections in the herd (Nielsen *et al.*, 1995). This high prevalence affirms brucellosis as a major health concern on the investigated farm. Moreover, the observed disparity between the RBPT and c-ELISA results, as illustrated in Table 3, highlights the differences in detection sensitivity between the two methods. RBPT detected 41.94% (13/31) positivity, while c-ELISA identified 74.19% (23/31). Both tests agreed on 41.94% (13/31) positivity. Of the five males tested, two (40%) were positive on c-ELISA but negative on RBPT, suggesting differing test sensitivities. The agreement between the two serological tests was assessed using Cohen's Kappa statistic, which yielded a value of 0.40, indicating moderate agreement. This suggests that while both tests identify overlapping cases,

c-ELISA detects a higher number of seropositive animals compared to RBPT. This finding aligns with a study conducted in Zimbabwe, where c-ELISA also demonstrated a higher seroprevalence in the cattle herd (Matope et al., 2011). Nonetheless, the study conducted in Mbeya region revealed that the general seroprevalence across the herd was 32.0%; 50.5% [95% CI: 40.9-59.9] in indigenous cattle and 4.2% [95% CI: 1.3-12.4] in exotic cattle (Sagamiko *et al.,* 2018). Likewise, the Kasulu District of Tanzania study indicated that the total seroprevalence of anti-Brucella antibodies in individual animals was 30.8% [95% CI: 25.5 - 36.2] (Swai *et al.,* 2021). While our study revealed a notably higher seroprevalence of brucellosis on the dairy farm compared to other studies, it was important to consider several contributing factors. Differences in herd size, management practices, and the implementation of biosecurity measures can have a substantial effect on seroprevalence rates. In this farm, several poor management practices were identified that may have contributed to the persistence and spread of the infection. For example, although farm workers reported that aborted fetuses and placental tissues were buried, the absence of standardized protocols for deep burial, disinfection, and supervised disposal likely reduced the effectiveness of this practice in containing Brucella organisms. Inadequate burial depth or exposure during handling may have allowed environmental contamination and subsequent infection of other animals. Furthermore, aborting and sick animals were not isolated from the rest of the herd, increasing the risk of direct transmission through contact or shared resources. This is particularly concerning, as infected cattle shed large quantities of Brucella through genital discharges following abortion or calving, heavily contaminating the environment and facilitating rapid spread to susceptible animals. Although shedding typically decreases two to three months after abortion or calving, some animals may continue to excrete the pathogen intermittently throughout life (Godfroid *et al.,* 2011b; Wang *et al.,* 2022). In addition to these management failures, the farm was exposed to other livestock and wildlife, which may serve as reservoirs or carriers of Brucella, contributing to reinfection or introduction of new strains. The absence of routine health monitoring further compromised early detection and control efforts, allowing infected animals to remain undetected and potentially infectious for extended periods. Moreover, the lack of basic biosecurity measures, such as controlled animal movement, disinfection protocols, and protective gear for workers, created an environment in which Brucella could spread easily within the herd. These combined shortcomings likely played a central role in the high seroprevalence observed in this study. Moreover, the large difference in seroprevalence between this study (n=36) and other studies conducted in Mbeya (n= 1211 from 178 farms) and Kasulu District (n= 285 from 27 herds) (Sagamiko *et al.,* 2018; Swai *et al.,* 2021), may reflect both the influence of sample size and the presence of unique farm-level risk practices.

Identification of Brucella Species

Real-time PCR provided specific confirmation of Brucella DNA in serum samples, as demonstrated in Table 4, including the identification of Brucella abortus. This molecular evidence supported the serological findings but indicated that not all seropositive cases corresponded to detectable Brucella DNA, possibly due to the stage of infection or the type of sample analyzed. Studies have shown that Brucella bacteria are not consistently present in the bloodstream or milk, especially in chronic cases, potentially leading to false-negative PCR results when using these sample types (Sreevatsan et al., 2000). Similar observations were made by Capparelli et al. (2009), who reported that while serological tests for Brucella detection demonstrated high sensitivity (100%), their specificity was lower, ranging from 40% to 48% compared to bacteriological tests like culture and PCR. This variation suggests that animals may test positive serologically but not bacteriologically due to the intermittent presence of Brucella bacteria in samples such as milk or blood. To improve detection accuracy, repeated sampling over time or using alternative sample types such as reproductive tissues, lymph nodes, or vaginal swabs could enhance PCR sensitivity. These approaches may improve diagnostic accuracy, particularly when investigating brucellosis prevalence in livestock herds, where serological tests alone may not capture all active infections (Capparelli *et al.,* 2009). Molecular techniques, such as real-time PCR, are crucial in Brucella detection by targeting specific DNA segments. They are especially valuable for confirming Brucella diagnoses and are widely used in

