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Bukar Tijjani

University of Maiduguri

Saleh Mohammed Jajere

drmsjajere@unimaid.edu.ng

University of Maiduguri

Abdulyekeen Olawale Tijjani

University of Maiduguri

Muhammad Abubakar Sadiq

University of Maiduguri

Wendy Anne Beauvais

Purdue University

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High seroprevalence of *Brucella* spp. antibodies in small-ruminant milk and poor brucellosis-related awareness among herders in Maiduguri, northeastern Nigeria

Bukar Tijjani¹, Saleh Mohammed Jajere ^{*1}, Abdulyekeen Olawale Tijjani¹, Muhammad Abubakar Sadiq¹, Wendy Anne Beauvais²

¹ Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Borno State, Nigeria

² Department of Comparative Pathobiology, College of Veterinary Medicine, Purdue University, West Lafayette, US

* Corresponding author: drmsjajere@unimaid.edu.ng

Highlights

- High anti-*Brucella* seroprevalence occurred in small-ruminant milk.
- *B. melitensis* DNA was detected in selected seropositive samples.
- Animal and flock factors were associated with *Brucella* seropositivity.
- Herders had poor knowledge and risky brucellosis-related practices.
- One Health interventions are needed to reduce transmission risks.

ABSTRACT

Background: Brucellosis is an important bacterial zoonosis affecting livestock production and public health, particularly in resource-limited settings where contact between humans and infected animals and consumption of unpasteurized animal products are common. In Nigeria, information on *Brucella* exposure among small ruminants and the knowledge, attitudes and practices (KAPs) of their handler's remains limited. This study assessed the seroprevalence

of anti-*Brucella* antibodies in milk from sheep and goats and evaluated brucellosis-related KAPs among small-ruminant herders in Maiduguri, northeastern Nigeria.

Methods: A cross-sectional study was conducted among small-ruminant flocks and herders in Maiduguri. A total of 184 fresh, unpasteurized milk samples, comprising 92 from sheep and 92 from goats, were collected from 14 purposively selected flocks using simple random sampling within flocks. Samples were screened for anti-*Brucella* antibodies using a commercial indirect enzyme-linked immunosorbent assay (iELISA). Because the iELISA does not differentiate among *Brucella* species, species-specific molecular confirmation was undertaken on 14 randomly selected ELISA-positive samples using polymerase chain reaction (PCR), owing to financial constraints. A separate KAP survey was conducted among 360 randomly selected small-ruminant herders drawn from a broader operational sampling frame comprising herders associated with the 60 identified flocks and additional larger flocks. Data were analysed using descriptive statistics, chi-square tests, and binary and multivariable logistic regression.

Results: Anti-*Brucella* antibodies were detected in 32.1% (95% CI: 25.4–39.3) of the 184 milk samples, with significantly higher seroprevalence among goats (54.4%; 95% CI: 43.6–64.8) than sheep (10.0%; 95% CI: 4.6–17.8). At the flock level, 71.4% (95% CI: 41.9–91.6) of the 14 sampled flocks had at least one ELISA-positive animal. Seropositivity was significantly associated with species, source of animals, parity, history of abortion, body condition score, and reported flock-level antibiotic use. However, these associations should be interpreted cautiously because the study was cross-sectional, antibiotic use was assessed at flock level based on herder recall, and the animal-level regression analysis did not explicitly account for clustering within flocks. Of the 14 ELISA-positive samples tested by PCR, four

(28.6%) were positive for *B. melitensis* DNA, all from sheep, while 10 were PCR-negative. Thus, molecular evidence of *B. melitensis* was restricted to the tested subset and was not used to estimate population-level *B. melitensis* prevalence. The KAP assessment revealed generally poor levels, with only 20.8%, 17.2%, and 16.9% of herders demonstrating good knowledge, positive attitudes, and appropriate practices, respectively. Breeding system, years of experience, economic status, educational level, and farming system were independently associated with KAP outcomes.

Conclusions: The high seroprevalence of anti-*Brucella* antibodies indicates substantial *Brucella* exposure among small ruminants in the study area, while detection of *B. melitensis* DNA in a limited subset provides species-specific molecular evidence of *B. melitensis* among some tested animals. The combination of substantial serological evidence and poor brucellosis-related KAPs highlights an important One Health concern, particularly in relation to raw milk consumption and unsafe handling of potentially infectious materials. Strengthening veterinary extension and surveillance services, improving herder awareness, promoting milk pasteurization and safer handling of abortion materials, and strengthening collaboration between veterinary and public health services are warranted to reduce the potential risk of brucellosis transmission in the study area.

Key words: Brucellosis; Sheep; Goats; Knowledge—attitude—practice; Maiduguri

1. BACKGROUND

Small ruminants are integral to livestock production systems in low- and middle-income countries (LMICs), including Nigeria and other sub-Saharan African nations, where they contribute substantially to food security, nutrition, and household livelihoods through the provision of meat, milk, wool, manure, and income from livestock sales. Their products have also been used in biomedical research, including studies related to diabetes and insulin production (Bala et al., 2019). Compared with large ruminants, sheep and goats are characterized by relatively low production costs, rapid turnover rates, ease of management, and greater resilience to harsh environmental conditions such as drought, poor-quality pasture, heat stress, and emerging infectious diseases (Armson et al., 2020; Joy et al., 2020; Birhanu et al., 2022). Nevertheless, their productivity remains constrained by seasonal feed shortages, inadequate veterinary services, infectious diseases, poor nutrition, and suboptimal husbandry practices (FAO, 2025; Dereje et al., 2022). Among these diseases, brucellosis is one of the most important bacterial zoonoses affecting both livestock and humans. Caused by bacteria of the genus *Brucella*, brucellosis is widely distributed throughout sub-Saharan Africa and imposes substantial public health and economic burdens through reproductive losses in livestock and occupational and foodborne transmission to humans (Gul and Khan, 2007; Moreno, 2014; Celli, 2015; Golshani and Buozari, 2017; Cadmus et al., 2020; Natesan et al., 2021; WHO, 2021; Kurmanov et al., 2022; Desa et al., 2025). Of the recognized *Brucella* species, *Brucella melitensis* is the principal cause of brucellosis in sheep and goats, whereas *Brucella ovis* primarily affects sheep and *Brucella abortus*, although predominantly associated with cattle, may also infect small ruminants (Tekle et al., 2019; USDA, 2023). Human infection occurs mainly through direct contact with infected animals or the consumption of contaminated animal products, particularly unpasteurized milk and undercooked meat, placing livestock keepers, veterinarians, slaughterhouse workers, hunters,

and laboratory personnel at increased occupational risk (Cadmus et al., 2020; Golshani and Buozari, 2017). In livestock, brucellosis causes abortion, infertility, reduced milk production, and poor reproductive performance, while transmission is influenced by herd management, animal density, breed, herd size, and herd immunity (Kenneth et al., 2020; Adamu et al., 2022).

Nigeria possesses the largest small ruminant population in Africa, followed by Sudan, Chad, Ethiopia, and Kenya (FAO, 2025). The national herd comprises approximately 42.1 million sheep and 73.8 million goats, most of which are indigenous breeds reared by pastoralists and smallholder farmers, particularly in northern Nigeria, for meat, hides, wool, and, to a lesser extent, milk production (NASS, 2011; FAO, 2023). The economic importance of this sector continues to increase, with substantial production values for mutton and chevon and projected increases in demand by 2050 (FAO, 2025). Brucellosis is endemic among small ruminants in Nigeria, with several studies reporting its occurrence across different regions (Adamu et al., 2016; Agada et al., 2018; Olufemi et al., 2018). National seroprevalence estimates of 23.3% in sheep and 10.2% in goats have been reported (Akinyemi et al., 2022), while studies conducted in northeastern Nigeria documented seroprevalence rates of 2.06% in Borno State and 10.4% in neighboring Yobe State (Oguagu et al., 2014; Adamu et al., 2022). Northeastern Nigeria represents an important livestock production and trading corridor with extensive transboundary animal movement across the porous borders shared with Niger, Chad, and Cameroon, where brucellosis is also endemic (Awah-Ndukum et al., 2018; Boukary et al., 2013; Laine et al., 2020; Özcelik et al., 2023). These transhumance practices increase opportunities for disease transmission and complicate efforts toward effective disease control. Despite the endemic nature of brucellosis in the region, available studies have largely focused on serological investigations in livestock and humans, with limited

information on the occurrence of *Brucella* species in small-ruminant milk and the level of awareness among herders.

Effective prevention and control of brucellosis in endemic settings depend not only on reliable disease surveillance and diagnostic capacity but also on farmers' knowledge, attitudes, and practices regarding disease transmission and prevention (Pinn-Woodcock et al., 2023; Hussain et al., 2021). Although previous studies have investigated brucellosis in cattle, sheep, goats, and humans in Maiduguri, no study has simultaneously determined the prevalence of *Brucella* species in small-ruminant milk and assessed herders' knowledge, attitudes, and practices regarding the disease. This knowledge gap is particularly important because the widespread consumption of raw milk and traditional milk products in Maiduguri may facilitate the transmission of *Brucella* infection to humans (Zeb et al., 2025). Therefore, this study aimed to i) determine the seroprevalence of anti-*Brucella* antibodies in milk from sheep and goats and assess the occurrence of *Brucella melitensis* DNA in a subset of ELISA-positive samples; and (ii) assess the knowledge, attitudes, and practices of small-ruminant herders regarding brucellosis in Maiduguri, northeastern Nigeria.

2. MATERIALS AND METHODS

2.1 Description of the study area

The study was conducted in Maiduguri, the capital city of Borno State, located in northeastern Nigeria. Maiduguri shares extensive and porous international borders with the Republics of Niger, Chad, and Cameroon, as well as domestic boundaries with Yobe, Gombe, and Adamawa States. Geographically, the city lies between latitudes 11°46'18" N and 11°53'21" N and longitudes 13°03'23" E and 13°14'19" E. According to the National

148 Population Commission, Maiduguri had an estimated population of 732,696 in 2006 (NPC,
149 2009).

150

151 The Maiduguri metropolis comprises Maiduguri Metropolitan Council (MMC) and parts of
152 Jere and Mafa Local Government Areas (LGAs) (Mustapha et al., 2024). The major
153 occupations of the inhabitants include mixed crop–livestock farming, trading, and civil
154 service. Commonly cultivated crops include maize, millet, groundnuts, cowpeas, and
155 vegetables, while sheep, goats, and cattle constitute the major livestock species reared.
156 Agricultural commodities such as grains, hides, skins, and gum arabic are among the
157 principal goods traded in the area.

