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# Risk perception, seroprevalence, and real-time PCR detection of *Brucella* among pyretic patients and domestic animals in Kwara State, Nigeria

Nusirat Elelu[1,5]✉, Bilqees A. Yakub-Obalowu[1,5], Nwachukwu Raymond Chinedu[1], Ismail A. Odetokun[1] & Ahmad I. Al-Mustapha[2,3,4]✉

**Brucellosis is a neglected, reportable, and under-reported zoonosis that poses a significant public health challenge in endemic countries like Nigeria. In this study, the risk perception was assessed and 103 pyretic patients from three local government areas of Kwara State, Nigeria were screened by using Rose Bengal plate test (RBPT) and confirmed the analytical sensitivity through real-time PCR (qPCR). We further screened 150 cattle and 52 goats from within a 1-km radius of pyretic patients to determine disease burden in domestic animal reservoirs. Our findings revealed that most of the farmers have heard of brucellosis (89.3%, *n* = 92/103) while 38.8% (*n* = 40/103) reported they could identify the symptoms of brucellosis in their herds. The most important risk factors were the consumption of raw milk (92.2%, *n* = 95/103) and animal abortion within the last year (19.4%, *n* = 20/103). Also, most study participants (94.2%, *n* = 97/103) did not believe that they could be infected with the disease. The seroprevalence of brucellosis in humans was 5.8% (*n* = 6/103) and there was 100% concordance between the results of the RBPT and qPCR in humans. The seroprevalence was 18% and 25% in cattle and goats respectively. The qPCR revealed a lower prevalence rate of 11.3% and 15.4% in cattle and goats respectively. There was high discordance in sensitivity between the RBPT and qPCR in animal samples screened, although both tests had an analytical specificity of 100%. There was no association between awareness and positivity for brucellosis among the study participants and none of the sociodemographic variables significantly influenced the brucellosis positivity rate of humans. Public health physicians and community health workers should consider brucellosis as a differential diagnosis when clerking pyretic patients. In addition, we advocate for mass animal vaccinations and public education with an emphasis on identified risks.**

**Keywords** Brucellosis, Zoonoses, Foodborne pathogen

Brucellosis is a neglected zoonotic disease that causes an estimated 1.6–2.1 million new cases with approximately 25% (500,000) new cases in Africa annually[1]. The disease is typically characterized by nonspecific influenza-like illness manifesting as undulating fever, sweats, fatigue, and malaise, which are similar signs and symptoms to those of malaria, one of the most commonly acquired infectious diseases in resource-limited regions[2–4]. Furthermore, undulant fever, arthritis, myocarditis, and neuropathies can occur among chronic cases of human brucellosis[1,5].

In most low and middle-income countries, the true burden of brucellosis is poorly understood and misconceptions about its true incidence often arise from gross underreporting and insufficient epidemiological

[1]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Kwara State, Nigeria. [2]Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland. [3]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria. [4]Department of Veterinary Services, Kwara State Ministry of Agriculture and Rural Development, Ilorin, Kwara State, Nigeria. [5]Nusirat Elelu and Bilqees A. Yakub-Obalowu are co-first authors. ✉email: elelu.n@unilorin.edu.ng; ahmad.al-mustapha@helsinki.fi

monitoring data, lack of financial resources and technical capacity, non-availability of brucellosis diagnostic tests at rural health facilities, and poor one-health integrated disease surveillance[6]. These factors have allowed the disease to increase unchallenged in the marginalized human and animal populations of the world.

In Nigeria, the results of a recent meta-analysis of the national seroprevalence of human and animal brucellosis revealed a prevalence of 17.6% ($n = 554/3144$) and 13.3% ($n = 8547/64,435$), respectively[6]. The study further reported a higher brucellosis burden (15.8%) in northern Nigeria as against 8.7% in the southern states of Nigeria[6]. In Kwara State, Bamidele et al.[7] reported 22.2% among abattoir workers and 9.6% among cattle slaughtered at the Ilorin Metropolitan abattoir. Another study also reported a prevalence of 14.2% in cattle using the rose Bengal plate test (RBPT)[8].

