Application of the Fluorescence Polarization Assay for Detecting Bovine Brucellosis Antibodies in Kasulu and Kibondo Districts, Western Tanzania

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SUMMARY

A cross-sectional study on bovine brucellosis was carried out between November 2021 and January 2022 in Kasulu and Kibondo Districts of Kigoma Region, Western Tanzania, to determine the disease prevalence. The Fluorescence Polarization Assay (FPA), a highly sensitive and specific diagnostic tool for detecting *Brucella* antibodies, was employed to enhance diagnostic accuracy. A total of 388 cattle were examined, yielding an overall seroprevalence of 7.73%. The prevalence was significantly higher in Kasulu District (10.98%) compared to Kibondo District (0.81%) ($p = 0.002$). Female cattle recorded a higher seroprevalence (10.95%) than males (0%) ($p = 0.001$). Moreover, the Ankole breed exhibited greater susceptibility, with adult Ankole cattle showing a significantly higher prevalence (9.4%) compared to indigenous breeds (4.5%) ($p < 0.001$). These results indicate that bovine brucellosis remains an important concern in both districts, with Kasulu particularly Kagerankanda village emerging as a hotspot due to its prominence in livestock keeping. Given the zoonotic nature of brucellosis, these findings underscore the urgent need for targeted and strategic control measures to mitigate both animal and public health risks.

Keywords

Cattle, Brucellosis seroprevalence, Fluorescence polarization Assay (FPA), Kigoma, Tanzania.

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INTRODUCTION

Brucellosis is a zoonotic bacterial disease of significant public health and economic importance, affecting domestic animals, wildlife, and humans (Berhanu and Pal, 2020). It is caused by Gram-negative bacteria of the genus *Brucella*, which include 12 species known to infect both humans and animals, including cattle, dogs, sheep and goats (Sulayman *et al*., 2020; Djangwani *et al*., 2021). Among these, *Brucella melitensis*, *Brucella abortus*, and *Brucella suis* are the most common in in humans and livestock infections (Doganay and Aygen, 2003). Brucellosis spreads between animals through exposure to infected animals' secretions, aborted fetuses, contaminated environments, pasture, and in some cases, via artificial insemination with infected semen (Corbel, 2006). Brucellosis in human is primarily transmitted by direct contact with infected animals or indirect contact with contaminated materials (Khurana *et al*., 2021). The primary route of human infection remains the consumption of unpasteurized or contaminated milk (Lemos *et al*., 2015).

While brucellosis has been eradicated in some countries such as Denmark, Australia, Canada and the United Kingdom, it remains prevalent in developing countries. Globally, it ranks as the second most important zoonotic disease after rabies with an estimated 5 to 12.5 million human cases each year (Seleem *et al*., 2010; Khurana *et al*., 2021). In sub-Saharan Africa and other developing regions, brucellosis is endemic and poses substantial public health challenges. In Tanzania, it is one of the six prioritized zoonotic diseases, with a national protocol already in place for its prevention and control in livestock. Economically, the disease causes severe losses to livestock keeping communities who largely depend on livestock. These losses arise from-reduced fertility in cattle, stillbirths, abortions, and decreased milk production, often necessitating culling or replacement of affected animals. In humans, brucellosis leads to high treatment costs and reduced productivity, which in turn impedes socioeconomic development.

Bovine brucellosis remains a major livestock health challenge in Tanzania. Multiple studies have documented its prevalence across the country, with variations linked to production systems: ranging from 0% to 22.1% in cattle, 5.1% to 11% in goats, and 3.4% to 7.7% in sheep (Sijapenda et al., 2017; Sagamiko, 2019; Katandukila et al., 2021; Elisha et al., 2023; Mengele et al., 2023). Despite these efforts, surveillance and research in the western regions particularly Kigoma remain limited.

Diagnosis of bovine brucellosis in Tanzania has traditionally relied on serological tests such as the Rose Bengal Test (RBT), Complement Fixation Test (CFT), and Enzyme-Linked Immunosorbent Assay (ELISA). More recently, the Fluorescence Polarization Assay (FPA) has gained recognition as a reliable method for both screening and confirmation.

