African Journal of Laboratory Medicine
ISSN: (Online) 2225-2010, (Print) 2225-2002

Seroprevalence and risk factors of *Brucella* antibodies among patients seeking medical attention at health facilities in selected districts of Western Province in Zambia

Authors:
Armand M. Mambote[1]
Ruth L. Mfune[2]
Victor Daka[2]
Melai Mubanga[3]
Mary Mubiana[1]
Flavien N. Bumbangi[4]
Chitalu Chanda[5]
John B. Muma[1]

Affiliations:
[1]Department of Disease Control, Faculty of Veterinary Medicine, University of Zambia, Lusaka, Zambia

[2]Department of Public Health, Faculty of Medicine, Copperbelt University, Ndola, Zambia

[3]Department of Environmental Health, School of Medicine, Eden University, Lusaka, Zambia

[4]Department of Medicine and Clinical Sciences, Faculty of Medicine, Eden University, Lusaka, Zambia

[5]Ministry of Health, Lusaka, Zambia

Corresponding author:
Ruth Mfune,
lindizyani@gmail.com

Dates:
Received: 31 May 2025
Accepted: 09 Sept. 2025
Published: 11 Dec. 2025

Background: Brucellosis is a neglected zoonosis that impacts public health and livestock productivity. It is endemic in Zambia, particularly in regions with extensive livestock farming, such as the Western Province.

Objective: This study aimed to determine the seroprevalence of *Brucella* antibodies and identify risk factors for *Brucella* exposure among healthcare-seeking patients at health facilities in Western Province, Zambia.

Methods: A cross-sectional study was conducted from 16 December 2022 to 31 December 2023 among 197 patients at health facilities in Mongu, Senanga, and Limulunga districts of Western Province. Serum samples were collected and tested for *Brucella* antibodies using the Rose Bengal test (RBT) and competitive-enzyme linked immunosorbent assay. According to the serial interpretation of results, only sera that tested positive on RBT and competitive-enzyme linked immunosorbent assay were considered positive. A structured questionnaire was used to gather epidemiological data.

Results: Most study participants were women (55.8%), married (54.31%), and had a primary level of education (55.33%). There was a 4.57% seroprevalence of *Brucella* antibodies. Most participants (67.01%) were unaware that *Brucella* affects humans, and 91.88% lacked knowledge about its mode of transmission to humans. Multivariable logistic regression model showed that the number of animals kept was a statistically significant risk factor ($p < 0.039$) associated with human *Brucella* seropositivity.

Conclusion: *Brucella* antibodies were detected among patients visiting health facilities in Western Province, Zambia. The number of animals kept was significantly associated with *Brucella* antibodies.

What this study adds: This is the first study on the seroprevalence of *Brucella* antibodies in patients at health facilities in Zambia, addressing a research gap that has largely centred on livestock. This information will help to develop public health strategies for preventing and controlling human brucellosis intervention.

Keywords: *Brucella* antibodies; human brucellosis intervention; risk factors; seroprevalence; Western Province; Zambia.

Introduction

Brucellosis is an infectious zoonotic infection caused by intracellular gram-negative coccobacilli bacteria of the genus *Brucella,* and is among the seven neglected zoonotic diseases.[1,2] The disease is widespread, affecting humans, a wide range of wild animals, and economically viable domestic livestock such as cattle, goats, sheep, donkeys, camels, swine, and dogs.[3] Currently, 12 *Brucella* species have been identified; however, only *B. melitensis*, *B. abortus*, *B. suis,* and, on rare occasions, *B. canis,* are classified as causing human infection.[4] Humans can become infected by consuming unpasteurised dairy products, or through direct contact with secretions from infected animals.[4] Typical symptoms observed in most high-risk occupational groups affected, such as veterinarians,

How to cite this article: Mambote AM, Mfune RL, Daka V, et al. Seroprevalence and risk factors of *Brucella* antibodies among patients seeking medical attention at health facilities in selected districts of Western Province in Zambia. Afr J Lab Med. 2025;14(1), a2885. https://doi.org/10.4102/ajlm.v14i1.2885

laboratory workers, abattoir workers, slaughterhouse workers, livestock workers, and farmers, include fever, headaches, physical weakness, sweats, and back pain.[5] The disease profoundly impacts developing countries with weak public and animal health systems.[2]

According to the World Health Organization (WHO), more than 500 000 new human cases are reported yearly worldwide.[5] Human brucellosis studies have reported varying seroprevalences across Africa: 31.3% in Egypt,[6] 24.1% in Nigeria,[7] 5.6% in Cameroon,[8] 5.7% and 31.8% in Kenya,[9] 18.7% in Uganda,[10] 6.1 % in Rwanda,[11] and 1.41% in Tanzania.[12] However, there is scarce information on human brucellosis in Zambia, as more studies have focused on animals than humans.[13,14] The findings are intended to improve stakeholder awareness and to strengthen brucellosis diagnosis, surveillance, and prevention efforts in endemic regions.