epidemiological research to identify active infections accurately (Navarro *et al.,* 2002; Probert *et al.,* 2004). The detection of Brucella DNA in 8.3% of the cattle indicated an active infection on the farm during the study period. Notably, all positive cases were cows without a history of abortion, with one testing positive in both RBPT and c-ELISA, one in c-ELISA only, and one seronegative in both tests. These findings highlight the potential for subclinical infections and the limitations of relying solely on serology, emphasizing the value of molecular diagnostics like real-time PCR in identifying carriers and enhancing brucellosis control strategies. Importantly, no DNA of *Brucella melitensis* was detected, suggesting its absence in the tested herd. Moreover, this finding aligns with reported brucellosis prevalence rates in Tanzania. Alonso *et al.* (2016) reported an overall brucellosis prevalence of 8.2% [95% CI: 6.5-10.2] in Tanzania, while other studies conducted in Dar es Salaam and Lugoba observed higher rates of 14.1% and 12.3%, respectively (Weinhäupl *et al.,* 2000).

This study presents some limitations. The sample size was relatively small (n = 36), which limited the statistical power. It was also restricted to a single dairy farm, so the findings may not be generalizable to other farms or regions with different management practices or epidemiological conditions. Lastly, the cross-sectional design and use of only serum, blood, and milk samples for PCR detection may have missed some cases, particularly in animals with chronic infections or localized bacterial presence, potentially resulting in false-negative results.

Conclusion

This study investigated the presence of Brucella spp. in a dairy cattle herd in Morogoro, revealing a high seroprevalence of brucellosis, with 74.19% of cattle older than six months testing positive using competitive ELISA. Real-time PCR detected Brucella DNA in 8.30% of the cattle, all of which were cows without a history of abortion, suggesting subclinical infections. The persistence of infection appears to be strongly associated with specific management shortcomings, including the failure to isolate aborting and sick animals, inconsistent disposal practices for aborted materials, exposure to other livestock and wildlife, inadequate biosecurity measures, and lack of routine health monitoring. These practices likely facilitated environmental contamination and ongoing transmission.

Recommendation

This study provided valuable insights into Brucella seropositivity and abortion history, but future research with a larger population could enhance understanding and generalizability while building on these findings.

Based on the detection of Brucella DNA in asymptomatic cattle and the high seroprevalence observed, regular serological testing and molecular diagnostics should be implemented in dairy herds. These measures would enhance early detection and management of brucellosis, reducing the potential for subclinical infections to act as reservoirs and mitigating the risk of widespread transmission and associated economic losses.

In addition, farm-level biosecurity practices should be strengthened, particularly through the isolation of aborting and sick animals, proper supervision of disposal methods for aborted materials, and restricting access to livestock areas, to prevent environmental contamination and disease spread within the herd.

Acknowledgment

We acknowledge the cattle owner for granting permission to use their animals in this study. We also appreciate the financial support provided by the Congregation of the Holy Ghost Fathers (Cssp) in the Tanzania Province and the BruSTAR Project at the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture.

Ethical considerations

The study adhered to established research clearance and ethical protocols to protect participants' rights and ensure regulatory compliance. Research clearance was granted by Sokoine University of Agriculture identified by reference number DPRTC/R/186/31.

References

Alonso, S., Dohoo, I., Lindahl, J., Verdugo, C., Akuku, I., & Grace, D. (2016). Prevalence of tuberculosis, brucellosis andtrypanosomiasis in cattle in Tanzania: A systematic review and meta-analysis. Animal Health Research Reviews, 17(1), 16–27.https://doi.org/10.1017/S146625231600013X

Assenga, J. A., Matemba, L. E., Muller, S. K., Malakalinga, J. J., & Kazwala, R. R. (2015). Epidemiology of Brucella infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem, Tanzania. BMC Veterinary Research, 11, 1–11. https://doi.org/10.1186/s12917-015-0504-8

Capparelli, R., Parlato, M., Iannaccone, M., Roperto, S., Marabelli, R., Roperto, F., & Iannelli, D. (2009). Heterogeneous shedding of Brucella abortus in milk and its effect on the control of animal brucellosis. Journal of Applied Microbiology, 106(6), 2041–2047. https://doi.org/10.1111/j.1365-2672.2009.04177.x

Corbel, M. J. (2006). Brucellosis in humans and animals. World Health Organization.

de Figueiredo, P., Ficht, T. A., Rice-Ficht, A., Rossetti, C. A., & Adams, L. G. (2015). Pathogenesis and immunobiology of brucellosis: Review of Brucella–Host Interactions. The American Journal of Pathology, 185(6), 1505–1517. 10.1016/j.ajpath.2015.03.003