Previous investigations in Kasulu and Kibondo districts have highlighted significant prevalence levels. Chitupila et al. (2015) reported a 1% prevalence in Kibondo, along with insights into associated risk factors and community knowledge, attitudes, and practices (KAP) regarding brucellosis. A subsequent study conducted in Kasulu in 2019, published by Swai et al. (2021), revealed a much higher prevalence of 30%, based on RBT and ELISA testing. This study also employed participatory epidemiology approaches to raise awareness among livestock farmers in Kagerankanda village, Kasulu District.

This survey started at the end of 2021 in Kasulu and Kibondo districts to reassess the prevalence of brucellosis following a partial awareness campaign reported by Swai et al. (2021). The study employed the Fluorescence Polarization Assay (FPA), a diagnostic tool recognized for its high sensitivity and specificity compared to conventional tests (Dong et al., 2001). FPA detects *Brucella* antibodies by measuring changes in the polarization of fluorescent light emitted by labeled antigens when bound to antibodies. This method offers distinct advantages, including improved diagnostic accuracy, the ability to differentiate between acute and chronic infections, and suitability for high-throughput testing of serum, milk, or plasma samples (Nielsen et al., 2001). By using FPA, the study was aimed to generate more precise diagnostic insights into brucellosis and to establish the current status of the disease in these districts.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Kasulu and Kibondo districts within the Kigoma Region of western Tanzania (Figure 1). These areas were specifically chosen based on previous reports of high brucellosis prevalence. The study aimed to reassess the current status of brucellosis in the region following earlier findings that highlighted its high prevalence and raised awareness of the disease among livestock farmers.

Kasulu District is located in the north-western part of the region lying between latitudes 4^0 25’ and 6^0 24’ S and longitudes 30^0 20’ and 32^0 26’ E. The district’s livestock population is estimated at 292,465 cattle, 134,048 goats, 52,230 sheep and 21,256 pigs. (Kigoma Region socio-economic profile, 2016). The district is characterised by mean annual rainfall varying from 600 to 1500 mm/year. The mean daily ambient temperatures range from 25°C in December- January to 28°C in September.

On the other hand, Kibondo district is located in north-western Tanzania, sharing an international border with Burundi to the northwest, Kakonko district to the north, Kasulu district to the west, Tabora region to the east and Uvinza district to the south. The district is located at 4°S and 31°E at an elevation of 1254 m above the sea level. The district receives an annual rainfall of about 900 mm (ranging between 600 and 1,000 mm) with an average temperature of 24.6 °C. It has a total of 35,241 cattle, 64,570 goats, 2,278 sheep and 3,650 pigs (Kigoma Region socio-economic profile, 2016).

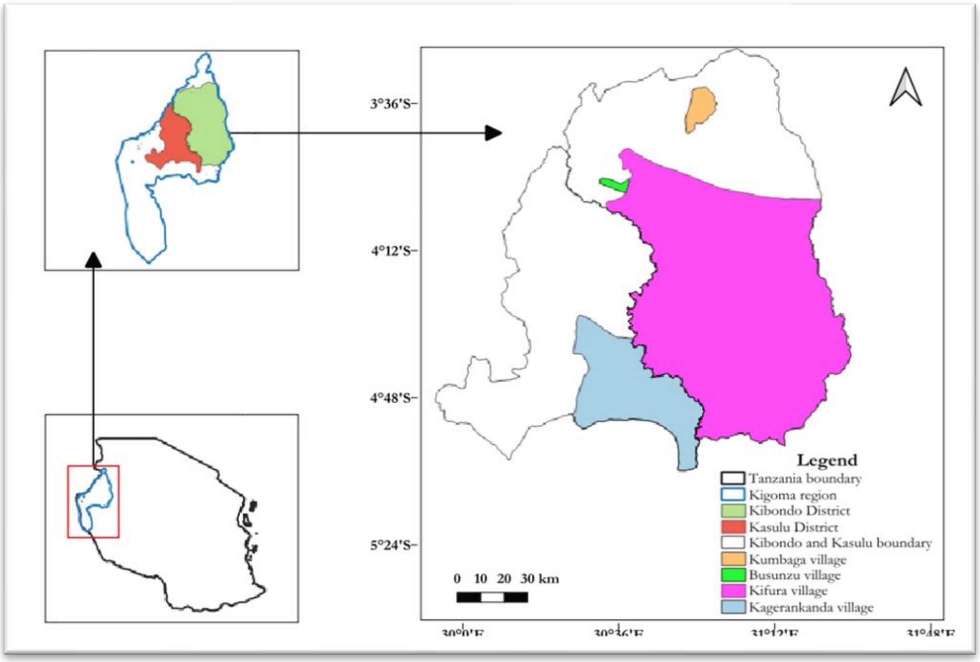

Figure 1. A map showing the study area. Source Draw by using QGIS 3.38.2

Study design and sample size determination

The study employed a cross-sectional design conducted from November 2021 to January 2022 involving cattle under agropastoral farming systems. The study was carried out in four villages; Kagerankanda in Kasulu and Busunzu, Kumbanga and Kifura in Kibodno district. The study involved a total of 80 herds (50 from Kasulu and 30 from Kibondo districts). The sample size was calculated using Lwanga (1991) statistical formula;