158

159 Maiduguri is a major livestock production and trading hub in Nigeria and hosts one of the
160 largest livestock markets in the country, *Kasuwan Shanu*, which serves as a production,
161 trading, and transit center for both small and large ruminants (Bila and Bulama, 2007). The
162 high volume of livestock trading has prompted the establishment of a modern abattoir by the
163 Borno State Government, with a processing capacity of approximately 400 cattle per day
164 (Mayomi and Mohammed, 2014). A substantial proportion of the population is employed
165 along the livestock value chain, including production, fattening, trading, brokerage, and
166 processing activities.

167 Small ruminants in Maiduguri are predominantly raised under extensive production systems,
168 where animals graze freely on natural pastures with minimal housing, veterinary care, and
169 management inputs (Egwu et al., 1995). Some herders practice semi-intensive systems,
170 allowing animals to graze during the day while providing supplementary feeding, controlled

breeding, and periodic veterinary care. Intensive production systems, characterized by confinement, complete reliance on supplied feed and water, and high capital investment, are less commonly practiced in the area (Egwu et al., 1995).

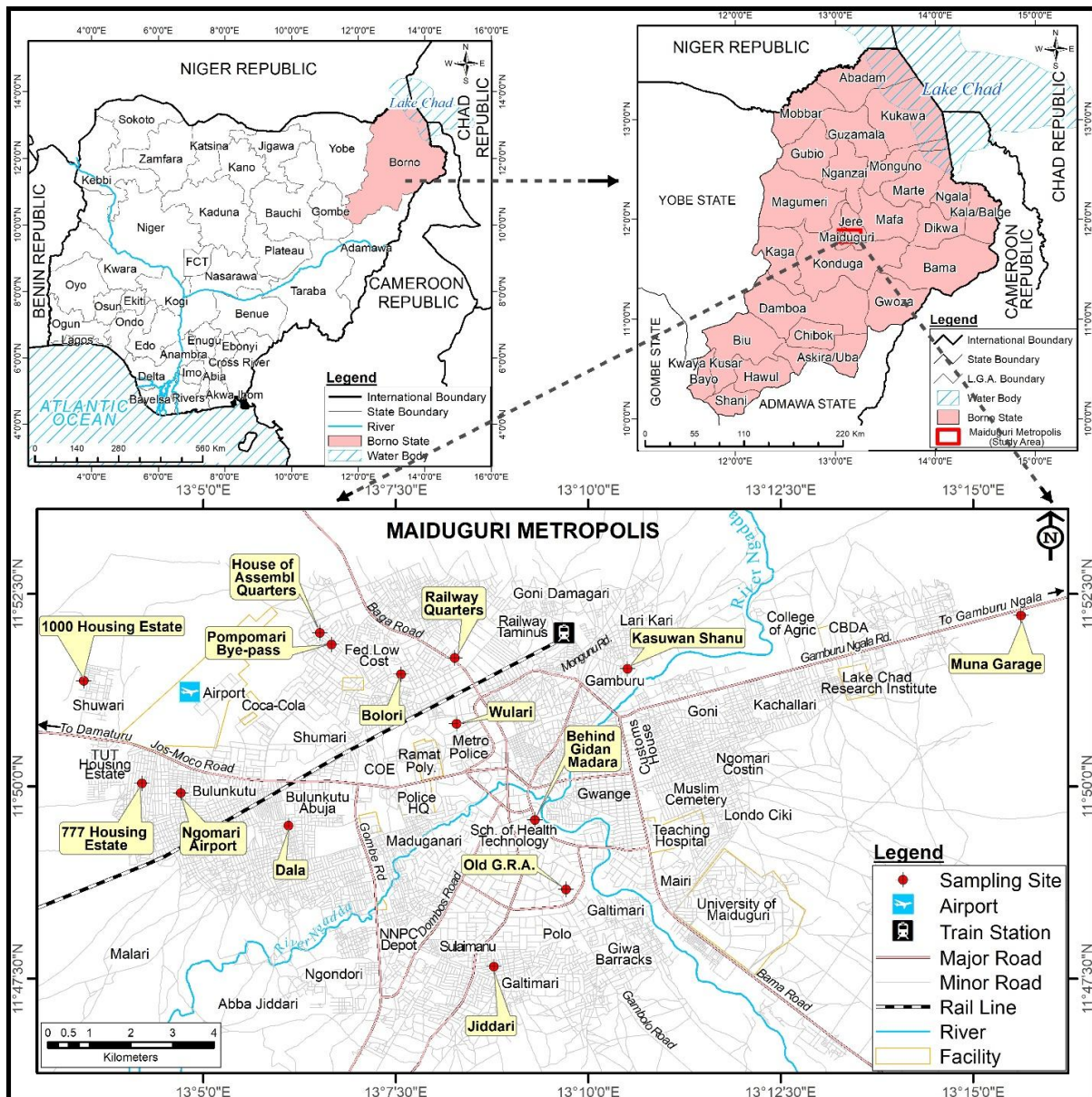


Figure 1: Map of Nigeria, Borno and Maiduguri Metropolis with Sampling Sites. Source: Modified from <https://www.openstreetmap.org/#map=6/9.117/8.674> (<https://www.openstreetmap.org/>)

Study design and study sites

A cross-sectional study was conducted between February and September 2023 to determine the animal- and flock-level prevalence of *Brucella melitensis* in the milk of lactating sheep and goats in Maiduguri metropolis, northeastern Nigeria. In addition, a pre-tested and validated semi-structured questionnaire was administered to assess the knowledge, attitudes, and practices (KAP) of small-ruminant herders regarding brucellosis.

The study was conducted in fourteen districts distributed across ten wards within Maiduguri Metropolitan Council, Jere, and Konduga Local Government Areas (LGAs), Borno State (Figure 1). The study districts included Kasuwan Shanu, Bolori, 1000 Housing Estate, Pompomari Bypass, Old Government Reserved Area (GRA), 707 Housing Estate, House of Assembly Quarters, Muna Garage, Dala, Ngomari Airport, Railway Quarters, Gidan Madara, Wulari, and Jiddari.

Study and target populations

The study comprised two complementary cross-sectional components: (i) a laboratory-based survey to determine the prevalence of *Brucella melitensis* in milk from small ruminants and (ii) a questionnaire survey to assess herders' knowledge, attitudes, and practices regarding brucellosis.

Milk survey

The study population for the laboratory survey consisted of sheep and goat flocks maintained under extensive and semi-intensive production systems within Maiduguri Metropolitan Council, Jere, and Konduga LGAs. A total of 60 small-ruminant flocks were identified within

the study area during the preparatory phase of the study. Fourteen flocks were purposively selected for milk sampling based on predefined eligibility criteria, principally the presence of lactating ewes and does available for sampling during the study period, as well as accessibility to the flocks. The selected flocks were distributed across Maiduguri Metropolitan Council (n = 7), Jere (n = 4), and Konduga (n = 3) LGAs. The mean flock size among the 14 selected flocks was 87.1 animals, ranging from 26 to 705 animals (Table 1). The remaining 46 identified flocks were not included in the milk-sampling component because they did not meet the study requirements or could not be accessed during the sampling period due to insecurity. Detailed comparative information on the non-selected flocks was not systematically collected; therefore, potential differences between selected and non-selected flocks could not be assessed.

All breeds of sheep (predominantly Yankasa, and less frequently Balami, Uda, and Sudanese) and goats (mainly Red Sokoto and Sahel) present in the selected flocks were eligible for inclusion. The study units were lactating ewes and does randomly selected from the selected flocks. Animal age was determined using dentition patterns as described by Abegaz and Awgichew (2009) and categorized as young (6–18 months) or adults (>18 months). Eligible flocks were required to have at least 10 lactating female animals and be located within the study area.

During milk sampling, both animal-level and flock-level information were recorded. Animal-level variables included species, breed, age, and reproductive status. Flock-level variables included flock size, production system, history of abortion, introduction of new animals into the flock, contact with other livestock species, grazing and watering practices, and flock-level

history of antibiotic use. Information on flock management practices was obtained from the herders during sample collection. Antibiotic use was assessed at the flock level and referred to the reported administration of antibiotics to one or more animals within the flock, rather than to the individual animal from which the milk sample was collected.

Questionnaire survey

The target population for the KAP survey comprised all eligible small-ruminant herders within Maiduguri Metropolitan Council, Jere, and Konduga LGAs. Eligible participants were herders' aged ≥ 18 years who had actively managed sheep and/or goats for at least one year, owned or managed flocks within the study area, and voluntarily provided informed consent. Herders who declined participation or were unable to provide reliable information regarding their flocks were excluded.

In Nigeria, no formal national policy currently exists for the control of brucellosis through routine vaccination or test-and-slaughter programmes (Ducrotoy et al., 2014). Although vaccination against brucellosis was initiated during the colonial era and production of the S19 vaccine commenced in 1950, these control efforts declined and vaccine production ceased in 1954 (Ocholi et al., 1993).

Sampling technique and sample size determination

Milk survey

A combination of purposive and simple random sampling techniques was employed for the milk survey. A list of eligible small-ruminant flocks was compiled with the assistance of the University of Maiduguri Veterinary Teaching Hospital, Ali Modu Sheriff Veterinary

Hospital, and private veterinary practitioners. From this list, 14 of the 60 identified flocks were purposively selected based on the availability of lactating sheep and/or goats and accessibility during the study period. Subsequently, lactating animals within each selected flock were selected by simple random sampling using the lottery method. Briefly, all eligible lactating animals were assigned unique identification numbers, which were placed in a container, and numbers were drawn without replacement until the required number of animals had been selected.

Using the Thrusfield (2018) formula for simple random sampling, the minimum required sample size was estimated at 121 animals, based on an expected prevalence of 12.96% (Buhari et al., 2020), a 95% confidence level, and 6% desired precision. To ensure balanced representation of the two target species and facilitate comparison of serological findings between sheep and goats, the final sample comprised 184 lactating animals, with equal allocation of 92 sheep and 92 goats. The species-specific estimates were interpreted with their corresponding 95% confidence intervals, recognizing that the precision of each estimate was based on 92 animals per species. An equal allocation strategy was adopted to ensure balanced representation of both species. In mixed-species flocks, sheep and goats were sampled separately according to the predetermined allocation. In flocks containing only one species, animals of the available species were sampled, while the required number for the other species was obtained from additional eligible flocks until the target sample size of 92 animals per species was achieved.