Díaz, R., Casanova, A., Ariza, J., & Moriyon, I. (2011). The Rose Bengal Test in human brucellosis: A neglected test for the diagnosis of a neglected disease. PLOS Neglected Tropical Diseases, 5(4), e950

Dubey, J., Schares, G., & Ortega-Mora, L. (2007). Epidemiology and control of neosporosis and Neospora caninum. Clinical Microbiology Reviews, 20(2), 323–367

El-Sayed, A., & Awad, W. (2018). Brucellosis: Evolution and expected comeback. International Journal of Veterinary Science and Medicine, 6, S31–S35. https://doi.org/10.1016/j.ijvsm.2018.01.008

Fonseca, F. C., de Oliveira, M. V. M., Luz, D. F., Ferraz, A. L. J., Simões, A. R. P., da Silva, E. R. F., & de Oliveira, D. M. (2023). Assessment of different bovine blood collection methods. CONTRIBUCIONES A LAS CIENCIAS SOCIALES, 16(10), 18794–18808. https://doi.org/10.55905/revconv.16n.10-005

Godfroid, J., Cloeckaert, A., Liautard, J.-P., Kohler, S., Fretin, D., Walravens, K., Garin-Bastuji, B., & Letesson, J.-J. (2005). From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Veterinary Research, 36(3), 313–326. https://doi.org/10.1051/vetres:2005003

Godfroid, J., Scholz, H., Barbier, T., Nicolas, C., Wattiau, P., Fretin, D., Whatmore, A., Cloeckaert, A., Blasco, J., & Moriyon, I.

(2011a). Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Preventive Veterinary Medicine, 102(2), 118–131. https://doi.org/10.1016/j.prevetmed.2011.04.007

Godfroid, J., Scholz, H. C., Barbier, T., Nicolas, C., Wattiau, P., Fretin, D., Whatmore, A. M., Cloeckaert, A., Blasco, J. M., & Moriyon, I. (2011b). Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Preventive Veterinary Medicine, 102(2), 118–131. https://doi.org/10.1016/j.prevetmed.2011.04.007

Godfroid, J., De Bolle, X., Roop, R., O'Callaghan, D., Tsolis, R. M., Baldwin, C. L., Santos, R. L., McGiven, J. A., Olsen, S. C., & Nymo, I. H. (2014). The quest for a true One Health perspective of brucellosis. http://dx.doi.org/10.20506/rst.33.2.2290

Hurley, W., & Doane, R. (1989). Recent developments in the roles of vitamins and minerals in reproduction. Journal of Dairy Science, 72(3), 784–804. https://www.journalofdairyscience.org/article/S0022-0302(89)79170-0/pdf

Kılıç, S., Çelebi, B., & Turan, M. (2021). Brucella melitensis and Brucella abortus genotyping via real-time PCR targeting 21 variable genome loci. Journal of Microbiological Methods, 180, 106125. https://doi.org/10.1016/j.mimet.2020.106125

Kubuafor, D., Awumbila, B., & Akanmori, B. (2000). Seroprevalence of brucellosis in cattle and humans in the Akwapim-South district of Ghana: Public health implications. Acta Tropica, 76(1), 45–48. https://doi.org/10.1016/S0001-706X(00)00088-7

Kumar, V., Maan, S., Kumar, A., Batra, K., Chaudhary, D., Dalal, A., Gupta, A. K., Bansal, N., Sheoran, N., & Maan, N. (2018). Real time PCR assay for differentiation of Brucella abortus and Brucella melitensis. Indian Journal of Animal Research, 52(7), 1037–1042. https://arccjournals.com/journal/indian-journal-of-animal-research/B-3349

Masawe, P., ** Cundall, EP and Callgari, PDS. (1999). Pedological characterization, clay mineralogy and classification of some soiles of Mikese area, Morogoro district, Tanzania. Tanzania Journal of Agricultural Sciences, 2(1), 1–6.

Mathew, C. M. (2017). Infections associated with reproductive disorders in cattle in Tanzania: Occurrence, characterization and impact. https://nmbu.brage.unit.no/nmbuxmlui/bitstream/handle/11250/2500603/Thesis%20Coletha%20Mathew-Printed%20version.pdf?sequence=1&isAllowed=y

Matope, G., Muma, J., Toft, N., Gori, E., Lund, A., Nielsen, K., & Skjerve, E. (2011). Evaluation of sensitivity and specificity of

RBT, c-ELISA and fluorescence polarisation assay for diagnosis of brucellosis in cattle using latent class analysis. Veterinary Immunology and Immunopathology, 141(1–2), 58–63. https://doi.org/10.1016/j.vetimm.2011.02.005

Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J., & Skjerve, E. (2011). Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. Tropical Animal Health and Production, 43 ,651–656. https://link.springer.com/article/10.1007/s11250-010-9748-2