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

Where:

N= Sample size, P= Estimated prevalence (0.31), Z = Level of confidence as 1.96 for 95% and c = Desired precision level = 0.05. A margin error of 5% was considered and prevalence of 31% in smallholder dairy cattle (Chitupila *et al.*, , 2015, Swai *et al*., 2021).

Therefore, the calculated minimum sample size was 328. However, the researcher managed to sample a total of 388 Ankole and indigenous cattle from the study areas.

Cattle sampling procedures

Cattle were sampled randomly with selection guided by taking note of the skin color of the animals present in each herd. A numbering system was used to categorize skin colors, such as 1 for black, 2 for brown, and so on, up to 5. Upon arrival at a herd, a random number corresponding to a skin color was selected, ensuring an unbiased selection process. Between one and five animals were chosen from each herd depending on the total number of cattle present. This approach accounted for the mixed farming practices in the region, where crop farming predominates leading to smaller herd sizes. The method ensured a representative sample of the cattle population across the study areas.

Blood sample collection

During blood sample collection, cattle were manually and humanly restrained using a halter to avoid harm. Using a plain vacutainer and needle, 10 ml of blood was drawn from the jugular vein. Blood samples were collected into plain vacutainer tubes and labelled according to the animal's identity and put in a cool box and transported to the laboratory (World Health Organization, 2010).

Serological tests

Fluorescence polarization assay (FPA).

The serological test for this study was carried out at the Tanzania Veterinary Laboratory Agency (TVLA) -Northern Zone. Collected blood samples were left overnight at 4 °C in an upright position. The clotted blood was were centrifuged by using a BHG S Segurita- centrifuge (Germany) at 3000 rpm for 10 minutes to obtain blood sera. The supernatant (serum) was transferred to a new 15 ml tube with screw cap and labelled accordingly. The sera were analyzed by a Fluorescence polarization Instrument (Polarimeter) (Sentry ®200, Ellie LLC 229 N1870 West wood, W153186, United States) using a fluorescence polarization test tube antibody detection kit and results were interpreted as recommended by the manufacturer's guidelines. This technique measures the changes in the polarization of fluorescent light emitted by labeled antigens when they bind to antibodies (Nielsen, 2010). The degree of polarization changes depending on whether or not the antigen is bound to the antibody. Compared to other methods, this technique offers high sensitivity and specificity, can differentiate between acute and chronic infections. In this study, we aimed to utilize high specificity and sensitivity ϵ to better understand the magnitude of brucellosis in these districts.

Data analysis

Data were first entered in the Microsoft Excel version 2502 (Build 18526.20168). The dataset was cleaned and imported into R version 4.4.0 (2024-04-24 Puppy Cup) for analysis. Descriptive statistics, particularly percentages were computed for proportions of positive animals. The relationship between the two districts and outcome variable was individually investigated in univariate analysis through which collinearity assessment was done through Pearson's Chi-square test.

RESULTS

Results indicated that adult cattle were the mostly sampled animals in this study compared to young ones. The Ankole breed was the dominant animals in the study area thus were mostly sampled compared to indigenous animals. The study involved mostly female animals compared to male (Table 1).

Table 1: Overall demographic characteristics of sampled cattle in the study areas (Kasulu and Kibondo Districts)

Variable	Category	n	%
Sex	M	114	29.4
	F	274	70.6
	Total	**388**	
Breeds	Ankole	255	65.7
	Indigenous	133	34.3
	Total	**388**	
Age	Adult	329	84.8
	Young	59	15.2
	Total	**388**	

Bovine seroprevalence of brucellosis

The overall seroprevalence of bovine brucellosis at animal level in the study area was 7.73% (95% CI: 5.07% - 10.39%). Table 2 presents the seroprevalence of bovine brucellosis or the proportion of individual cattle testing positive for brucellosis in the two districts, Kasulu and Kibondo. In Kasulu District, out of 264 tested animals, 29 tested positives, resulting in a seroprevalence of 10.98% with a 95% (CI: 22%-14.74%). A chi-square test showed a significant difference in prevalence with a P-value of 0.002, indicating that the observed seroprevalence in Kasulu was statistically significant.