Questionnaire survey

The sample size for the questionnaire survey was calculated using a maximum expected proportion of 50%, a 95% confidence level, and a desired precision of 6%, as no previous study had assessed knowledge, attitudes, and practices regarding brucellosis among small-ruminant herders in the study area. The minimum required sample size was 267 herders. To compensate for potential non-response and improve the precision of the estimates, 360 herders were ultimately recruited.

For the KAP component, a broader sampling frame of small-ruminant herders was developed separately from the flock-level sampling frame used for milk collection. Community leaders and representatives of livestock associations assisted the research team in identifying eligible herders within the study area. The sampling frame included herders associated with the 60 flocks initially identified during the animal-sampling component, as well as herders from additional larger flocks within the study area. Eligible herders were those actively involved in small-ruminant production and who met the predefined inclusion criteria. From the resulting operational sampling list, 360 herders were selected using simple random sampling and interviewed using the pre-tested semi-structured questionnaire. The herders participating in the KAP component were not necessarily from the 14 flocks selected for milk sampling; therefore, individual KAP responses could not be directly linked to the serological status of a specific sampled animal or flock.

2.2 Milk sample collection and serological screening for anti-*Brucella* antibodies

Milk samples were collected aseptically from individual lactating animals after discarding the initial foremilk. Udders were washed, and teats were disinfected using 70% ethanol.

Approximately 5 mL of fresh, unpasteurized milk was collected into sterile Falcon® conical tubes (Thermo Fisher Scientific, South Africa), labeled, and transported on ice to the Veterinary Virology Laboratory, University of Maiduguri. Milk samples were centrifuged at 3,000 rpm for 10 minutes to obtain lactosera, which were stored at -20°C until analysis. Screening for anti-*Brucella* antibodies was conducted using a commercial indirect ELISA kit (ID Screen® BRUMILK ver. 0415 EN, IDvet, France) following the manufacturer's instructions. The assay detects antibodies against *Brucella* spp. and does not differentiate among *B. abortus*, *B. melitensis*, and *B. ovis*. The assay has a reported specificity of approximately 100% and a sensitivity ranging from 85% to 95%, comparable to other validated commercial ELISA kits (Garcia-Bocanegra et al., 2014).

2.3 Molecular analysis

2.3.1 DNA extraction

Genomic DNA was extracted from a randomly selected subset of 14 ELISA-positive milk samples, comprising seven sheep and seven goat samples. The subset was selected to provide equal representation of both species for molecular confirmation while accommodating the financial resources available for PCR analysis. DNA extraction was performed using the Wizard® Genomic DNA Extraction Kit (Promega, USA) according to the manufacturer's protocol. DNA concentration and purity were assessed spectrophotometrically at 260 and 280 nm using a GeneQuant™ spectrophotometer (Life Sciences Ltd., UK).

2.3.2 PCR amplification

Polymerase chain reaction (PCR) was performed to detect *Brucella melitensis* DNA by amplifying a 731 bp fragment of the IS711 gene. Forward (5'-TGCCGATCACTTAAGGGCCTTCAT-3') and reverse (5'-

AAATCGCGTCCTTGCTGGTCTGA-3') primers used were synthesized by Inqaba Biotec (South Africa). The PCR reactions were carried out in a 25 µL volume containing 12.5µL of OneTaq® 2× Master Mix (New England Bio labs, USA), 0.5µL of each forward and reverse primer (20µM working solution), 5µL of DNA template and 6.5µL of nuclease-free water. Amplification was performed under standard cycling conditions in a MultiGene™ OptiMax Thermal Cycler (Labnet International Inc., Edison, NJ, USA) at an initial denaturation temperature of 95°C for 5 mins, followed by 40 cycles of denaturation at 95°C for 1 mins, annealing at 52°C for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 5 mins. Appropriate positive and negative controls were included in each PCR assay as quality-control measures. The positive control consisted of genomic DNA from the *Brucella melitensis* vaccine strain acquired from the Sokoine University of Agriculture, Morogoro, Tanzania, while the negative control was included to monitor potential contamination. The PCR products were visualized on 1.5% agarose gel stained with SYBR Safe DNA Gel Stain (Invitrogen, Thermo Fisher Scientific, USA) and viewed using a UV transilluminator gel documentation system (BioRAD, Hercules, CA). Samples showing the expected amplification product were considered positive for *B. melitensis* DNA.

2.4 KAPs Questionnaire design and data collection

A semi-structured questionnaire comprising 34 items was administered through face-to-face interviews. The questionnaire included sections on informed consent, socio-demographic characteristics, and KAPs related to brucellosis. It was prepared in English and translated into Hausa, Kanuri, Fulfulde, and Shuwa Arabic. The economic status was categorized into below poverty (\$784.75/₦601,443.05 per annum), lower middle (\$1,146 - \$4,515/₦878,982 – ₦3,463,005 per annum), upper middle (\$4,516 – \$14,005/₦3,463,772 – ₦10,741,835 per annum) and upper levels (> \$14,005/₦10,741,835 per annum) as previously described

(Barnes et al., 2023). The questionnaire was pre-tested among 20 herders, and the final version demonstrated acceptable reliability with Cronbach's alpha coefficients of 0.71, 0.74, and 0.79 for knowledge, attitude, and practice domains, respectively.

KAPs scoring and categorization

Responses to the KAP questionnaire were scored using a structured and standardized scoring system. For the knowledge component, correct responses were assigned a score of one (1), while incorrect or "do not know" responses were assigned a score of zero (0). Attitude-related questions were scored using a Likert-type scale, with positive attitudes assigned higher scores and negative or indifferent attitudes assigned lower scores (Allen and Seaman, 2007). Practice-related questions were scored based on the adoption of recommended behaviors, with appropriate practices receiving higher scores than inappropriate practices.

Individual scores for knowledge, attitudes, and practices were summed separately and converted into percentage scores. Respondents were then categorized into three levels—good, moderate, and poor—based on modified Bloom's cut-off points (Bloom, 1956): scores $\geq 80\%$ were classified as good, scores between 60–79% as moderate, and scores $<60\%$ as poor. For inferential statistical analysis, KAP scores were further dichotomized into adequate and inadequate categories. Respondents with good or moderate scores were classified as having adequate KAPs, while those with poor scores were classified as having inadequate KAPs. This categorization facilitated regression analyses to identify factors associated with KAP levels.

2.5 Data Management and Statistical Analysis

Completed questionnaires and laboratory data were checked daily for completeness and consistency. Data were entered into Microsoft Excel (Microsoft Inc., USA 2019), cleaned, and coded prior to analysis. Cleaned datasets were then imported into SPSS Statistics version 21.0 (IBM®, Armonk, NY: IBM Corp.) for analysis. Descriptive statistics, including frequencies, percentages, means, and standard deviations, were used to summarize socio-demographic characteristics, KAP responses, and laboratory findings. Animal- and flock-level prevalence estimates were calculated with corresponding 95% confidence intervals.

Data were analysed using descriptive statistics, chi-square tests, and binary logistic regression. For the animal-level analysis, *Brucella* seropositivity detected by iELISA was used as the binary outcome variable. Univariable logistic regression was initially performed to assess the association between candidate explanatory variables and seropositivity. Variables meeting the predefined screening criterion of $p < 0.20$ were considered for inclusion in the multivariable logistic regression model. Adjusted odds ratios (AORs) with corresponding 95% confidence intervals (CIs) and p-values were reported. Categorical variables were entered using appropriate reference categories. Statistical significance in the final model was assessed at $p < 0.05$. The animal-level regression analysis did not explicitly account for clustering of animals within flocks, which may have affected the precision of the estimated associations and this is acknowledged as a limitation of the study. Model fitness was assessed using appropriate goodness-of-fit tests. Spearman's rank correlation was used to determine the relationship between KAPs domains. Results were presented in tables and figures where appropriate.

3. RESULTS

3.1 Small ruminant flock characteristics

The general characteristics of the sampled small ruminant flocks are presented in Table 1. The median flock size was 55.0 animals (interquartile range [IQR]: 38.0–83.8). Sheep and goats accounted for median flock sizes of 23.0 (IQR: 15.8–44.5) and 19.0 (IQR: 16.8–32.3), respectively. A total of 331 lactating small ruminants were recorded across the sampled flocks, with a mean $\pm$ SD of 6.7 ± 5.6 lactating animals per flock, ranging from 1 to 33 animals (Table 1).

Table 1: General description of small ruminants flocks in Maiduguri Metropolis, northeast Nigeria

Variables	Total	Mean	SD	Minimum	1 st Quartile	Median	3 rd Quartile	Maximum
Flock size*	5224	87.1	114	26.0	38.0	55.0	83.8	705
Sheep	2106	35.1	28.6	10.0	15.8	23.0	44.5	126
Goats	3118	52.0	110.5	11.0	16.8	19.0	32.3	650.0
Lactating ruminants	331	6.70	5.60	1.0	2.0	4.0	6.30	33.0

* Flock size was defined as the total number of sheep and goats present on the farm during sample collection

Location-, individual-, and flock-level seroprevalence of anti-Brucella antibodies in milk

Location-specific seroprevalence varied markedly across the study sites (Table 2). No positive milk samples were detected in House of Assembly Quarters, *Bolori*, 1000 Housing Estate, or *Gidan Madara*, whereas the highest prevalence was observed in Old Government Reserved Area (GRA) at 90.0% (95% CI: 68.3–98.8). At the individual animal level, goats exhibited a significantly higher seroprevalence of anti-*Brucella* antibodies (54.4%; 95% CI: 43.6–64.8) compared to sheep (10.0%; 95% CI: 4.6–17.8). The overall individual-level seroprevalence of anti-*Brucella* antibodies across both species was 32.1% (95% CI: 25.4–39.3). At the flock level, 71.4% (95% CI: 41.9–91.6) of the flocks had at least one

423 seropositive animal, indicating widespread distribution of brucellosis among small ruminant
 424 flocks in the study area.
 425
 426 **Table 2:** Location – wise seroprevalence of anti-*Brucella* antibodies in small ruminant’s milk
 427 from Maiduguri Metropolis, northeast Nigeria.