The RBPT is the most common diagnostic tool used in brucellosis screening studies in Nigeria[6] and only one study previously used PCR as a molecular diagnostic tool for brucellosis in Nigeria[9]. Therefore, this study aimed to detect the presence of *Brucella* spp. in blood samples from pyretic patients presenting at pre-selected health centers (PHCs) in three pilot Local Government Areas (LGAs) of Kwara State. We also wanted to differentiate chronic from acute infections by screening all RBPT-positive samples using real-time polymerase chain reaction (qPCR). Understanding the true prevalence of *Brucella* spp. in pyretic patients is crucial to its immediate reporting and the establishment of effective control and prevention strategies in addition to its use in patient care[10]. In animals, this study emphasizes the public health threat posed by food-borne pathogens and underscores the need to safeguard public health in Nigeria.

## Methods

### Ethical considerations

The ethical approval for the study was granted by the University of Ilorin ethical review committee (Approval number: UERC/ASN/2018/1387) and the Kwara State Ministry of Agriculture and Rural Development as well as the Kwara State Ministry of Health. A personalized written informed consent was obtained from each respondent after brief information on the purpose of the study was provided to them by the attending healthcare worker. These medical personnel had already been trained on the objectives of the study, the survey instrument, and the volume of blood samples needed. We made sure that study participants knew that participation was voluntary and without prejudice as specified in the World Medical Association Declaration of Helsinki Ethical principles. The study participants were also pre-informed that they were free to withdraw from the survey at any time and no personal identifying information was obtained from any of the study participants. Finally, we informed consenting participants that we will visit their household to sample domestic animals prior to blood sampling.

The animal sampling was conducted in accordance with the ARRIVE guidelines. An informed consent was obtained from each animal owner prior to blood sampling. In certain cases, the consent was obtained from the head of the settlement.

### Risk perception survey

The study was conducted as a cross-sectional risk perception survey of pyretic patients who presented at any of the pre-selected health centres in three LGAs in Kwara State, North Central Nigeria (Fig. 1) All participants were adults who were 18 years or above and presented to these health centres with a fever greater than 38.3 °C. The semi-structured survey administered to each participant obtained information about the socio-demographics, exposure to brucellosis, and awareness of brucellosis. The instrument was pre-tested among 10 patients from two health centres prior to its final deployment for data collection. The questionnaire was prepared in English and translated into the local dialect (Fulfulde, Hausa, or Yoruba) of the respondents during the face-to-face interviews. The objective and purpose of the study were explicitly described to the respondents prior to the

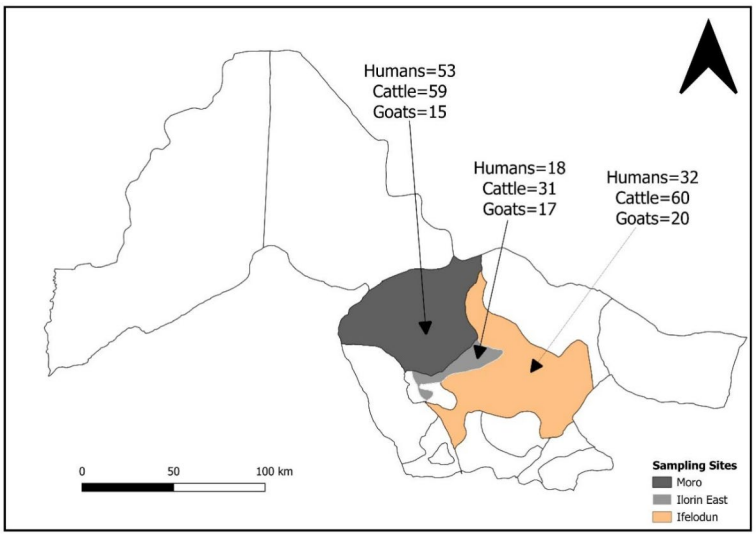


**Fig. 1**. Map of Kwara State showing the sampling locations.

interview, and their oral consent to participate in the study was obtained. The survey response was only obtained from those who provided consent and participated voluntarily in the study.