Table 2. Seroprevalence of bovine brucellosis at the individual animal level in Kasulu and Kibondo Districts

Study District	**Animal level seroprevalence**		
	n (+ve)	Prevalence, % (95% CI)	P-value
Kasulu	264 (29)	10.98 (7.22 – 14.74)	
Kibondo	124 (1)	0.81 (0.02-4.43)	0.0021
Overall results	388 (30)	7.73 (5.07-10.39)	

n = Number of animals tested; +ve = number of positive samples

Seroprevalence of bovine brucellosis by sex, age, and breed

Table 3 summarizes the variation in brucellosis seroprevalence by sex, age, and breed of cattle. None of the sampled male animals tested positive (0%), whereas 10.95% of females were seropositive. The chi-square test confirmed a significant association between sex and brucellosis prevalence (p = 0.002), indicating higher susceptibility in females. Adult cattle showed a seroprevalence of 7.9% compared to 6.78% in younger animals; however, this difference was not statistically significant (p = 0.79). Breed-wise, Ankole cattle exhibited a notably higher prevalence (9.4%) compared to indigenous breeds (4.5%), with a strong statistical association (p < 0.001).

At the district level, only one out of 123 animals in Kibondo tested positive, resulting in a seroprevalence of 0.81%. The overall seroprevalence across both Kasulu and Kibondo Districts was 7.73%.

Table 3. Seroprevalence of bovine brucellosis in relation to sex, breed and age.

Variable	Category	n (+ve)	Prevalence, % (95% CI)	Chi- square test	P-value
Sex	Male	114 (0)	0	14.816	0.001
	Female	274 (30)	10.95		
Age	Adult	329 (26)	7.9	3.135	0.79
	Young	59 (4)	6.78		
Breed	Ankole	255 (24)	9.4	6.9	< 0.001
	Indigenous	133 (6)	4.5		

n= Number of animals tested; +ve = number of positive samples

DISCUSSION

Brucellosis is an endemic disease of cattle widely distributed in Tanzania, albeit inconsistent prevalence. Several studies across the country have reported high prevalence of bovine brucellosis. Reports of brucellosis in Kigoma region are limited, with two recent studies indicating variable prevalence of the disease in the region (Swai *et al*., 2021; Chitupila *et al*., 2015. This study was conducted using a more sensitive and specific test method for the detection of brucella antibodies in the two districts. The method is reported to be more sensitive and specific for the brucellosis antibody detection compared to the previously used Rose Bengal and ELISA tests. Female Ankole cattle were the most prevalent breed sampled, comprising 274 out of 388 total cattle tested (70.6%). This indicates that Ankole breed is dominant in the study area and majority of these animals were seen in Kagerankanda village in Kasulu district, were more livestock keeping communities were found.

The overall seroprevalence of bovine brucellosis in the study areas was 7.73% (95% CI: 5.07%-10.39%) indicating a substantial presence of the disease in Kasulu and Kibondo Districts. The prevalence aligns closely with earlier reports in Tanzania such as Mellau and Wambura, 2009 (7.5%); Chota *et al*., 2016 (7.19%); Shirima and Kunda, 2016, (7.77%), and Ukita *et al*., 2021 (7.0%). Comparable findings have also been documented internationally, such as in South Africa (7.7%; Lesley et al., 2017), Saudi Arabia (7.7%; Al Anaz et al., 2019), and Ethiopia (7.59%; Tulu et al., 2024). However, relative to earlier studies specifically conducted in the same districts (Chitupila et al., 2019; Swai et al., 2021), the present study recorded a slightly lower prevalence. Such differences may be attributed to variations in sample size, geographic coverage, diagnostic methods employed, or the characteristics of cattle populations sampled across studies.

In contrast, the prevalence of brucellosis reported in this study differs from findings of previous investigations in Tanzania (Swai et al., 2021; Mengele et al., 2023). Such variations between studies and across countries may be explained by differences in diagnostic methods applied in sero-surveys, study designs employed, and livestock management systems.