Methods

Ethical considerations

Ethical approval was obtained from Excellence in Research Ethics and Science (reference number: 2018-Dec-004), the National Health Research Authority, and the Ministry of Health prior to the study. Written informed consent was obtained from all participants. For participants below 18 years, written informed consent was obtained from their parents or legal guardians, followed by their assent. The participants were informed of the purpose of the study, procedures, risk of minimal pain and discomfort at the injection site during blood sample collection, their benefits, and the right to withdraw at any time during the study. All data were restricted to the investigators and treated confidentially; no participant identifiers were used. To ensure participant data confidentiality, all personal identifiers were deleted from the data set and replaced with unique codes. The data, which was stored on password-protected computers, could only be accessed by the research team. Any published results are given in aggregate form, ensuring that individual individuals are not recognised.

Study design and setting

A cross-sectional study was conducted between 16 December 2022 and 31 December 2023 among patients seeking care at health facilities in three districts of Western Province, Zambia: Senanga, Limulunga, and Mongu. The study involved collecting blood samples from consenting symptomatic patients and epidemiological data using a pretested structured questionnaire.

The province and districts were selected based on the fact that Western Province is one of the major livestock-producing provinces, where bovine brucellosis has been reported.[15] Western Province covers an area of 126 386 km^2 and lies between the latitudes 14 °S and 17 °S and longitudes 22 °E and 25 °E.[16] It is characterised by the Barotse floodplains of the Zambezi River and the sandy soils. The main economic activity in the province is livestock production, followed by fishing and crop production.

Three health facilities were selected in Senanga District: Ngundi, Sikumbi, and Lui Wanya Health Centres. Two facilities were included from Mongu District (Lealui Mini Hospital and Sefula Rural Health Centre) and two from Limulunga District (Limulunga Mini Hospital and Ikwichi Rural Health Centre).

Study population and sampling strategy

The sample size was estimated using the Ausvetepitool software (http://epitools.ausvet.com.au/), assuming an expected prevalence of 20%,[17] a desired absolute precision of 5% and a confidence level of 95%. A sample size of 225 participants was considered after increasing the required sample size (n = 195) by 15% to account for sample haemolysis and data incompleteness. Sample haemolysis was accounted for because of the distance (approximately two days) between the study area where samples were collected and the laboratory in Lusaka. The 15% was considered based on previous literature.[18] The sample size was distributed in the three districts according to the weight index (Table 1).

Included in the study were all consenting patients at the health facilities during the study period who presented any of the following signs and symptoms: intermittent or persistent fever, headache, weakness, profuse sweating, chills, arthralgia, weight loss and joint pain, with a negative result for. The patients with another confirmed diagnosis, such as smear-positive tuberculosis, malaria, salmonella, febrile disease, and those who did not consent, were excluded from the study. Patients who presented with different signs and symptoms, or were unwilling to participate, were excluded from the study.

Sample and data collection

A clinical officer or nurse collected 4 mL of blood from each consenting participant into a labelled sterile plain tube and kept at +4 °C for 24 h. The serum samples were separated using a portable field centrifuge (TOMy Digital MX-300, Japan) and stored in labelled cryovial tubes at −20 °C until transportation to the University of Zambia School of Veterinary Medicine (UNZAVET) for laboratory analysis. Epidemiological data were collected from participants using a structured questionnaire adopted from a similar study.[17] The questionnaire had four parts: (1) socio-demographic characteristics, (2) knowledge and attitude of participants regarding brucellosis, (3) practices of participants towards brucellosis, and (4) risk factors.

TABLE 1: Distribution of sample size of humans weighted by district populations.