Study sites	No. of flocks	Relative %	Flock size	No. Positive/No. Sampled	Prevalence	
					%	95% CI
Old GRA	5	8.33	1288	18/20	90.0	68.30 – 98.76
<i>Kasuwan Shanu</i>	7	11.67	402	9/20	45.0	23.06 – 68.47
<i>Wulari</i>	4	6.67	234	6/20	30.0	11.89 – 54.28
<i>Jiddari</i>	6	10.00	77	8/20	40.0	19.12 – 63.95
<i>Dala</i>	4	6.67	70	3/10	30.0	6.67 – 65.25
<i>Pompomari Bypass</i>	4	6.67	105	3/10	30.0	6.67 – 65.25
777 Housing Estate	4	6.67	75	2/10	20.0	2.52 – 55.61
Railway Quarters	4	6.67	157	3/10	30.0	6.67 – 65.25
<i>Muna Garage</i>	6	10.00	265	2/20	10.0	1.24 – 31.70
Ngomari Airport	2	3.33	57	5/10	50.0	18.71 – 81.29
House of Assembly Quarters	2	3.33	58	0/10	0	0
<i>Bolori</i>	4	6.67	46	0/10	0	0
1000 Housing Estate	4	6.67	36	0/10	0	0
<i>Gidan Madara</i>	4	6.67	48	0/4	0	0
Overall	60	100	5 224	59/184	32.1	25.4 – 39.3

428

429 **Socio-demographic characteristics and risk factors associated with *Brucella***
 430 **seropositivity**

431 A total of 184 lactating small ruminants, comprising equal numbers of sheep (n = 92) and
 432 goats (n = 92), were sampled (Table 3). Most animals were sourced from households (n = 86)

433 and livestock markets (n = 68). The predominant sheep breed was Yankasa (n = 61), while
 434 Sahel goats constituted the majority among goats (n = 86). Most animals were multiparous (n
 435 = 123), had no history of abortion (n = 167), and animals originating from flocks with no
 436 reported history of antibiotic use (n = 138).

437 In the univariable logistic regression analysis, several factors were significantly associated
 438 with *Brucella* seropositivity. Goats were more likely to be seropositive than sheep (OR =
 439 10.98; $p < 0.0001$). Animals sourced from outside Maiduguri (OR = 27.28; $p < 0.0001$) and
 440 livestock markets (OR = 3.72; $p = 0.002$) showed significantly higher odds of *Brucella*
 441 seropositivity compared to household-sourced animals. Multiparity (OR = 3.34; $p = 0.001$),
 442 animals from flocks with a history of abortion (OR = 6.12; $p = 0.001$), fair (OR = 27.50; $p <$
 443 0.001) and moderate (OR = 5.75; $p = 0.002$) body condition scores, and flocks with a
 444 reported history of no antibiotic use (OR = 4.16; $p = 0.002$) were also significantly associated
 445 with increased odds of *Brucella* seropositivity.

446
 447 **Table 3:** Univariable logistic regression analysis of factors associated with *Brucella*
 448 seropositivity in milk from small ruminants in Maiduguri Metropolis, northeast Nigeria.
 449

Variables	No. of animals tested	ELISA-positive (%)	COR (95% CI)	<i>p</i> - value
Species				
Sheep	92	83 (90.2)	1.0	
Goat	92	42 (45.7)	10.98 (4.93 – 24.46)	0.0001
Breeds of Sheep				
Yankasa	61	53 (86.9)	1.0	
Others *	31	30 (96.8)	0.22 (0.03 – 1.85)	0.2637
Breeds of Goats				
Red Sokoto	6	4 (66.7)	1.0	
Sahel	86	38 (44.2)	2.53 (0.44 – 14.54)	0.4064
Source of Animals				
Household	86	75 (87.2)	1.0	

Outside Maiduguri	30	6 (20.0)	27.28 (9.12 – 81.59)	0.0001
Livestock Market	68	44 (64.7)	3.72 (1.65 – 8.832)	0.0017
Parity				
Primiparous	61	51 (83.6)	1.0	
Multiparous	123	74 (60.2)	3.34 (1.57 – 7.28)	0.0014
Flock history of abortion				
No	167	120 (71.9)	1.0	
Yes	17	5 (29.4)	6.12 (2.05 – 18.34)	0.0007
Body Condition Score				
Good	36	33 (91.7)	1.0	
Fair	14	4 (28.6)	27.50 (5.25 – 144.02)	0.0001
Moderate	134	88 (65.7)	5.75 (1.67 – 19.76)	0.0017
Flock – level history of antibiotic use				
Yes	46	40 (87.0)	1.0	
No	138	85 (61.6)	4.16 (1.65 – 10.46)	0.0017
Age of Animals				
Young adult	80	59 (73.8)	1.0	
Adult	104	66 (63.5)	1.62 (0.85 – 3.06)	0.1539

450 *Others include Balami, Uda, and Sudanese; COR, Crude Odds ratio
451

452 Predictors of *Brucella* seropositivity in milk

453 In the multivariable logistic regression model (Table 4), all variables that were significant in
454 the univariable analysis remained independent predictors of *Brucella* seropositivity. Goats
455 were 8.84 times more likely to be seropositive compared to sheep (AOR = 8.84; 95% CI:
456 2.97–26.23; $p = 0.001$). Compared with animals sourced from households, animals
457 originating from outside Maiduguri (AOR = 8.26; 95% CI: 1.49–45.66; $p = 0.016$) and
458 livestock markets (AOR = 89.78; 95% CI: 13.08–616.37; $p = 0.001$) showed significantly
459 higher odds of *Brucella* seropositivity. However, the wide confidence interval suggests that
460 the magnitude of this association should be interpreted with caution.

461

Primiparous animals had significantly lower odds of *Brucella* seropositivity compared to multiparous animals (AOR = 0.03; 95% CI: 0.01–0.20; $p = 0.001$). Animals from flocks with a history of abortion were more likely to be seropositive (AOR = 6.79; 95% CI: 1.28–36.01; $p = 0.024$). Similarly, animals with fair (AOR = 33.29; 95% CI: 2.46–450.88; $p = 0.008$) and moderate (AOR = 347.99; 95% CI: 11.32–10698.00; $p = 0.001$) body condition showed higher odds of *Brucella* seropositivity than animals in good body condition, although the very wide confidence interval indicates considerable uncertainty around the magnitude of the estimate. Conversely, animals from flocks with a reported history of antibiotic use within the three months preceding sampling had lower odds of *Brucella* seropositivity than animals from flocks without reported antibiotic use (AOR = 0.09; 95% CI: 0.02–0.38; $p = 0.001$). Because antibiotic use was assessed at the flock level and information on the specific antimicrobial, indication, treatment duration, and individual animals treated was not available, this association should be interpreted cautiously.

475

Table 4: Multivariable logistic regression analysis of factors associated with *Brucella* seropositivity in milk of small ruminants in Maiduguri Metropolis, northeast Nigeria.

Variable	No. positive	β	SE	df	AOR (95% CI)	$p - value$
Species						
Sheep	83				1.0	
Goat	42	2.18	0.56	1	8.84 (2.97 – 26.23)	0.001
Source of Animals						
Household	75				1.0	
Outside Maiduguri	6	2.11	0.87	1	8.26 (1.49 – 45.66)	0.016
Livestock Market	44	4.49	0.98	1	89.78 (13.08 – 616.37)	0.001
Parity						
Multiparous	51				1.0	
Primiparous	74	-3.39	0.92	1	0.03 (0.01 – 0.20)	0.001
Flock history of abortion						

No	120				1.0	
Yes	5	1.92	0.85	1	6.79 (1.28 – 36.01)	0.024
Body Condition Score						
Good	33				1.0	
Fair	4	3.51	1.33	1	33.29 (2.46 – 450.88)	0.008
Moderate	88	5.85	1.75	1	347.99 (11.32 – 10698.00)	0.001
Flock – level history of antibiotic use						
No	85				1.0	
Yes	40	-2.37	0.72	1	0.09 (0.02 – 0.38)	0.001

β, Regression coefficient; SE, Standard Error; df, degrees of freedom; AOR, Adjusted Odds ratio; CI, Confidence interval

3.2 Polymerase chain reaction (PCR)

Figure 2 shows the amplified PCR products for *Brucella melitensis* ELISA positive milk samples from sheep samples in the study area. Of the 14 randomly selected ELISA-positive milk samples subjected to PCR, four (28.6%) were positive for *B. melitensis* DNA, while 10 (71.4%) were negative. All four PCR-positive samples were from sheep. Thus, PCR provided species-specific molecular confirmation in a subset of the ELISA-positive samples, whereas the remaining 10 samples showed serological evidence of *Brucella* exposure without molecular confirmation of *B. melitensis*. Of the 14 randomly selected ELISA-positive milk samples subjected to PCR, four (28.6%) were positive for *B. melitensis* DNA. The four PCR-positive samples were from sheep. These represented 6.8% (4/59) of all ELISA-positive milk samples and 2.2% (4/184) of all milk samples collected; however, the latter proportion does not represent a PCR-based prevalence estimate because PCR was not performed on all samples.

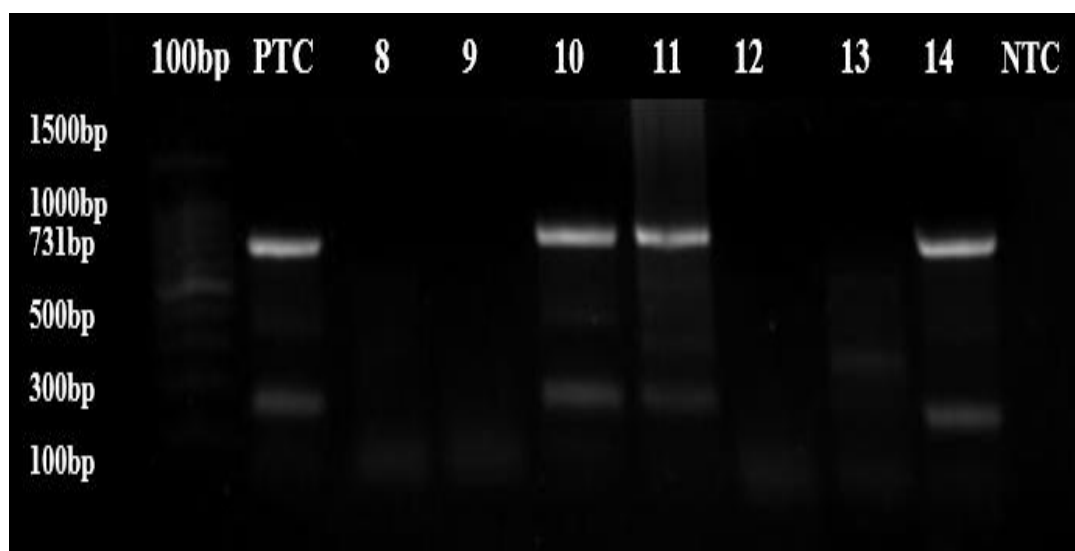


Figure 2: Ethidium bromide stained agarose gel electrophoresis of the amplified Polymerase Chain Reaction (PCR) Product for *Brucella melitensis* DNA of the ELISA positive milk samples in small ruminants (Lanes 8 – 14) in Borno State, Nigeria (PCR was performed on 14 randomly selected ELISA-positive milk samples).