At the individual animal level, the current study revealed a significantly higher seroprevalence of bovine brucellosis in Kasulu District compared to Kibondo District ($p < 0.05$). This significant disparity warrants further well-structured research in Kasulu to identify the underlying factors contributing to the relatively higher disease burden. Nevertheless, the prevalence observed in Kasulu was lower than that reported by Swai et al. (2021). This discrepancy may be largely attributed to the diagnostic methods used in the respective studies. The present study utilized the Fluorescence Polarization Assay (FPA), which offers greater sensitivity and specificity than conventional methods such as the Rose Bengal Test (RBT) or Serum Agglutination Test (SAT).

By contrast, the earlier study employed the Rose Bengal Test. Differences in diagnostic accuracy are known to substantially influence prevalence estimates, as less sensitive methods may either under- or over-estimate disease occurrence (Jolley, 2013; Eremin et al., 2024). Additionally, temporal factors including changes in livestock management practices, increased farmer awareness, and enhanced disease control interventions may have contributed to the lower prevalence detected in this study. These contextual differences underscore the importance of adopting standardized diagnostic approaches to allow more reliable comparisons across studies.

Breed differences may also influence brucellosis prevalence, as certain cattle breeds exhibit varying susceptibility to infection. In this study, a strong association was observed between the Ankole breed and brucellosis seropositivity. Previous studies have suggested that indigenous breeds tend to be more resistant to brucellosis than exotic breeds (Naveenkumar et al., 2017; Mangi, 2018; Matope et al., 2011). The higher prevalence in Ankole cattle may be linked to genetic, behavioral, or management-related factors that increase their vulnerability to *Brucella* infection. Notably, Ankole cattle in Kasulu, particularly in Kagerankanda village, are often reared in larger herds and graze extensively, conditions that elevate exposure risks (Ntivuguruzwa et al., 2020). These cattle, which are dominant in Kagerankanda, may have originated from Rwanda, where the breed is recognized for its hardiness and general disease resistance (Swai et al., 2021). This finding highlights the need to consider breed-specific factors in brucellosis control strategies, with interventions tailored more aggressively for herds dominated by Ankole cattle. Additionally, improved diagnostic sensitivity over time may explain differences in detection, with more recent tests identifying subclinical cases that earlier studies might have missed.

The study also found a significantly higher prevalence of brucellosis in female cattle compared to males. This result may partly reflect the smaller sample size of males (n = 114) relative to females (n = 274). Similar observations have been reported by other researchers (Terefe et al., 2017; Mellau and Wambura, 2009). Female cattle are generally more susceptible due to their reproductive physiology, including pregnancy and lactation, which increase exposure to infectious materials during calving and abortion (Mfune, 2015). This raises important concerns about reproductive losses and reduced productivity, which can have major economic consequences for livestock keepers. Control programs should therefore prioritize herds with high numbers of breeding females to reduce both transmission and economic losses. Male cattle, in contrast, may be less susceptible to infection because they produce lower levels of erythritol (Ferede et al., 2011). Moreover, females pose a greater risk of spreading infection, as they excrete *Brucella* organisms in vaginal discharges and retained fetal membranes following abortion (Kabi et al., 2015).

No significant difference in brucellosis prevalence was detected between young and adult cattle in the study area. This may be explained by the free-range grazing system practiced locally, which exposes cattle of all ages to similar infection risks. However, this finding contrasts with Kabi et al. (2015), who reported increasing seropositivity with age, likely due to cumulative exposure under traditional husbandry systems.

The findings emphasize the urgent need for targeted control interventions in areas with higher seroprevalence, particularly Kasulu District. Compared to Kibondo, Kasulu consistently shows higher prevalence rates, likely due to its larger cattle population and reliance on livestock rather than crop farming. A high livestock density increases opportunities for disease transmission both within herds and between animals and humans. Educational campaigns for livestock keepers are therefore critical to improve knowledge about brucellosis, its zoonotic transmission routes, and prevention measures such as vaccination with *Brucella* strain 19 (B. S19). Strengthening community awareness on safe animal handling, proper husbandry practices, and the importance of testing and culling infected animals could substantially reduce transmission.

Given the zoonotic nature of brucellosis, these findings also highlight significant public health risks in Kasulu, especially in villages such as Kagerankanda. Residents are at risk through direct contact with infected animals, consumption of unpasteurized dairy products, or exposure to contaminated environments. Public health initiatives should therefore prioritize awareness campaigns on the importance of milk pasteurization, use of protective equipment, and regular veterinary screening to protect both livestock and human populations.