District	Weighting index (human population)	Number of participants to be sampled
Mongu	197 816	120
Senanga	12 040	68
Limulunga	61 102	37
Total	370 958	225

Source: Central Statistical office (CSO, 2020)

Laboratory analysis

Serum samples were screened for *Brucella* antibodies using the Rose Bengal test (RBT, ID. Vet, and innovative Diagnostics, France) as described elsewhere. Only RBT-positive serum samples were subjected to competitive-enzyme linked immunosorbent assay (c-ELISA). Briefly, for the RBT, 30 µL of serum was mixed with 30 µL of Rose Bengal antigen on a slide. The mixture was gently rocked for 4 min and observed for agglutination. The presence of agglutination was considered positive. The c-ELISA analysis was done according to the manufacturer's guidelines and reagent kit manual (SVANOVIR® *Brucella*–Ab c-ELISA, Boehringer Ingelheim Svanova, Sweden). Briefly, 50 µL of Mab-solution was added into all wells used for controls and samples (time difference between controls: a positive, weak positive, and negative control were included in a 96-well plate; samples and Mab-solution addition did not exceed 10 min). The plate was sealed, and the reagents were thoroughly rinsed for 5 min using a plate shaker. The plate was then incubated at room temperature (25 °C) for 30 min, rinsed four times with phosphate-buffered saline–Tween buffer. The wells were filled up at each rinse. The plate was then emptied and tapped hard to remove all remaining fluid. Then, 100 µL of the conjugate solution was added to each well, and the plate was sealed and then incubated at room temperature for 30 min. Rinsing was repeated, as explained above. Then, 100 µL substrate solution was added to each well and incubated for 10 min at room temperature (timing began after the first well was filled). The reaction was stopped by mixing 50 µL of stop solution in the same order as the substrate solution. Then, the optic density (OD) of controls and samples was measured at 450 nm in a microplate photometer (the air was used as a blank). The OD was measured within 15 min after the addition of the stop solution to prevent fluctuation in OD values. The OD of the positive control was the one with which the OD of each test serum was compared to establish the final result (negative or positive). Determination of the positive and negative tests using the cut-off was provided in the c-ELISA kit guide. Negative results were determined by a percent inhibition of < 30%, while positive results were ≥ 30%. In this study, serial interpretation of the results was used. Therefore, only sera testing positive on both RBT and c-ELISA were regarded as positive.

Data analysis

The data were entered, coded and cleaned using Microsoft Excel 2016® (Microsoft, Redmond, Washington, United States) and analysed using STATA version 17 (StataCorp LLC, College Station, Texas, United States). Categorical data were expressed as a percentage, and seroprevalence was calculated by dividing the number of positive serum samples by the total samples examined. Descriptive statistics were used to summarise participant characteristics and seroprevalence. Categorical variables were presented as frequencies and proportions (%), and continuous variables were summarised using medians (interquartile range) based on their distribution. The seroprevalence of *Brucella* antibodies, defined as a sample positive on both the RBT and confirmatory c-ELISA, was calculated as a proportion with a 95% confidence interval (CI) derived from the Wilson Score method to ensure accuracy for proportions near zero. The odds ratio, 95% CI, and Fisher's exact tests were computed to see the degree of association of the risk factors with *Brucella* seropositivity. Using the cut-off, negative results were determined by a percent inhibition of < 30%, while positive results were ≥ 30% for c-ELISA.

The independent effects of risk factors on anti-*Brucella* spp. seropositivity were assessed using univariable analysis. Variables with a p-value ≤ 0.25 were used as variables in the multivariable logistic model, which was built using a backwards selection strategy. The odds ratio was calculated at 95% CI to see the degree of association between *Brucella* antibodies and the risk factors. The model's predictability was estimated by the receiver operating characteristic (ROC) curve analysis, while the Hosmer and Lemeshow test was used to test model fit.

Results

Socio-demographic characteristics of study participants

Out of the 225 samples collected, 197 were acceptable for analysis and 28 were haemolysed, hence could not be

TABLE 2: Socio-demographic variables of study participants (N = 197).

Variable	Category	*n*	%	95% CI	*p*-value†
Gender		-	-	-	0.582
	Male	110	55.84	55.76–55.92	-
	Female	87	44.16	44.08–44.24	-
Age (years)		-	-	-	0.811
	10–20	49	24.97	24.90–25.04	-
	21–35	52	26.40	26.33–26.47	-
	36–60	79	40.10	40.03–40.17	-
	> 60	17	8.63	8.59–8.67	-
Level of education		-	-	-	0.920
	None	15	7.61	7.57–7.65	-
	Primary	109	55.33	55.24–55.42	-
	Secondary	62	33.50	33.46–33.54	-
	Tertiary	7	3.55	3.53–3.57	-
Marital status		-	-	-	0.627
	Single	73	37.06	37.02–37.10	-
	Married	107	54.31	54.22–54.40	-
	Divorced	9	4.57	4.55–4.59	-
	Widowed	8	4.06	4.04–4.08	-
Occupation		-	-	-	0.225
	Abattoir worker	1	0.51	0.50–0.52	-
	Health worker	3	1.52	1.51–1.53	-
	Livestock farmer	25	12.69	12.64–12.74	-
	Student	26	13.19	13.16–13.22	-
	Other	8	4.06	4.04–4.08	-
	Unemployed	134	68.02	67.94–68.10	-
District		-	-	-	0.975
	Mongu	66	33.50	33.46–33.54	-
	Limulunga	38	19.30	19.15–19.45	-
	Senanga	93	47.20	46.95–47.50	-