3.3 Socio-demographic characteristics of herders

A total of 360 herders participated in the study. Most respondents were male (83.9%), married (83.6%), and belonged to the lower middle-income category (69.2%). Over half of the herders practiced semi-intensive farming (58.1%) and maintained flock sizes of 31–50 animals (56.4%). Approximately 36.7% of respondents were aged 31–40 years, 47.2% had informal education, and 42.2% had 3–5 years of farming experience (Table 5).

Table 5: Socio-demographic characteristics of herders in Maiduguri Metropolis.

Variables	Categories	Frequency (n)	Percentage % (95% CI)
Gender	Male	302	83.9 (79.7 – 87.5)
	Female	58	16.1 (12.5 – 20.3)
Age (years)	Mean $\pm$ SD	41.6 $\pm$ 9.5	
	< 30	45	12.5 (9.3 – 16.4)
	31 – 40	132	36.7 (31.7 – 41.9)
	41 – 50	111	30.8 (26.1 – 35.9)

	51 – 60	56	15.6 (12.0 – 19.7)
	≥ 61	16	4.4 (2.6 – 7.1)
Farming system			
	Extensive/Pastoral	74	20.6 (16.5 – 25.1)
	Semi – intensive	209	58.1 (52.8 – 63.2)
	Intensive	77	21.4 (17.3 – 26.0)
Breeding system			
	Single species	108	30.0 (25.3 – 35.0)
	Small and large ruminants	115	31.9 (27.2 – 37.0)
	Mixed breeding	137	38.1 (33.0 – 43.3)
Years of experience			
	0 – 2	53	14.7 (11.2 – 18.8)
	3 – 5	152	42.2 (37.1 – 47.5)
	6 – 8	95	26.4 (21.9 – 31.3)
	9 – 11	38	10.6 (7.6 – 14.2)
	≥ 12	22	6.1 (3.9 – 9.1)
Level of education			
	Informal	170	47.2 (42.0 – 52.5)
	Primary school	24	6.7 (4.3 – 9.8)
	Secondary school	124	34.4 (29.5 – 39.6)
	Graduate	42	11.7 (8.5 – 15.4)
Economic status *			
	Below poverty	15	4.2 (2.4 – 6.8)
	Lower middle	249	69.2 (64.1 – 73.9)
	Upper middle	63	17.5 (13.7 – 21.8)
	Upper	33	9.2 (6.4 – 12.6)
Marital status			
	Single	46	12.8 (9.5 – 16.7)
	Married	301	83.6 (79.4 – 87.3)
	Widow	13	3.6 (1.9 – 6.1)
Farm size			
	10 – 30	131	36.4 (31.4 – 41.6)
	31 – 50	203	56.4 (51.1 – 61.6)
	51 – 70	20	5.6 (3.4 – 8.5)
	≥ 71	6	1.7 (0.6 – 3.6)

* Below poverty (\$784.75/₦601,443.05 per annum); Lower Middle (\$1,146 - \$4,515/₦878,982 – ₦3,463,005 per annum); Upper Middle (\$4,516 – \$14,005/₦3,463,772 – ₦10,741,835 per annum); Upper (above \$14,005/₦10,741,835 per annum)

3.4 Knowledge, attitudes and practices regarding brucellosis

Responses to the knowledge questions revealed generally poor awareness of brucellosis. Only 22.5% of herders had previously heard of the disease, while fewer than one-third correctly identified its zoonotic nature, transmission routes, or bacterial cause (Table 6). Slightly over half of the respondents recognized that the disease is preventable, and most believed that boiling milk does not reduce its nutritional value (Table 6).

Table 6: Small ruminant farmer's knowledge regarding brucellosis in Maiduguri Metropolis

Items	No. (%) of respondents	
	Correct	Incorrect
K1. Have you heard about the disease brucellosis in animal?	81 (22.5)	279 (77.5)
K2. Are you aware that brucellosis can be transmitted from animal to man?	74 (20.6)	286 (79.4)
K3. Do you know that contact with aborted fetuses or placental membrane poses a risk of brucella transmission?	151 (41.9)	209 (58.1)
K4. Are you aware that drinking raw milk without boiling can cause Brucellosis?	110 (30.6)	250 (69.4)
K5. Are you aware that Brucellosis can be prevented?	188 (52.1)	172 (47.8)
K6. Do you think that boiling the milk affects its nutritional value?	336 (93.3)	24. (6.7)
K7. Did you ever observe any of these clinical sign(s) in your farm (abortion, infertility, decrease milk yield, death)?	121 (33.6)	239 (66.4)
K8. Brucellosis is caused by (bacteria)	79 (21.9)	281 (78.1)
K9. How can you prevent brucellosis (Vaccination, Quarantine)	143 (39.3)	217 (60.7)
K10. Where did you heard about brucellosis (veterinarian, Animal health worker, fellow farmer, social media, television)	78 (21.7)	282 (78.3)

Regarding attitudes, only 17.2% of respondents exhibited positive attitudes toward brucellosis prevention (Table 7). While most agreed that milk consumption is beneficial to

526 health, a substantial proportion acknowledged the risks associated with consuming
527 unpasteurized milk and contact with aborted materials (Table 7).

528 **Table 7:** Small ruminant farmer's attitude regarding brucellosis in Maiduguri Metropolis

Items	No. (%) of Respondents				
	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
A1. Do you agree that consumption of milk is good for health?	35 (9.7)	20 (5.6)	19 (5.3)	127 (35.3)	159 (44.2)
A2. Do you agree that consumption of unpasteurized milk is beneficial to health?	3 (0.8)	192 (53.3)	93 (25.8)	68 (18.9)	4 (1.1)
A3. Do you agree that boiling milk will reduce its nutritional value?	25 (6.9)	253 (70.3)	41 (11.4)	41 (11.4)	0 (0.0)
A4. Do you agree that consumption of raw milk pose a risk for contracting infectious diseases like brucellosis?	0 (0.0)	119 (33.1)	44 (12.2)	193 (53.6)	4 (1.1)
A5. Do you agree that contact with placental membrane and/ aborted fetuses can put you at risk of contracting brucellosis?	7 (1.9)	209 (58.1)	39 (10.8)	103 (28.6)	2 (0.6)

529

530 In terms of practices, 67.5% of herders reported separating sick animals from healthy ones;
531 however, vaccination against brucellosis was extremely rare (0.6%). Less than one-quarter
532 used protective equipment during animal delivery or disposed of aborted materials safely.
533 Overall, only 16.9% of herders demonstrated good practices related to brucellosis prevention
534 (Table 8).

535

536 **Table 8:** Small ruminant farmer's practices regarding brucellosis in Maiduguri Metropolis

Items	No. (%) of Respondents		
	Yes	Sometimes	No
P1. Do you sell diseased animals to butcher?	165 (45.8)	76 (21.1)	119 (33.1)
P2. Do you separate diseased animals from healthy animal?	243 (67.5)	23 (6.4)	94 (26.1)
P3. Do you vaccinate your animals against brucellosis?	2 (0.6)	0 (0.0)	358 (99.4)
P4. Do you assist your animals during delivery?	106 (29.4)	204 (56.7)	50 (13.9)

P5. Do you wear protective equipment when helping your animals during delivery?	84 (23.3)	92 (25.6)	184 (51.1)
P6. Do you send your animals to open grazing?	109 (30.3)	78 (21.7)	173 (48.1)
P7. Do you throw aborted fetus and placental membrane in water canals or streets?	74 (20.6)	76 (21.1)	210 (58.3)
P8. Do you call a veterinarian when animals aborted in your farm?	99 (27.5)	144 (40.0)	117 (32.5)
P9. Do you bury or burn aborted fetus?	211 (58.6)	71 (19.7)	78 (21.7)
P10. Do you clean your flock dung, feed and water trough every day?	94 (26.1)	117 (32.5)	149 (41.4)

537

538 Overall KAPs score levels

539 The overall KAP assessment revealed predominantly poor scores across all domains (Table
540 9). Good knowledge, positive attitudes, and good practices were observed in only 20.8%
541 (95% CI: 16.8 – 25.4), 17.2% (95% CI: 13.5 – 21.5), and 16.9% (95% CI: 13.2 – 21.2) of
542 respondents, respectively. The median scores for knowledge, attitude, and practice were 2.0
543 (IQR: 1.0, 3.03), 10.0 (IQR: 8.0, 12.0), and 8.0 (IQR: 7.0, 10.0), respectively (Table 9).

544

545 **Table 9:** Overall score levels for KAPs of herders regarding brucellosis in Maiduguri
546 Metropolis, northeast Nigeria.

Items	Knowledge		Attitude		Practice	
	No. (%)	95% CI	No. (%)	95% CI	No. (%)	95% CI
Good ($\geq 80\%$)	75 (20.8)	16.8 – 25.4	62 (17.2)	13.5 – 21.5	61 (16.9)	13.2 – 21.2
Moderate (60–79%)	62 (17.2)	13.5 – 21.5	89 (24.7)	20.4 – 29.5	103 (28.6)	24.0 – 33.6
Poor ($< 60\%$)	223 (61.9)	56.7 – 67.0	209 (58.1)	52.8 – 63.2	196 (54.4)	49.1 – 59.7
Mean $\pm$ SD	27.2 $\pm$ 19.3		61.4 $\pm$ 17.7		58.5 $\pm$ 18.0	
Median (IQR)	2 (1.0, 3.0)		10.0 (8.0, 12.0)		8.0 (7.0, 10.0)	

547 CI, Confidence interval; SD, Standard deviation; IQR, Inter-quartile range

548

549 3.5 Association between socio-demographic factors and KAPs levels

550 Knowledge levels were significantly associated with age, breeding system, years of
551 experience, education level, economic status, marital status, and farm size (Table 10).
552 Similarly, attitude levels varied significantly by age, breeding system, years of experience,

education level, and economic status (Table 10). Farming system, years of experience, education level, economic status, and farm size, significantly influenced practice levels. In the multivariable model, breeding system, years of experience, and economic status emerged as significant predictors of knowledge levels (Table 11). Similarly, education level and breeding system were independent predictors of attitude scores (Table 11). Lastly, farming system and years of experience appeared as strong predictors of good practices toward brucellosis prevention (Table 11).