CONCLUSION AND RECOMMENDATIONS

The present study confirms that bovine brucellosis remains prevalent in Kasulu and Kibondo Districts of Kigoma Region. There is an urgent need for in-depth investigations to identify the risk factors contributing to the persistence of the disease in these areas, which will be essential for improving its management. Strengthening farmers' knowledge and awareness in both districts could play a key role in reducing the risks brucellosis poses to animal health and public health. Notably, the findings point to critical factors driving the disease's prevalence in Kasulu, while also underscoring the need for further research in Kibondo to inform strategic action plans for effective brucellosis control in this zone.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

Al Anaz M, AlFayyad I, AlOtaibi R, Abu-Shaheen Epidemiology of Brucellosis in Saudi Arabia. *Saudi Med J* 40(10): 981–988, 2019.

Berhanu G, Pal M. Brucellosis: A highly infectious zoonosis of public health and economic importance. J Emerg Environ Technol Health Prot 3:17–28, 2020.

Caine Nwodo, UU, Okoh AI, Green E.. Molecular characterization of Brucella species in cattle, sheep and goats obtained from selected municipalities in the Eastern Cape, South Africa. *Asian pacific Journal of Tropical Disease* .*7*(5): 293–298, 2017.

Chitupila GY., Komba EVG, Mtui-Malamsha NJ. Epidemiological study of bovine brucellosis in indigenous cattle population in Kibondo and Kakonko Districts , Western Tanzania. *Livest Res Rural Dev* 27(6): 1–13, 2015.

Chota A., Magwisha H., Stella B., Bunuma E., Shirima G., Mugambi J., Omwenga S., Wesonga H., Mbatha P., Gathogo S. Prevalence of brucellosis in livestock and incidences in humans in east Africa. 24(1): 45, 2016. https://doi.org/10.4314/acsj.v24i1.5s

Djangwani J, Wekesa C, Gitao G. Brucellosis: Prevalence with reference to East African Community countries—A rapid review. *Vet Med Sci* 7(6):2295–2307, 2021. https://doi.org/10.1002/vms3.425

Doganay M, Aygen B. Human brucellosis: An overview. *Int J Infect Dis* 7(3):173–182, 2003.Elisha, P., Kimaro, E., Shirima, G. (2023). Seroprevalence of bovine brucellosis and associated risk factors among smallholder dairy cattle farmers in Hai and Meru District Councils Northern Tanzania.. *SVU-International Journal of Veterinary Sciences*, *6*(4), 38-54. doi: 10.21608/svu.2023.225444.1286

Eremin SA., Mukhametova LI., Krylov VB., Nifantiev NE. Fluorescence Polarization Assay for Infection Diagnostics: A Review. *Molecules* 29(19): 4712, 2024.

Ferede Y, Mengesha D, Mekonen G. Study on the seroprevalence of small ruminant brucellosis in and around Bahir Dar , North West. *Ethiop Vet J* 15(2):35-44, 2011.

Jolley M E, Nasir MS The Use of Fluorescence Polarization Assays for the Detection of Infectious Diseases. *Comb Chem High Throughput Screen* 6(3): 235-244, 2003. https://doi.org/10.2174/138620703106298419

Kabi F, Muwanika V, Masembe C. Spatial distribution of Brucella antibodies with reference to indigenous cattle populations among contrasting agro-ecological zones of Uganda. *Prev Vet Med* 121(1–2): 56–63, 2015. doi:10.1016/j.prevetmed.2015.06.007

Katandukila, J. V, Chuhila, Y. J., & Chibwana, F. D.. *Serological Analyses of Human Brucellosis in Ngara and Kibondo Districts* ,. *47*(3), 1225–1235.(2021)

Kumar VN, Bharathi MV, Porteen K, Risk factors associated with prevalence of bovine brucellosis in milk from Tamil Nadu, India. *Int J Curr Microbiol Appl Sci* 6:2604-2609, 2017

Lwanga, S. K., & Lemeshow, S. (1991). *Sample size determination in health studies: A practical manual*. World Health Organization.

Lemos TS, Cequinel JC, Costa TP, Navarro AB, Sprada A, Shibata FK, Gondolfo R, Tuon FF Outbreak of human brucellosis in Southern Brazil and historical review of data from 2009 to 2018. *PLoS Neglected Tropical Diseases* 12(9): e0006770, 2018.