CI, confidence interval.
†, Fisher's exact test.

included in the study. Out of the 197 participants, the study had more women (55.8%, *n* = 110) than men (44.2%, *n* = 87). The participants' mean age was 36 years, ranging from 10 to 81 years. About (54.31%, *n* = 107) were married, (55.33%, *n* = 109) had a primary education, and (68.02%, *n* = 134) were unemployed. Senanga District had more participant (48.23%, *n* = 93), than Mongu and Limulunga districts (Table 2).

Seroprevalence of human *Brucella* antibodies

From the 197 serum samples that were acceptable for analysis, the estimated seroprevalence of *Brucella* antibodies among the patients attending the health facilities in Western Province was 4.57% (*n* = 9, 95% CI: 2.34–8.49). The seroprevalence was higher in Senanga and Mongu (2.03%) than Limulunga district (Table 3).

Knowledge and attitudes of participants regarding brucellosis

Most of the participants, 114 (57.87%) had obtained their information on brucellosis from veterinary officers, while only a few, 13 (6.60%), were aware that brucellosis can affect humans. Most participants, 132 (67.01%), were ignorant about the mode of transmission to humans, while only 16 (8.12%) had the symptoms of brucellosis, as shown in Table 4.

TABLE 3: Distribution of *Brucella* antibodies per study district.

District	Number tested	RBT positive	RBT		c-ELISA		
			%	95% CI	*n*	%	95% CI
Mongu	66	10	5.08	2.50–9.01	4	2.03	0.58–5.05
Senanga	93	19	9.64	6.05–14.45	4	2.03	0.58–5.05
Limulunga	38	7	3.55	1.45–7.14	1	0.51	0.01–2.84
Total	**197**	**36**	**18.27**	**13.0–24.0**	**9**	**4.57**	**2.34–8.49**

CI, confidence interval; c-ELISA, competitive-enzyme linked immuno sorbent assay; RBT, Rose Bengal test.

TABLE 4: Knowledge and attitude of participants about brucellosis (*N* = 197).

Variable	Category	*n*	%	95% CI	*p*-value
Information about brucellosis		-	-	-	0.8
	Yes	114	57.87	51.09–64.65	-
	No	83	42.13	35.35–48.91	-
Source of information		-	-	-	0.734
	Veterinary officer	53	46.49	36.96–56.02	-
	Neighbour	12	10.53	6.20–14.87	-
	Health worker	39	34.21	26.77–41.65	-
	Media	4	3.50	1.36–5.66	-
	Patients with brucellosis	6	5.26	2.52–7.99	-
Can humans be affected?		-	-	-	0.663
	Yes	13	6.60	3.79–9.41	-
	No	67	34.01	29.06–38.97	-
	I do not know	117	59.39	52.92–65.86	-
Mode of transmission		-	-	-	0.167
	I do not know	132	67.01	60.78–73.24	-
	Contact with an infected animal	25	12.69	9.48–15.90	-
	Eating undercooked meat and drinking raw milk	40	20.30	16.50–24.11	-
Symptoms of brucellosis		-	-	-	0.004
	Yes	16	8.12	5.56–10.68	-
	No	181	91.88	88.88–94.88	-

CI, confidence interval.

TABLE 5: Univariable analysis of risk factors associated with *Brucella* antibodies.