3.6 Correlation among knowledge, attitudes and practices

The Spearman's rank correlation analysis showed weak but statistically significant positive correlations between knowledge and attitudes ($\rho = 0.205$; $p < 0.001$) and between knowledge and practices ($\rho = 0.390$; $p = 0.008$) (Table 12). No significant correlation was observed between attitudes and practices.

567 **Table 10:** Pearson's Chi - square analysis of the association between the socio-demographic factors and categorized knowledge,
568 attitudes and practices scores regarding brucellosis of herders in Maiduguri metropolis, northeast Nigeria.

Variables	Knowledge n (%)		χ^2 (<i>p</i> – value)	Attitude n (%)		χ^2 (<i>p</i> – value)	Practices n (%)		χ^2 (<i>p</i> – value)
	Poor	Good		Negative	Positive		Poor	Good	
Gender									
Male	181 (59.9)	121 (40.1)	3.22 (0.073)	173 (57.3)	129 (42.7)	0.46 (0.457)	166 (55.0)	136 (45.0)	0.21 (0.650)
Female	42 (72.4)	16 (27.6)		36 (62.1)	22 (37.1)		30 (51.7)	28 (48.3)	
Age (years)									
< 30	37 (82.2)	8 (17.8)	16.33 (0.003)	24 (53.3)	21 (46.7)	19.10 (0.0001)	32 (71.1)	13 (28.9)	8.21 (0.084)
31 – 40	69 (52.3)	63 (47.7)		60 (42.5)	72 (54.5)		70 (53.0)	62 (47.0)	
41 – 50	74 (66.7)	37 (33.3)		71 (64.0)	40 (36.0)		61 (55.0)	50 (45.0)	
51 – 60	31 (55.4)	25 (44.6)		42 (75.0)	14 (25.0)		24 (42.9)	32 (57.1)	
≥ 61	12 (75.0)	4 (25.0)		12 (75.0)	4 (25.0)		9 (56.3)	7 (43.7)	
Farming system									
Extensive/pastoral	55 (74.3)	19 (25.7)	7.41 (0.025)	45 (60.8)	29 (39.2)	0.54 (0.764)	54 (73.0)	20 (27.0)	13.44 (0.0001)
Semi-intensive	127 (60.8)	82 (39.2)		118 (56.5)	91 (43.5)		101 (48.3)	108 (51.7)	
Intensive	41 (53.2)	36 (46.8)		46 (59.7)	31 (40.3)		41 (53.2)	36 (46.8)	
Breeding system									
Single species	45 (41.7)	63 (58.3)	35.47 (0.0001)	44 (40.7)	64 (59.3)	21.95 (0.0001)	63 (58.3)	45 (41.7)	1.65 (0.439)
Small & Large ruminants	70 (60.9)	45 (39.1)		82 (71.3)	33 (28.7)		64 (55.7)	51 (44.3)	
Mixed Breeding	108 (78.8)	29 (21.2)		83 (60.6)	54 (39.4)		69 (50.4)	68 (49.6)	
Years of experience									
0 – 2	43 (81.1)	10 (18.9)	27.70 (0.0001)	29 (54.7)	24 (45.3)	9.40 (0.052)	45 (84.9)	8 (15.1)	50.80 (0.0001)
3 – 5	80 (52.6)	72 (47.4)		91 (59.9)	61 (40.1)		93 (61.2)	59 (38.8)	
6 – 8	51 (53.7)	44 (46.3)		60 (63.2)	35 (36.8)		26 (27.4)	69 (72.6)	
9 – 11	33 (86.8)	5 (13.2)		14 (36.8)	24 (63.2)		21 (55.3)	17 (44.7)	
≥ 12	16 (72.7)	6 (27.3)		15 (68.2)	7 (31.8)		11 (50.0)	11 (50.0)	
Level of education									
Informal	121 (71.2)	49 (28.8)	14.41 (0.002)	108 (63.5)	62 (36.5)	17.40 (0.0001)	108 (63.5)	62 (36.5)	13.65 (0.003)
Primary school	13 (54.2)	11 (45.8)		15 (62.5)	9 (37.5)		9 (37.5)	15 (62.5)	

Secondary school	71 (57.3)	53 (42.7)		74 (59.7)	50 (40.3)		55 (44.4)	69 (55.6)	
Graduate	18 (42.9)	24 (57.1)		12 (28.6)	30 (71.4)		24 (57.1)	18 (42.9)	
Economic status									
Below poverty	10 (66.7)	5 (33.3)	35.55 (0.0001) *	1 (6.7)	14 (93.3)	31.37 (0.0001) *	7 (46.7)	8 (53.3)	9.41 (0.024)
Lower middle	164 (65.9)	85 (34.1)		140 (56.2)	109 (43.8)		139 (55.8)	110 (44.2)	
Upper middle	20 (31.7)	43 (68.3)		38 (60.3)	25 (39.7)		26 (41.3)	37 (58.7)	
Upper	29 (87.9)	4 (12.1)		30 (90.9)	3 (9.1)		24 (72.7)	9 (27.3)	
Marital status									
Single	40 (87.0)	6 (13.0)	14.69 (0.0001) *	29 (60.9)	18 (39.1)	0.26 (0.880) *	39 (84.8)	7 (15.2)	20.30 (0.880) *
Married	174 (57.8)	127 (42.2)		173 (57.5)	128 (42.5)		152 (50.5)	149 (49.5)	
Widow	9 (69.2)	4 (30.8)		8 (61.5)	5 (38.5)		5 (38.5)	8 (61.5)	
Farm size (No. of animals)									
10 – 30	78 (58.0)	53 (40.5)	8.17 (0.043) *	78 (58.0)	55 (42.0)	5.40 (0.145) *	66 (50.4)	65 (49.6)	18.60 (0.006) *
31 – 50	118 (58.1)	69 (34.0)		118 (58.1)	85 (41.9)		122 (60.1)	81 (39.9)	
51 – 70	14 (70.0)	10 (50.0)		14 (70.0)	6 (30.0)		3 (15.0)	17 (85.0)	
≥ 71	1 (16.7)	5 (83.3)		1 (16.7)	5 (83.3)		5 (83.3)	1 (16.7)	

569 * Fisher's Exact test

570

571

572

573

574 **Table 11:** Multivariable logistic regression analysis of the socio-demographic factors associated
575 with good knowledge, positive attitudes and good practices regarding brucellosis of herders in
576 Maiduguri Metropolis, northeast Nigeria.

KAPs theme	Variables	No. of observations	β	SE	df	AOR (95% CI)	<i>p</i> – value
Good knowledge	Breeding system						
	Single species	63				1.0	
	SLR	45	3.59	0.67	1	36.30 (9.71 – 135.67)	0.000
	Mixed breeding	29	1.04	0.48	1	2.82 (1.10 – 7.25)	0.031
	Years of experience						
	0 – 2	10				1.0	
	3 – 5	72	2.36	1.09	1	10.62 (1.26 – 89.38)	0.030
	6 – 8	44	2.37	0.86	1	10.21 (1.88 – 55.36)	0.007
	9 – 11	5	- 0.33	0.89	1	0.72 (0.23 – 4.11)	0.710
	≥ 12	6	- 2.75	1.18	1	0.06 (0.01 – 0.65)	0.020
	Economic status						
	Below poverty	5				1.0	
	Lower middle	85	2.41	1.23	1	11.13 (1.01 – 122.85)	0.049
	Upper middle	43	3.66	0.80	1	38.96 (7.99 – 189.98)	0.000
	Upper	4	4.55	0.89	1	94.42 (16.67 – 534.85)	0.000
Positive attitudes	Breeding system						
	Single species	64				1.0	
	SLR	33	- 2.02	0.39	1	0.13 (0.06 – 0.29)	0.001
	Mixed Breeding	54	- 0.84	0.41	1	0.43 (0.19 – 0.96)	0.039
	Level of education						
	Informal	62				1.0	
	Primary school	9	- 1.83	0.76	1	1.60 (1.40 – 8.72)	0.016
	Secondary school	50	0.05	0.34	1	1.05 (0.54 – 2.04)	0.877
	Graduate	30	0.99	0.50	1	2.70 (1.11 – 7.27)	0.048
Good practices	Years of experience						
	0–2years	8				1.0	
	3–5years	59	2.11	0.56	1	8.27 (2.78 – 24.62)	0.001
	6–8years	69	4.28	0.71	1	72.23 (17.85 – 292.23)	0.001
	9–11years	17	2.11	0.72	1	8.27 (2.02 – 33.76)	0.003
	≥ 12 years	11	3.51	0.89	1	33.52 (5.83 – 192.79)	0.001
	Farming system						

	Extensive/pastoral	20				1.0	
	Semi-intensive	108	1.29	0.42	1	3.66 (1.59 – 8.38)	0.002
	Intensive	36	2.03	0.48	1	7.63 (3.01– 19.34)	0.001

577 β , Regression coefficient; SE, Standard error; df, degrees of freedom; AOR, Adjusted odds ratio; CI, Confidence
578 interval; SLR, Small and large ruminants

579

580

581 **Table 12:** Spearman’s rank correlation of small ruminant farmer’s knowledge, attitudes and
582 practices levels towards brucellosis

583

Associations	Correlation coefficient	<i>p</i> value
Knowledge – Attitudes	0.205	< 0.001
Knowledge – Practices	0.390	0.008
Attitudes – Practices	0.022	0.847

584 Bold *p* values are statistically significant

585

586

587 **4. Discussion**

588 Brucellosis is widely recognized as an endemic zoonotic disease in Nigeria (Akinyemi et al.,
589 2022; Agada et al., 2018; Adamu et al., 2016; Olufemi et al., 2018), with numerous studies
590 documenting its occurrence across diverse livestock production systems. In the present study, a
591 high flock-level seroprevalence (71.4%) of anti-*Brucella* antibodies was observed, which was
592 approximately twice the overall individual-level seroprevalence (32.1%) detected in the milk of
593 sheep and goats. These findings suggest widespread exposure to *Brucella* spp. among small
594 ruminant flocks in the study area, where no formal brucellosis control or vaccination programme
595 exists. The detection of *B. melitensis* DNA in four randomly selected ELISA-positive milk
596 samples provides evidence that *B. melitensis* was present among the sampled small-ruminant
597 population; however, the limited number of samples subjected to PCR precludes estimation of its
598 population-level prevalence or inference regarding its wider distribution in the study area.
599 However, because the indirect ELISA used in this study detects antibodies against multiple

Brucella species without species differentiation and PCR was performed on only a subset of ELISA-positive samples, the serological findings should be interpreted as evidence of exposure to *Brucella* spp. rather than *B. melitensis* infection in all seropositive animals.