### Sampling method and sample collection

The study was designed to screen pyretic patients presenting in selected health centres in three LGAs of Kwara State. This selection was motivated by an increasing report of malaria-negative pyretic patients across health facilities in Kwara State. The addresses of these patients were obtained, and we further screened domestic animals within a 1 km radius of these patients. The human sampling was purposively conducted from six health centres (two from each LGA) and targeted towards patients presenting with fever above 38.3 °C. An informed consent from each patient was obtained by the attending medical officer in the health facility before sample collection. A total of 103 pyretic patients were included in this study. For each study participant, blood samples were collected into heparinized bottles, labeled and preserved in ice packs, and transported to the molecular laboratory of the Department of Veterinary Public Health and Preventive Medicine at the University of Ilorin for further processing. The collected blood samples were centrifuged at 2000 rpm for 5 min for plasma separation. The resultant plasma samples were dispensed into well labelled cryovials and stored in a freezer at − 20 °C until used for further assay.

The animal sampling was conducted as a simple randomized sampling of 150 samples from cattle and 52 samples from small ruminants from the three LGAs: Moro, Ifelodun, and Ilorin East. All field samplings were conducted between November 2023 and February 2024 in domestic animals that were in close proximity to pyretic patients.

### Rose Bengal plate test

The Rose Bengal Plate Test was utilized as the initial screening of the sera collected from both humans and ruminants using the ready-to-use *Brucella* species antigen (Veterinary Laboratory Agency (VLA), Surrey, United Kingdom), according to the manufacturer's instructions. Briefly, 30 µl of plasma was taken using a clean Pasteur pipette and placed on a test tile. An equal drop of RBT antigen was added using another clean Pasteur pipette. Then it was mixed well using a sterile applicator stick and spread onto entire circles about 2 cm in diameter. Cards were manually rotated for four minutes according to a previously described procedure[11]. The presence of any degree of clumping or distinct pink granules (agglutination) was recorded as a positive result while samples that appeared clear without agglutination granules were recorded as negative.

### DNA extraction

DNA was also extracted from an aliquot of the plasma samples collected from both animal handlers and ruminants in their close proximity. This was carried out using the commercial DNeasy Blood and Tissue Kit (Qiagen, Milan, Italy) according to the manufacturer's instructions. The plasma was resuspended in 220 µl of phosphate-buffered saline (PBS). Some 200 µl of lysis buffer (AL) was added, mixed thoroughly by vortexing, and incubated for 10 min at 56 °C. Afterwards, 200 µl of 100% ethanol was added and thoroughly mixed. The mixture was then transferred into a mini spin column placed in a 2 mls collection tube and centrifuged at 8000 rpm for 1 min, then the flow through and collection tube was discarded. The spin column was then placed in a new 2 ml collection tube, and 500 µl of wash buffer (AW2) was added and centrifuged at 8000 rpm for 1 min. The flow through and collection tube were discarded. The spin column was placed in new collection tube, 500 µl wash buffer was added and centrifuged at 14,000 for 3 min. The flow through and collection tube were discarded. Finally, the spin column was transferred to a 2 ml micro-centrifuge tube. The DNA was eluted by adding 200 µl of elution buffer to the spin column membrane for 1 min at room temperature and then centrifuged at 8000 rpm for 1 min.

### Real-time PCR assay conditions

The real-time PCR for the *Brucella genus* was performed using the Biobase LEIA-X4 instrument for genomic amplification using the following parameters: initial denaturation at 95 °C for 10 min followed by 45 cycles of 95 °C for 15 s and 58 °C for 1 min. Each 20 µl qPCR reaction mix consisted of 10 µl of the Sso Advanced Universal SYBR Green Supermix (BioRad, Italy), 6 µl of the *Brucella genus* primer (forward; 5′-CATATCGTT GCGCGTAAGGA-3′, reverse; 5′-GAAACGCGCTTGCCTTTC-3′)[12], and 4 µl of purified DNA template. Data were analyzed with the Biobase qPCR software. We used double distilled water as a negative control throughout the reactions and used the DNA of an in-house isolate as the positive control. A cycle threshold (Ct) value of 36 or lower was used as the threshold for the analysis of isolates.