Mugizi, D. R., Muradrasoli, S., Boqvist, S., Erume, J., Nasinyama, G. W., Waiswa, C., Mboowa, G., Klint, M., & Magnusson, U. (2015). Isolation and molecular characterization of Brucella isolates in cattle milk in Uganda. BioMed Research International, 2015. https://doi.org/10.1155/2015/720413

Navarro, E., Escribano, J., Fernandez, J., & Solera, J. (2002). Comparison of three different PCR methods for detection of Brucella spp. In human blood samples. FEMS Immunology & Medical Microbiology, 34(2),147–151. https://doi.org/10.1111/j.1574-

Nielsen, K., Kelly, L., Gall, D., Nicoletti, P. & Kelly, W. (1995). Improved competitive enzyme immunoassay for the diagnosis of bovine brucellosis. Veterinary Immunology and Immunopathology, 46(3–4), 285–291. https://doi.org/10.1016/0165-2427(94)05361-U

Otlu, S., Sahin, M., Atabay, H., & Unver, A. (2008). Serological investigations of brucellosis in cattle, farmers and veterinarians in the Kars district of Turkey. Acta Veterinaria Brno, 77(1), 117–121

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., & Tsianos, E. V. (2006). The new global map of human brucellosis. The Lancet Infectious Diseases, 6(2), 91–99.

Probert, W. S., Schrader, K. N., Khuong, N. Y., Bystrom, S. L., & Graves, M. H. (2004). Real-time multiplex PCR assay for detection of Brucella spp., B. abortus, and B. melitensis. Journal of Clinical Microbiology, 42(3), 1290–1293. https://doi.org/10.1128/jcm.42.3.1290-1293.2004

Sagamiko, F. D., Muma, J. B., Karimuribo, E. D., Mwanza, A. M., Sindato, C., & Hang'Ombe, B. M. (2018). Sero-prevalence of Bovine Brucellosis and associated risk factors in mbeya region, Southern highlands of Tanzania. Acta Tropica, 178, 169–175. https://doi.org/10.1016/j.actatropica.2017.11.022

Singh, B., Dhand, N. K., & Gill, J. (2015). Economic losses occurring due to

brucellosis in Indian livestock populations. Preventive Veterinary Medicine, 119(3–4), 211–215. https://doi.org/10.1016/j.prevetmed.2015.03.013

Sreevatsan, S., Bookout, J. B., Ringpis, F., Perumaalla, V. S., Ficht, T. A., Adams, L. G., Hagius, S. D., Elzer, P. H., Bricker, B. J., & Kumar, G. K. (2000). A multiplex approach to molecular detection of Brucella abortus and/or Mycobacterium bovis infection in cattle. Journal of Clinical Microbiology, 38(7), 2602–2610.

Swai, E. S., Mkumbukwa, A. J., Chaula, S. L., & Leba, B. G. (2021). Focus: Zoonotic Disease: Epidemiological Investigation of Bovine Brucellosis in Indigenous Cattle Herds in Kasulu District of Tanzania. The Yale Journal of Biology and Medicine, 94(2), 285. https://pmc.ncbi.nlm.nih.gov/articles/PMC8223540/

Ukita, M., Hozé, N., Nemoto, T., Cauchemez, S., Asakura, S., Makingi, G., Kazwala, R., & Makita, K. (2021). Quantitative evaluation of the infection dynamics of bovine brucellosis in Tanzania. Preventive Veterinary Medicine, 194, 105425. https://doi.org/10.1016/j.prevetmed.2021.105425

Wang, Y., Wang, Y., Peng, Q., Xiang, Z., Chen, Y., Wang, G., Wu, X., Guo, A., & Robertson, I. D. (2022). A case study investigating the effects of emergency vaccination with Brucella abortus A19 vaccine on a dairy farm undergoing an abortion outbreak in China. Animal Diseases, 2(1), 24. https://link.springer.com/article/10.1186/s44149-022-00056-6

Weinhäupl, I., Schöpf, K., Khaschabi, D., Kapaga, A., & Msami, H. (2000). Investigations on the prevalence of bovine tuberculosis and brucellosis in dairy cattle in Dar es Salaam region and in zebu cattle in Lugoba area, Tanzania. Tropical Animal Health and Production, 32, 147–154. https://doi.org/10.1023/A:1005231514467

Xiang, Z., Zheng, W. & He, Y. (2006). BBP: Brucella genome annotation with literature mining and curation. BMC Bioinformatics, 7, 1–14. https://doi.org/10.1186/1471-2105-7-347

Zvizdić, Š., Čengić, D., Bratić, M., Mehanić, S., Pinjo, F., & Hamzić, S. (2006). Brucella melitensis review of the human infection case. Bosnian Journal of Basic Medical Sciences, 6(1), 15. https://doi.org/10.17305/bjbms.2006.3203