The discordance between the ELISA and PCR findings also warrants consideration. Of the 14 randomly selected ELISA-positive milk samples tested by PCR, 10 were negative for *B. melitensis* DNA. Because the ELISA detects antibodies against *Brucella* spp. without species differentiation, these samples cannot be interpreted as negative for *Brucella* infection or exposure. The discordant results may reflect exposure to *Brucella* species other than *B. melitensis*, including *B. abortus* or *B. ovis*, although these possibilities could not be evaluated because species-specific molecular assays for these organisms were not performed. Alternatively, the absence of detectable DNA may reflect low or intermittent bacterial shedding, limitations associated with the milk matrix and PCR inhibition, or degradation or insufficient quantity of target DNA. These possibilities cannot be distinguished from the present data. Therefore, the PCR results should be interpreted as confirming *B. melitensis* DNA only in the four tested samples, rather than as excluding other *Brucella* species or infection in the remaining ELISA-positive samples.

The prevalence of anti-*Brucella* antibodies was significantly higher in goats (54.4%) than in sheep (10.0%). Furthermore, species, source of animals, parity, history of flock abortion, body condition score, and flock-level history of antibiotic use was significantly associated with *Brucella* seropositivity. In addition, assessment of herders' knowledge, attitudes, and practices (KAPs) revealed that a substantial proportion demonstrated poor scores across all domains,

although several socio-demographic and farming-related factors were identified as significant predictors of good KAPs.

The overall seroprevalence of anti-*Brucella* antibodies (32.1%) observed in this study is relatively high and has important public health and economic implications, particularly in view of the widespread consumption of raw and unpasteurized milk in the study area. Previous studies in Nigeria have reported variable prevalence estimates. Similar prevalences of 38.1% and 25.8% were reported in Kaduna State (Dogo and Viv, 2016; Kaltungo et al., 2013). In contrast, lower prevalences of 2.06%, 6.0%, and 10.4% were reported in Borno and Yobe States (Brisibe et al., 1996; Tijjani et al., 2010; Adamu et al., 2022), while lower estimates have also been documented in Taraba, Kaduna, and Sokoto States (Olufemi et al., 2018; Buhari et al., 2020; Junaidu et al., 2010). These differences should be interpreted cautiously because studies varied considerably in their diagnostic methods, sample types, study populations, geographical settings, and sampling strategies. The indirect ELISA employed in the present study has been reported to possess higher sensitivity and specificity than RBPT and MSAT for detecting anti-*Brucella* antibodies in small ruminants (McDermott and Arimi, 2002; Legesse et al., 2023). Furthermore, the use of lactoserum instead of mastitic milk, cream, colostrum, or late lactation milk, which are more prone to false-positive reactions, may have improved the diagnostic performance of the assay (OIE, 2019).

Comparable seroprevalence estimates have been reported in Jordan (34.3%), Mali (32.5%), and India (32.9%) (Mussalam et al., 2015; Mussalam et al., 2019; Holt et al., 2021), whereas higher prevalences have been documented in Tanzania (44.4%) and Togo (62.0%) (Asakura et al.,

2018; Mussalam et al., 2019). Conversely, lower prevalences have been reported in Pakistan (18.6%) and Myanmar (17.2%) (Ali et al., 2017; Win et al., 2023). As with studies conducted in Nigeria, direct comparisons should be interpreted with caution because variations in diagnostic techniques, livestock management systems, animal species, breed composition, sample size, geographical location, introduction of replacement animals, and study design may substantially influence reported prevalence estimates (Dubie et al., 2025; Hussen et al., 2023).

The significantly higher seroprevalence observed among goats than sheep is consistent with reports from several countries (Kelkay et al., 2017; Tesfaye et al., 2012; Ullah et al., 2020; Geletu et al., 2021; Aloto et al., 2022; Kimera et al., 2025). This difference may reflect biological, behavioural, and management-related factors. Previous studies have suggested that goats are generally more susceptible to *Brucella* infection and may shed the organism in milk for longer periods than sheep (Poester et al., 2013; Aloto et al., 2022). Although sheep may also shed *Brucella* organisms, the duration of shedding is generally shorter, potentially limiting sustained transmission within sheep flocks (Radostits et al., 2007). Similarly, vaginal excretion following parturition or abortion has been reported to persist for up to three weeks in sheep but may continue for up to two months in goats (Aloto et al., 2022). In addition to these biological differences, management practices may have contributed to the higher seroprevalence observed among goats. Goats in the study area are more frequently grazed under extensive production systems, potentially increasing opportunities for exposure to contaminated environments. Furthermore, livestock restocking programmes implemented following the prolonged insurgency in northeastern Nigeria have distributed substantial numbers of goats to affected households. Many of these animals were reportedly sourced from neighbouring countries, including Niger,

Chad, and Cameroon, where brucellosis is endemic. Unregulated transboundary livestock movement has previously been identified as an important factor contributing to the persistence and spread of brucellosis in endemic regions (Refai et al., 2002; Cutler et al., 2005).

Animals originating from outside the study area and those sourced through livestock markets had significantly higher odds of *Brucella* seropositivity than household-bred animals. Similar associations have been reported in Nigeria and Ethiopia (Bertu et al., 2010; Asmare et al., 2013; Aloto et al., 2022). A possible explanation is that purchased animals may originate from herds with unknown health status, including herds affected by reproductive disorders compatible with brucellosis. The introduction of unscreened replacement animals has been recognized as an important mechanism for introducing *Brucella* infection into previously unaffected flocks (Hussen et al., 2023). In Maiduguri, livestock markets are characterized by unrestricted mixing of animals of different species, breeds, ages, and geographical origins, thereby increasing opportunities for disease transmission among animals from herds of unknown infection status.

Multiparous animals exhibited significantly higher odds of *Brucella* seropositivity than primiparous animals, consistent with previous reports (Hussen et al., 2023; Almuzaini, 2023). This association may be explained by the greater susceptibility of sexually mature and pregnant animals, in which elevated concentrations of erythritol and reproductive hormones create favourable conditions for the multiplication of *Brucella* organisms (Radostits et al., 2007). Similarly, animals originating from flocks with a reported history of abortion had significantly higher odds of seropositivity than those from flocks without such a history, supporting previous studies that identified abortion events as important indicators of *Brucella* circulation within

flocks (Aloto et al., 2022; Wadejo et al., 2015; Adamu et al., 2022). Animals with poorer body condition scores also demonstrated higher odds of seropositivity. This observation may reflect reduced immune competence, the chronic effects of infection, or other concurrent health and nutritional challenges that increase susceptibility to infectious diseases (Ullah et al., 2020; Tsegay et al., 2015). However, because some adjusted odds ratios were accompanied by wide confidence intervals, these estimates should be interpreted cautiously and confirmed in larger epidemiological studies.

An inverse association was observed between reported flock-level antibiotic use and *Brucella* seropositivity. However, this finding should not be interpreted as evidence that antibiotic treatment protects against brucellosis. Antibiotic use was reported at the flock level and did not establish whether the sampled animals had received treatment, which antimicrobial agents were used, or the indication and duration of treatment. Moreover, flocks reporting antibiotic use may differ from other flocks in veterinary access, socioeconomic status, husbandry, biosecurity, or other management characteristics that were not fully captured in the study. Such differences could confound the observed association. Therefore, the inverse association observed in this study should be regarded as an epidemiological association requiring further investigation rather than a causal or therapeutic relationship.

The present study demonstrated that most small ruminant herders had poor knowledge of brucellosis, with fewer than one-third having previously heard of the disease. Limited awareness was particularly evident regarding the zoonotic nature of brucellosis, its modes of transmission, clinical manifestations in animals and humans, and appropriate preventive measures. These findings are consistent with reports from other parts of Nigeria and several low- and middle-

income countries, where livestock keepers generally exhibit limited awareness of brucellosis despite living in endemic settings (Adamu et al., 2022; Musa et al., 2016; Lindahl et al., 2015; Holt et al., 2021). The low level of awareness observed among herders is of particular public health concern because inadequate knowledge may hinder early recognition of reproductive disorders associated with brucellosis, delay veterinary consultation, and perpetuate husbandry and food-handling practices that facilitate disease transmission within livestock populations and at the human–animal interface.

Several socio-demographic characteristics were significantly associated with knowledge of brucellosis. Herders with formal education, longer farming experience, previous contact with veterinary personnel, and access to animal health information demonstrated significantly better knowledge scores than their counterparts. Similar observations have been reported in Nigeria, Ethiopia, Pakistan, and Tanzania (Adamu et al., 2022; Hussen et al., 2023; Ali et al., 2017; Lindahl et al., 2015). Formal education may enhance the ability of herders to access, understand, and apply animal health information, while regular interaction with veterinary professionals provides opportunities for health education, disease recognition, and adoption of appropriate preventive measures. These findings highlight the importance of strengthening veterinary extension services and community-based awareness programmes as key components of brucellosis prevention and control in endemic areas.

Although attitudes towards brucellosis varied among respondents, a considerable proportion demonstrated attitudes that may undermine effective disease prevention and control. For example, reluctance to seek veterinary assistance following abortion events, failure to recognize

the public health importance of the disease, and limited perception of the risks associated with consuming raw milk and handling aborted materials may contribute to the continued circulation of *Brucella* spp. within livestock populations. Similar observations have been reported in previous studies conducted in Nigeria and elsewhere (Musa et al., 2016; Holt et al., 2021; Lindahl et al., 2015). Positive attitudes toward disease reporting, veterinary consultation, and implementation of biosecurity measures are essential for improving early detection and reducing opportunities for transmission.

The assessment of husbandry and food-handling practices also revealed behaviours that may increase the risk of *Brucella* transmission among animals and humans. Many herders reported consuming raw or unpasteurized milk, assisting animals during parturition without personal protective equipment, handling aborted fetuses and placental materials with bare hands, and disposing of aborted materials in open environments. These practices have consistently been identified as important pathways for zoonotic transmission of brucellosis in pastoral and agro-pastoral communities (Corbel, 2006; Ducrotoy et al., 2017; WHO, 2021). In the context of the present study, these behaviours are particularly important because serological evidence indicated widespread exposure of small ruminants to *Brucella* spp., while molecular analysis confirmed the presence of *Brucella melitensis* DNA in a subset of ELISA-positive sheep milk samples. Together, these findings underscore the potential public health risk associated with close human–animal contact and the consumption of unpasteurized dairy products in the study area.