Mangi MH, Kamboh AA, Rind R, Dewani P, Nizamani ZA, Mangi AR, Nizamani AR, Vistro WA. Seroprevalence of brucellosis in Holstein-Friesian and indigenous cattle breeds of Sindh Province, *Pakistan. J. Anim. Health Prod*. 3(4): 82-87, 2015.

Matope G, Bhebhe E, Muma JB, Oloya J, Madekurozwa RL, Lund A, Skjerve E. Seroprevalence of brucellosis and its associated risk factors in cattle from smallholder dairy farms in Zimbabwe. *Trop Anim Health Prod* 43(5): 975–982, 2011.

Mellau LSB, Kuya SL, Wambura PN. Seroprevalence of brucellosis in domestic ruminants in livestock-wildlife interface: A case study of Ngorongoro Conservation Area, Arusha, Tanzania. *Tanz Vet J* 26(1): 44–50, 2009.

Mengele IJ, Shirima GM, Bwatota SF, Motto SK, Bronsvoort B, Komwihangilo DM, Lyatuu E, Anne E, Cook J, Hernandez-castro LE. The status and risk factors of brucellosis in smallholder dairy cattle in selected regions of Tanzania. *Vet Sci* 10(2):155. 2023

Mfune, R.L. Epidemiological Study of Bovine Brucellosis in Smallholder Dairy Cattle in Lushoto and Rungwe Districts, Tanzania. Master’s Dissertation, Sokoine University of Agriculture, Morogoro, Tanzania, 2015.

Nielsen K. Serological diagnosis of brucellosis in cattle. *Scottish Med* 21(3): 134–135, 2010. https://doi.org/10.1177/003693307602100314

Ntivuguruzwa JB, Kolo,FB, Gashururu RS, Umurerwa L, Byaruhanga C, van Heerden H.—Seroprevalence and associated risk factors of bovine brucellosis at the wildlife-livestock-human interface in Rwanda. *Microorganisms* 8(10): 1–15, 2020. https://doi.org/10.3390/microorganisms8101553

Sagamiko, F. D. *Seroprevalence of human Brucellosis and associated risk factors among high risk occupations in Mbeya Region of Tanzania*. (*2019)*Shirima GM, Kunda JS. Prevalence of brucellosis in the human, livestock and wildlife interface areas of Serengeti National Park, Tanzania: research communication. *Onderstepoort J Vet Res* 83(1): 1–4, 2016. doi:10.4102/ojvr.v83i1.1014

Sijapenda, J. E., Komba, E. V. G., & Nonga, H. E. Studies on seroprevalence and risk factors for occurrence of bovine brucellosis in cattle in Lindi District, Tanzania. *Tanz Vet J*, *35*(Special Issue), 82–89. http://www.ajol.info/index.php/tvj. (2017).

Sulayman SMA, Bora RS, Sabir JSM, Ahmed MMM. Brucellosis: Current status of the disease and future perspectives. *Adv Microbiol* 59(4):337–344, 2020.

Swai ES, Mkumbukwa AJ, Chaula SL, Leba BG. Epidemiological investigation of bovine brucellosis in indigenous cattle herds in Kasulu District of Tanzania. *Yale J Biol Med* 94(2): 285–296, 2021.

Terefe Y, Girma S, Mekonnen N, ~~&~~ Asrade B. Brucellosis and associated risk factors in dairy cattle of eastern Ethiopia. *Trop Anim Health Prod* 49: 599-606, 2017. https://doi.org/10.1007/s11250-017-1242-7

Tulu D, Bogale A, Urge B, Aleme M. Seroprevalence of *Coxiella burnetii*, *Leptospira interrogans* serovar Hardjo, and *Brucella* species and associated reproductive disorders in cattle in southwest Ethiopia. *Heliyon* 10(3): e25558, 2024. doi:10.1016/j.heliyon.2024.e25558

Ukita M, Hozé N, Nemoto T, Cauchemez S, Asakura S, Makingi G, Kazwala R, Makita K. Quantitative evaluation of the infection dynamics of bovine brucellosis in Tanzania. *Prev Vet Med* 194:105425, 2021.

United Republic of Tanzania. *kigoma region socio-economic profile*. (2016)

World Health Organization. (2010). *WHO guidelines on drawing blood: Best practices in phlebotomy*. Geneva: World Health Organization