### Data analysis

The responses obtained from the risk perception survey were imported into SPSS software (version 28). We conducted descriptive statistics and presented the qualitative data as frequencies and percentages and the quantitative data as mean ± standard deviation. Additionally, we use the Chi-square analysis to test for association between the socio-demographic variables and the occurrence of brucellosis in humans and domestic respectively. The map was designed using QGIS v. 3.38.

## Results

### Structure of respondents

The information obtained from 103 persons from whom blood samples were also collected revealed that more respondents were of the female gender (70%, $n = 72/103$), had no formal education (77.7%, $n = 80/103$), and were farmers in addition to rearing livestock (68%, $n = 70/103$). Most of them were also married (Table 1). Most of the study participants had herds of cattle (95.1%, $n = 98/103$), small ruminants (61.2%, $n = 63/103$), or both

| Variable | Frequency (%) |
|---|---|
| 1. Age | |
| 18–30 | 42 (40.8) |
| 31–40 | 28 (27.2) |
| 41 and above | 33 (32) |
| 2. Gender | |
| Female | 72 (70) |
| Male | 31 (30) |
| 3. Location | |
| Ifelodun | 32 (31.1) |
| Ilorin East | 18 (17.5) |
| Moro | 53 (51.4) |
| 4. Level of education | |
| No formal education | 80 (77.7) |
| Primary school | 15 (14.6) |
| Secondary school | 8 (7.7) |
| 5. Occupation | |
| Farmer | 70 (68) |
| Trader | 16 (15.5) |
| Unemployed | 17 (16.5) |
| 6. Ethnicity | |
| Fulani | 60 (58.3) |
| Hausa | 28 (27.2) |
| Yoruba | 15 (14.5) |
| 7. Marital status | |
| Single | 12 (11.7) |
| Married | 85 (82.5) |
| Divorced | 6 (5.8) |

**Table 1**. Socio-demographic variables among study participants ($n = 103$).

(58.3%, $n = 60/103$). Approximately two-thirds (68%, $n = 102/150$) of the samples were from cows whereas 32% ($n = 48/150$) were from bulls. Also, more blood samples were obtained from doe (75%, $n = 39/52$) than from bucks (25%, $n = 13/52$). The samples were distributed between Ifelodun (20 goats and 60 cattle), Ilorin East (17 goats and 31 cattle), and Moro (15 goats and 59 cattle).

## Awareness of brucellosis

Most farmers have heard of brucellosis (89.3%, $n = 92/103$) and some of them could identify the symptoms of brucellosis in their herds (38.8%, $n = 40/103$). Most of them still regularly consume unpasteurized milk (92.2%, $n = 95/103$), and do not believe that they could be infected with the disease (94.2%, $n = 97/103$). In terms of disease burden, approximately 20% of the farmers have experienced abortion in the last 1 year and others have handled aborted animals either from their animals or from other herds (Table 2).

## Sero-prevalence and qPCR of brucellosis

The seroprevalence of brucellosis in humans was 5.8% as the RBPT revealed that only six samples were positive among the 103 human samples screened. There was 100% concordance between the results of the RBPT and qPCR in humans. The CT value in human samples ranged from 27.95 to 28.37 with a mean CT value of $27.73 \pm 0.26$.

On the contrary, there was a high discordance between the RBPT and qPCR results for cows with 27 of the 150 samples testing positive for the RBPT against 17 that tested positive by qPCR (Table 3). Also, 13 of the 52 samples tested positive by RBPT as against 8 samples that tested positive by qPCR. The CT value of the qPCR positive samples ranged from 18.52 to 34.45 ($25.85 \pm 5.43$) in goats and 22.4 to 32.34 with a mean CT value of $27 \pm 2.94$ in cattle respectively. The diagnostic specificity was 100% in cows, humans, and goats as none of the samples that tested negative in RBPT tested positive by qPCR (result not shown).