Several socio-demographic and management-related factors were identified as significant predictors of good knowledge, positive attitudes, and appropriate practices. These findings

suggest that improving awareness alone may not be sufficient to change behaviours unless accompanied by accessible veterinary services, sustained community engagement, and practical interventions that support adoption of recommended husbandry and biosecurity measures. Similar conclusions have been reached in previous studies evaluating brucellosis prevention among livestock-keeping communities (Lindahl et al., 2015; Holt et al., 2021; Hussen et al., 2023).

Taken together, the serological evidence of widespread exposure to *Brucella* spp., molecular confirmation of *Brucella melitensis* in a subset of milk samples, and the observed deficiencies in herders' knowledge, attitudes, and practices highlight the interconnected nature of animal and human health in northeastern Nigeria. The persistence of risky husbandry practices, unrestricted animal movement, inadequate veterinary engagement, and frequent consumption of raw milk may facilitate continued transmission of brucellosis within livestock populations and increase opportunities for zoonotic infection. These findings reinforce the need for coordinated One Health interventions involving veterinary services, public health authorities, livestock extension personnel, and local communities to strengthen surveillance, promote farmer education, improve biosecurity, encourage safe milk consumption, and reduce the burden of brucellosis at the livestock–human interface.

Research limitations

Despite the strengths of this study, several limitations should be considered when interpreting the findings. First, the cross-sectional design limits the ability to establish temporal or causal relationships between *Brucella* seropositivity and the animal- or management-related factors

investigated. Accordingly, the observed associations should be interpreted as epidemiological associations rather than evidence of causality. In addition, 14 flocks were purposively selected from the 60 flocks identified in the study area based primarily on the availability of lactating animals and accessibility. Detailed information on the 46 non-selected flocks was not systematically collected; therefore, differences between selected and non-selected flocks could not be evaluated. If the excluded flocks differed systematically in management, veterinary access, animal movement, production system, or *Brucella* exposure, the prevalence estimates may not be fully representative of the broader small-ruminant population in the study area. The findings should therefore be generalized with caution, particularly to less accessible rural and nomadic production systems.

Second, although the calculated minimum sample size was 121 animals, 184 animals were ultimately sampled, comprising equal numbers of sheep and goats (92 each). This larger overall sample allowed balanced comparison between species; however, precision for the species-specific estimates was determined by the sample size of 92 animals within each species rather than by the total sample of 184. Thus, the species-specific prevalence estimates should be interpreted with consideration of their corresponding confidence intervals. Furthermore, milk sampling was restricted to lactating animals, potentially excluding infected non-lactating females and males and thereby limiting the extent to which the findings represent the entire small-ruminant population.

Third, the indirect ELISA used in this study detects antibodies against *Brucella* spp. and does not differentiate among *B. melitensis*, *B. abortus*, and *B. ovis*. Although the assay was used

808 according to the manufacturer's instructions, it was not independently validated under the
809 specific field and laboratory conditions of the study population. Moreover, serological testing
810 detects antibody responses and cannot distinguish active infection from previous exposure.
811 Consequently, ELISA-positive animals should be interpreted as having evidence of *Brucella*
812 exposure rather than as having confirmed species-specific infection or active bacterial shedding
813 at the time of sampling.

814
815 Fourth, molecular confirmation was limited by financial constraints to 14 randomly selected
816 samples from the 59 ELISA-positive milk samples. Four of the 14 tested samples were positive
817 for *B. melitensis* DNA, whereas 10 were PCR-negative. Thus, the PCR findings provide species-
818 specific molecular evidence of *B. melitensis* only within the tested subset and cannot be used to
819 estimate the prevalence of *B. melitensis* in the entire study population or extrapolated to all
820 ELISA-positive samples. The 10 ELISA-positive but PCR-negative samples cannot be assumed
821 to represent false-positive serological results. Possible explanations include exposure to other
822 *Brucella* species, such as *B. abortus* or *B. ovis*, low or intermittent bacterial shedding, PCR
823 inhibition associated with the milk matrix, or degradation or insufficient quantity of target DNA.
824 However, these possibilities could not be distinguished because molecular assays targeting other
825 *Brucella* species were not performed. Bacterial culture was also not undertaken; therefore, viable
826 *Brucella* organisms were not isolated, and the PCR findings represent detection of bacterial DNA
827 rather than direct demonstration of viable organisms. Although both positive and negative PCR
828 controls were included as quality-control measures, the limited number of molecularly tested
829 samples remains an important constraint on species-level interpretation.

Fifth, the very large adjusted odds ratios observed for some variables, together with wide confidence intervals, indicate limited precision and possible sparse-data effects. These estimates should therefore not be interpreted as precise measures of effect. Similarly, the observed associations between animal characteristics, animal sources, reproductive history, body condition, and *Brucella* seropositivity do not establish that these factors independently caused infection. Rather, they identify factors associated with seropositivity within the study population and should be interpreted in the context of the study design and analytical limitations.

Sixth, antibiotic use was assessed at the flock level from herder recall and referred to the reported administration of antibiotics to one or more animals within the flock during the three months preceding sampling. Information was not available on the specific antimicrobial agents used, their indication, dosage, duration, or whether the individual animal sampled for milk had received treatment. Therefore, the inverse association observed between reported flock-level antibiotic use and *Brucella* seropositivity should not be interpreted as evidence of a protective or therapeutic effect of antibiotics against brucellosis or as evidence of suppression of bacterial shedding. The finding may also be influenced by recall or exposure misclassification and by unmeasured confounding related to veterinary access, socioeconomic circumstances, husbandry, biosecurity, management quality, or other factors associated with antibiotic use. Similarly, reported flock-level abortion history does not necessarily indicate abortion in the particular animal sampled and should not be interpreted as a direct causal determinant of seropositivity.

Finally, the KAP component had limitations related to the sampling frame and the nature of self-reported information. The 360 herders were selected from a broader operational sampling frame

that included herders associated with the 60 initially identified flocks as well as additional larger flocks. This KAP sampling population was separate from the 14 flocks purposively selected for milk sampling; consequently, individual KAP responses could not be directly linked to the laboratory status of a particular sampled animal or flock. Because a comprehensive official register of all small-ruminant herders in the study area was not available, the operational sampling frame was developed with assistance from community leaders and livestock association representatives. Although eligible participants were subsequently selected using simple random sampling from this operational frame, herders not captured during the identification process may have been excluded, potentially introducing selection bias and limiting representativeness. In addition, KAP responses were self-reported and may have been affected by recall and social desirability biases, particularly for questions concerning potentially undesirable practices. The study was also conducted within Maiduguri metropolis and may not fully reflect practices and disease patterns among herders in more remote rural or nomadic production systems across Borno State.

Despite these limitations, the study provides important epidemiological and behavioral evidence regarding *Brucella* exposure and brucellosis-related KAPs among small-ruminant herders in an urban setting of northeastern Nigeria. Future studies should consider probability-based selection of a larger and more geographically representative number of flocks, adequate species-specific sample sizes, explicit adjustment for flock-level clustering in statistical analyses, and broader molecular characterization of ELISA-positive samples using assays capable of detecting and differentiating multiple *Brucella* species. Where feasible, bacterial culture and complementary molecular approaches would further strengthen species attribution and characterization of viable

organisms. These approaches would improve the precision, generalizability, and epidemiological interpretation of future estimates of *Brucella* infection and exposure in small-ruminant populations.

5. Conclusion

This study demonstrated widespread exposure of small ruminants to *Brucella* spp. in Maiduguri metropolis, with a high flock-level seroprevalence and significantly higher individual-level seropositivity among goats than sheep. Molecular analysis further confirmed the presence of *Brucella melitensis* DNA in a subset of ELISA-positive sheep milk samples, providing evidence that this species is circulating within the study area. The detection of anti-*Brucella* antibodies in milk highlights the potential public health risks associated with the widespread consumption of raw and unpasteurized milk in this endemic setting.

Several animal- and flock-level factors were significantly associated with *Brucella* seropositivity, including species, source of animals, parity, flock history of abortion, body condition score, and flock-level history of antibiotic use. These findings suggest that livestock movement, reproductive events, and husbandry practices may contribute to the persistence and transmission of brucellosis within small ruminant flocks. Concurrently, the study revealed generally poor knowledge, attitudes, and practices regarding brucellosis among small ruminant herders, characterized by limited awareness of its zoonotic importance and widespread engagement in behaviours that may increase the risk of transmission, including the consumption of raw milk and the unprotected handling of aborted materials. The observed associations between KAPs and socio-demographic and farming-related characteristics further emphasize the

importance of education, veterinary extension services, and improved husbandry practices in promoting safer livestock management.

Overall, these findings underscore the need for integrated brucellosis prevention and control strategies in Maiduguri and other endemic settings. Such strategies should adopt a One Health approach that strengthens animal and public health surveillance, promotes routine farmer education, improves access to veterinary services, encourages milk pasteurization, and supports appropriate regulation of livestock movement. Addressing both the biological and behavioural drivers of transmission will be essential for reducing the burden of brucellosis in small ruminants, minimizing zoonotic transmission, and protecting the health and livelihoods of livestock-dependent communities.

Declaration section

Abbreviations

PCR: Polymerase Chain Reaction

ELISA: Enzyme-linked immunosorbent assay

KAPs: Knowledge-Attitudes-Practices

DNA: Deoxyribonucleic acid

OR: Odds ratio

AOR: Adjusted Odds ratio

SLR: Small and large ruminants

Ethical approval and consent to participate

Ethical approval for this study was obtained from the Scientific Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. A letter of

exemption (Reference No. FVM/UNIMAID/AUEC/2024-008) was issued, as the study was considered exempt under Nigerian Decree Law No. 113/2013 (Directive 2010/63/EU). Written informed consent was obtained from all participating herders before enrolment in the study. The study objectives and procedures were explained in the participants' preferred local language (*Hausa, Kanuri, or Shuwa Arabic*) to ensure informed participation. Participation was entirely voluntary, and respondents were informed of their right to withdraw from the study at any stage without any consequences. Confidentiality and anonymity were strictly maintained throughout the study, and all procedures involving human participants adhered to the ethical principles of the “Declaration of Helsinki”.

Consent for publication

Not applicable

Availability of data and materials

Data may be available upon reasonable request from the corresponding author.

Competing Interests

The authors declare no conflict of interests

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Authors' contributions

AOT, SMJ, MAS: Conceptualization, Investigation Methodology, Visualization, Formal analysis, Project administration, Supervision; BT: Field sampling, data curation, Laboratory analysis, Writing-original draft; AOT, SMJ, MAS, BT, WAB: Writing-review and editing; All authors read and approved the final manuscript.

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