Variable	Category	Total	Seroprevalence	%	*p*-value
Gender		-	-	-	0.592
	Male	110	5	4.5	-
	Female	87	4	4.6	-
Age (years)		-	-	-	0.811
	10–20	49	2	4.1	-
	21–35	52	2	3.8	-
	36–60	79	4	5.06	-
	> 60	17	1	5.8	-
Level of education		-	-	-	0.924
	None	15	1	6.6	-
	Primary	109	4	6.1	-
	Secondary	66	4	6.1	-
	Tertiary	7	0	0.0	-
Marital status		-	-	-	0.627
	Single	73	3	4.11	-
	Married	107	5	4.67	-
	Divorced	9	0	0.0	-
	Widowed	8	1	12.5	-
Occupation		-	-	-	0.225
	Abattoir worker	1	0	0.0	-
	Health worker	3	0	0.0	-
	Livestock farmer	25	4	16.0	-
	Unemployed	134	3	2.2	-
	Student	26	2	7.7	-
	Other	8	0	0.0	-
District		-	-	-	0.975
	Mongu	66	4	6.1	-
	Limulunga	38	1	2.8	-
	Senanga	93	4	4.3	-
Heard about brucellosis		-	-	-	0.800
	Yes	114	4	3.5	-
	No	83	5	6.2	-
Source of information		-	-	-	0.734
	Vet officer	53	1	1.9	-
	Neighbour	12	1	3.8	-
	Health worker	39	2	5.3	-
	Media	4	0	0.0	-
	Patients with brucellosis	6	0	0.0	-
Can humans become infected?		-	-	-	0.663
	Yes	13	0	0.0	-
	No	67	4	5.8	-
	I do not know	117	5	4.3	-
Mode of transmission		-	-	-	0.167
	I do not know	132	4	3.0	-
	Contact with the infected animal	32	1	3.1	-
	Eating raw meat and drinking raw milk	33	4	12.1	-
Knowledge about symptoms of brucellosis		-	-	-	0.310
	Yes	16	0	0.0	-
	No	181	9	4.8	-
Drinking raw milk and eating undercooked meat		-	-	-	0.647
	Yes	170	8	4.7	-
	No	27	1	3.7	-

Table 5 continues on the next page →

TABLE 5 (Continues...): Univariable analysis of risk factors associated with *Brucella* antibodies.

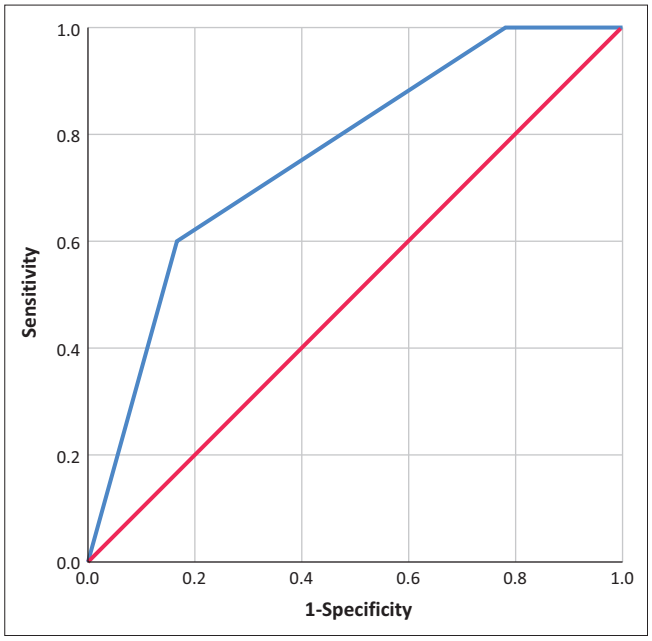
Variable	Category	Total	Seroprevalence	%	*p*-value
Keeping animal		-	-	-	0.818
	Yes	83	5	6.0	-
	No	114	6	3.5	-
Type of animal owned (*n* = 83)		-	-	-	0.308
	Goats	25	1	4.0	-
	Sheep	3	0	0.0	-
	Pigs	12	2	16.7	-
	Cattle	15	2	13.3	-
	Dogs	11	0	0.0	-
	Other	17	0	0.0	-
Number of animals kept (*n* = 83)		-	-	-	0.049*
	1	17	0	0.0	-
	1–10	50	2	4.0	-
	> 10	16	3	18.7	-
Vaccinated animal (*n* = 83)		-	-	-	0.421
	Yes	9	0	0.0	-
	No	74	5	6.8	-
Symptoms (*n* = 197)		-	-	-	0.004*
	Fever	161	5	3.1	-
	Malaise	17	1	11.8	-
	Weakness	5	2	40.0	-
	Weight loss	6	1	33.3	-
	Flu-like symptoms	8	0	0.0	-
Onset of symptoms (*n* = 197)		-	-	-	0.847
	1 day	28	1	14.8	-
	2–6 days	161	8	5.0	-
	7–13 days	5	0	0.0	-
	> 14 days	3	0	0.0	-

*, significant difference.

TABLE 6: Multivariable logistic regression analysis for risk factors associated with human *Brucella* antibodies.

Variable	Odds ratio	95% CI	*p*-value
Type of animal owned	0.72	0.42–1.23	0.226
Number of animals kept	6.49	1.10–38.13	0.039*

*, significance difference.
CI, confidence interval.



Note: Diagonal segments are produced by ties.

FIGURE 1: Receiver operating characteristic curve demonstrating the predictability of the model (area under the curve = 0.760).

Risk factors associated with *Brucella* antibodies

Initially, the univariable analysis looked at the relationship between possible risk factors and the dichotomous outcome variable, *Brucella* antibodies presence (Table 5). Some characteristics, including age, marital status, occupation, education level, and gender, did not correlate with *Brucella* antibodies presence (Table 5). The number of animals kept ($p < 0.049$) and exhibiting brucellosis symptoms ($p < 0.004$) were statistically significantly associated with *Brucella* antibodies.

As demonstrated by the multivariable logistic regression model (Table 6), the number of animals retained was a predictor of *Brucella* spp. seropositivity ($p < 0.039$). The odds of one keeping several animals being seropositive were 6.49 (95% CI: 1.10–38.13). The predictability of the model is indicated by the ROC curve (area under the curve = 0.760) (Figure 1). The area under the ROC indicated that the model can correctly predict up to 76% of the outcome. The Hosmer and Lemeshow test for goodness-of-fit (χ^2 = 0.76; with a *p*-value of 0.785) indicated that the model fit the data adequately.

Discussion

Seroprevalence of human *Brucella* antibodies

The study found a 4.57% seroprevalence of *Brucella* antibodies among the participants, which is close to the 6.1% reported among patients attending a district hospital in Rwanda.[11] *Brucella* antibodies were higher in patients from Senanga District than Mongu and Limulunga districts. This can be attributed to the fact that most of the samples collected in Senanga were from rural health centres, while most samples in Mongu and Limulunga districts were collected from mini-hospitals.

Our 4.57% finding is lower than the 20.2% reported in the Southern Province of Zambia.[17] However, our study focused on febrile patients seeking medical attention, while the former targeted occupationally exposed humans (herdsmen and abattoir workers). The study findings are also lower than the 14.9% prevalence observed among community hospital patients in Southwestern Uganda.[19] Southwestern Uganda has a high milk production and cultural practices of raw milk consumption, which increases brucellosis exposure. A similar study in Saudi Arabia reported a seroprevalence of 12.8% among febrile patients at a referral hospital.[20] The variations seen in results can be because of the various serodiagnostic methods used. The higher RBT positivity compared to c-ELISA may reflect cross-reactivity or past exposures; the use of c-ELISA improved specificity. Brucellosis serology showed a significant difference between the results of the RBT (18.27%) and confirmatory c-ELISA (4.57%). Because of its high sensitivity, the RBT can produce false positives by cross-reacting with antibodies against other Gram-negative bacteria with similar O-polysaccharide antigens, such as *Escherichia coli* O:157 or *Yersinia enterocolitica* O:9. Moreover, it may detect subclinical or low-level exposures that do not necessarily indicate

active illness. In this high-risk pastoralist community, RBT-positive/c-ELISA-negative results likely represent the background immunological stimulation, despite our serial testing approach (RBT screening followed by c-ELISA confirmation), which is the recommended standard to maximise diagnostic specificity. Therefore, the 4.57% seroprevalence is a precise and cautious estimate of actual *Brucella* exposure.

Knowledge and attitude of participants regarding brucellosis

About half of the respondents (57.87%) were knowledgeable about brucellosis, similar to findings in Uganda.[21] Low knowledge levels increase the risk of *Brucella* infection, weaken control measures and contribute to disease underreporting in the country. Only 6.60% of participants were aware that *Brucella* can affect humans, while the majority (67.01%) were ignorant about the mode of transmission to humans. The low awareness levels in our study may be attributed to the limited formal education received by participants and the lack of sensitisation on zoonotic diseases.

Risk factors associated with *Brucella* antibodies

The number of animals kept was a risk factor that was significantly associated with human *Brucella* seropositivity. The odds of someone keeping animals being seropositive were 6.49. A similar study conducted in Kenya found that herd size was a significant risk factor for exposure to *Brucella* spp.[22]

The model ROC curve demonstrated the predictability of the model (area under the curve = 0.760). The Hosmer and Lemeshow test showed that the model fitted the data, thus increasing its reliability in predicting, with ROC areas around 0.760, as confirmed by a $p > 0.78$. The reduced model predictability may be because of our small sample size, which reduces the ability to detect smaller effect sizes, or it could suggest that the level of livestock exposure is the main factor driving brucellosis seropositivity in this community, with other factors acting as mediators or being associated.

Study limitations

The sample size for the study was less than targeted, but enough to carry out the planned analysis without affecting its validity. A small sample size and few positive cases are reflected in some wide CIs, suggesting caution in interpreting associations. However, some samples were haemolysed and excluded from analysis; hence, other positive cases were possibly not detected. Our study did not present the bacteriological or molecular-based test results; hence, the seropositivity results might be caused by previous exposure to infection or cross-reactivity. The c-ELISA test used in this study is highly specific. Serial interpretation of RBT and c-ELISA increases the specificity of the test regime. Therefore, our reported 4.57% seroprevalence offers a precise and cautious estimate of actual *Brucella* exposure.

Recommendations

In rural livestock communities with endemic brucellosis, increasing routine testing and upholding a high index of clinical suspicion may enhance case discovery and lessen the burden of the disease. Furthermore, health authorities should organise public health awareness campaigns to educate healthcare workers and the communities about brucellosis, its risk factors, modes of transmission, and preventive measures. The practice of raw milk consumption and its products should be discouraged. Lastly, there is a need for further bacteriological and molecular studies to understand the epidemiology of brucellosis. Controlling brucellosis in animals will be the most efficient method of controlling it in humans.

Conclusion

A total of 4.57% of febrile patients seeking care at health facilities in Western Province tested positive for *Brucella* antibodies. Most participants were unaware that humans can contract *Brucella* or how the disease is transmitted. The number of animals kept was statistically associated with *Brucella* seropositivity. These findings underscore the need for public health interventions to enhance awareness, promote regular screening, and strengthen preventive measures for brucellosis in health facilities in Western Province, Zambia.

Acknowledgements

This article includes content that overlaps with research originally conducted as part of Armand M. Mambote's master's thesis titled 'Seroprevalence of human *Brucella* antibodies and associated risk factors among patients seeking medical attention in selected health facilities in Western Province of Zambia', submitted to the Department of Disease Control, University of Zambia, in 2024. The thesis was supervised by J.B. Muma and R.L. Mfune. Portions of the data, analysis, and discussion have been revised, updated, and adapted for journal publication. The original thesis is publicly available at: https://dspace.unza.zm/handle/123456789/9050. The author affirms that this submission complies with ethical standards for secondary publication, and appropriate acknowledgement has been made of the original work.

The authors would like to thank the provincial health officer, district health officers, clinical officers and nurses for their support during sample collection, and the laboratory staff at the Department of Disease Control, School of Veterinary Medicine, University of Zambia, for their assistance during laboratory analysis. While their contribution was important, it did not meet the criteria for authorship.

A preprint version of this work was previously published on medRxiv (https://doi.org/10.1101/2024.07.25.24311011) and we acknowledge its role in shaping the final manuscript.

Competing interests

The authors declare that no financial or personal relationships inappropriately influenced the writing of this article.

Authors' contributions

R.L.M and J.B.M participated in the conception of the idea, investigation and the writing, reviewing and editing of the manuscript. A.M.M. took part in data curation, formal analysis, investigation, methodology and prepared the original draft manuscript. V.D., M. Mubanga, M. Mubiana and C.C. participated in reviewing and editing of the manuscript. F.N.B. and A.M.M. validated the software, while R.L.M. and J.B.M. supervised the research activities.

Funding information

This work was supported by the International Foundation Sciences (grant number 13-B-6519-1), which funded the brucellosis project, and the Africa Centre of Excellence for Infectious Disease of Humans and Animals World Bank-funded project (grant number P151847).

Data availability

The data that support the findings of this study are not openly available because of reasons of sensitivity and are available from the corresponding author, R.L.M., upon reasonable request

Disclaimer

The views and opinions expressed in this article are those of the authors and are the product of professional research. The article does not necessarily reflect the official policy or position of any affiliated institution, funder, agency, or that of the publisher. The authors are responsible for this article's results, findings, and content.

References

1. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis. 2006;6(2):91–99. https://doi.org/10.1016/S1473-3099(06)70382-6
2. Ducrotoy M, Bertu WJ, Matope G, et al. Brucellosis in sub-Saharan Africa: Current challenges for management, diagnosis and control. Acta Trop. 2017;165:179–193. https://doi.org/10.1016/j.actatropica.2015.10.023
3. Ducrotoy MJ, Bertu WJ, Ocholi RA, et al. Brucellosis as an emerging threat in developing economies: Lessons from Nigeria. PLoS Negl Trop Dis. 2014;8(7):e3008. https://doi.org/10.1371/journal.pntd.0003008
4. El-Sayed A, Awad W. Brucellosis: Evolution and expected comeback. Int J Vet Sci Med. 2018;6:S31–S35. https://doi.org/10.1016/j.ijvsm.2018.01.008
5. World Health Organization. The burden of foodborne diseases is substantial WHO Estimates of the global burden of foodborne diseases. Geneva: World Health Organization, 2015; p. 3.
6. El-Moselhy EA, Zayet H, El-Khateeb AS, et al. Archives of clinical pathology human Brucellosis: Seroprevalence, risk factors, and barriers of protection among slaughterhouses' workers in El-Menia governorate, Egypt. Arch Clin Pathol J. 2018;1:1–10.
7. Aworh MK, Okolocha E, Kwaga J, et al. Human brucellosis: Seroprevalence and associated exposure factors among abattoir workers in Abuja, Nigeria – 2011. Pan Afr Med J. 2013;16. https://doi.org/10.11604/pamj.2013.16.103.2143
8. Awah-Ndukum J, Mouiche MMM, Kouonmo-Ngnoyum L, et al. Seroprevalence and risk factors of brucellosis among slaughtered indigenous cattle, abattoir personnel and pregnant women in Ngaoundéré, Cameroon. BMC Infect Dis. 2018;18(1):611. https://doi.org/10.1186/s12879-018-3522-x
9. Njeru J, Melzer F, Wareth G, et al. Human brucellosis in febrile patients seeking treatment at remote hospitals, northeastern Kenya, 2014–2015. Emerg Infect Dis. 2016;22(12):2160–2164. https://doi.org/10.3201/eid2212.160285
10. Muloki HN, Erume J, Owiny DO, et al. Prevalence and risk factors for brucellosis in prolonged fever patients in post-conflict Northern Uganda. Afr Health Sci. 2018;18(1):22–28. https://doi.org/10.4314/ahs.v18i1.4
11. Gafirita J, Kiiza G, Murekatete A, et al. Seroprevalence of brucellosis among patients attending a District Hospital in Rwanda. Am J Trop Med Hyg. 2017;97(3):831–835. https://doi.org/10.4269/ajtmh.16-0632
12. Sagamiko FD, Mfune RL, Hang'ombe BM, et al. Seroprevalence of human Brucellosis and associated risk factors among high-risk occupations in Mbeya Region of Tanzania. J Epidemiol Res. 2020;6(1):1. https://doi.org/10.5430/jer.v6n1p1
13. Muma JB, Pandey GS, Munyeme M, et al. Brucellosis among smallholder cattle farmers in Zambia. Trop Anim Health Prod. 2011;44(4):915–920. https://doi.org/10.1007/s11250-011-9987-x
14. Mfune RL, Mubanga M, Silwamba I, et al. Seroprevalence of bovine brucellosis in selected districts of Zambia. Int J Environ Res Public Health. 2021;18(4):1–8. https://doi.org/10.3390/ijerph18041436
15. Mfune RL, Muma SJB, Hangombe M, Godfroid J. Characterization of human and bovine Brucella species in Southern and Western provinces of Zambia. Lusaka: University of Zambia, 2017; p. 260.
16. Nyirenda SS, Sakala M, Moonde L, et al. Prevalence of bovine fascioliasis and economic impact associated with liver condemnation in abattoirs in Mongu district of Zambia. BMC Vet Res. 2019;15(1):33. https://doi.org/10.1186/s12917-019-1777-0
17. Mubanga M, Mfune RL, Kothowa J, et al. Brucella seroprevalence and associated risk factors in occupationally exposed humans in selected districts of Southern Province, Zambia. Front Public Health. 2021;9:754244. https://doi.org/10.3389/fpubh.2021.745244
18. Browner WS, Newman TB, Cummings SR, et al. Designing clinical research. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2022.
19. Migisha R, Nyehangane D, Boum Y, et al. Prevalence and risk factors of brucellosis among febrile patients attending a community hospital in south western Uganda. Sci Rep. 2018;8(1):1–8. https://doi.org/10.1038/s41598-018-33915-9
20. Alkahtani AM, Assiry MM, Chandramoorthy HC, Al-Hakami AM, Hamid ME. Seroprevalence and risk factors of brucellosis among suspected febrile patients attending a referral hospital in southern Saudi Arabia (2014–2018). BMC Infect Dis. 2020;20:26. https://doi.org/10.1186/s12879-020-4763-z
21. Kansiime C, Mugisha A, Makumbi F, et al. Knowledge and perceptions of brucellosis in the pastoral communities adjacent to Lake Mburo National Park, Uganda. BMC Public Health. 2014;14(1):242. https://doi.org/10.1186/1471-2458-14-242
22. Kairu-Wanyoike S, Nyamwaya D, Wainaina M, et al. Positive association between Brucella spp. Seroprevalences in livestock and humans from a cross-sectional study in Garissa and Tana River Counties, Kenya. PLoS Negl Trop Dis. 2019;13(10):e0007506. https://doi.org/10.1371/journal.pntd.0007506