## Factors associated with brucellosis prevalence

There was no association between awareness and positivity for brucellosis among the study participants ($p > 0.05$). In addition, none of the sociodemographic variables significantly influenced the brucellosis positivity rate of humans (Table 4), and cattle (Table 5). However, there was a statistically significant difference in the burden of brucellosis in goats between the three sampling sites (Table 6).

| Variable | Frequency (%) |
|---|---|
| Have you heard of brucellosis? | |
| No | 11 (10.7) |
| Yes | 92 (89.3) |
| Source of information | |
| Friends/family | 29 (28.1) |
| Animal health professionals | 20 (19.4) |
| Marketplace | 54 (52.4) |
| Can you identify symptoms of brucellosis in your animals? | |
| No | 63 (61.2) |
| Yes | 40 (38.8) |
| Can you or others be infected with brucellosis? | |
| No | 97 (94.2) |
| Yes | 6 (5.8) |
| Do you or your immediate family members drink unpasteurized milk? | |
| No | 8 (7.8) |
| Yes | 95 (92.2) |
| Did any of your animals abort in the last year? | |
| No | 83 (80.6) |
| Yes | 20 (19.4) |
| Did you handle aborted materials in the last year from your animals or others? | |
| No | 40 (38.8) |
| Yes | 63 (61.2) |
| Did you notice any other clinical signs? | |
| No | 33 (32) |
| Yes | 70 (68) |

**Table 2**. Awareness and risk perception of brucellosis among transhumance settlers in Ilorin, Kwara State, Nigeria ($n = 103$).

| Variable | RBPT | | qPCR | | Concordance (%) |
|---|---|---|---|---|---|
| | Negative | Positive | Negative | Positive | |
| Humans ($n = 103$) | 97 | 6 | 97 | 6 | 100 |
| Cattle ($n = 150$) | 123 | 27 | 133 | 17 | 63 |
| Small ruminant ($n = 52$) | 39 | 13 | 44 | 8 | 61.5 |

**Table 3**. Detection of *Brucella* spp. In humans and domestic animals, Kwara State, Nigeria.

## Discussion

To our knowledge, this is the first study to utilize both RBPT and qPCR for the detection of *Brucella* spp. in Nigeria. Our findings revealed *Brucella* could be easily missed in pyretic patients if unscreened. Hence, a public health challenge and a recipe for its underreporting. We do not have evidence to support whether any of the patients fall under the clinical definition of fever of unknown origin (FUO)[13]. We do know that brucellosis is not screened in clinics across the country and available data on the human population are from research and academic studies. Hence, routine screening of at-risk patients should be instituted across the country.

The RBPT has been reported to have a diagnostic sensitivity of 100% and a specificity of 98.69%[11]. Hence, it has maintained its scientific application and acceptance in the diagnosis of infectious diseases. In comparison to culturing or nucleic acid amplification methods, it is inexpensive and technically simple. Despite this, qPCR could also offer equally 100% diagnostic sensitivity and specificity for many infectious diseases[14,15]. This is primarily because qPCR is based on the extracted genomic materials usually present in active infections whereas seroprevalence may not show current infection or shedding of the pathogen since animals may seroconvert without detectable shedding and can remain seropositive for years post-infection or sometimes may not seroconvert during active shedding of infection[16]. In 2019, a study reported the diagnostic usefulness of qPCR in early diagnosis of infectious diseases in patients as opposed to the use of serological testing which requires antibody formation approximately 2 to 3 weeks after onset of symptoms before they could be detected[17]. Here, we considered all RBPT as true positives and the discordance between RBPT and qPCR (only in domestic animals) could be due to the stage of disease progression.

| Variable | qPCR+ | qPCR− | $X^2$ | *p*-value |
|---|---|---|---|---|
| Age | | | | |
| 18–30 | 2 | 40 | 1.76 | 0.439 |
| 31–40 | 3 | 25 | | |
| 41 and above | 1 | 32 | | |
| Gender | | | | |
| Female | 4 | 68 | 0.03 | 0.859 |
| Male | 2 | 29 | | |
| Location | | | | |
| Ifelodun | 2 | 30 | 2.434 | 0.296 |
| Ilorin East | 0 | 18 | | |
| Moro | 4 | 49 | | |
| Level of education | | | | |
| No formal education | 6 | 74 | – | – |
| Primary | 0 | 15 | | |
| Secondary | 0 | 8 | | |
| Consume raw milk? | | | | |
| No | 0 | 8 | – | – |
| Yes | 6 | 89 | | |

**Table 4**. Test of association between socio-demographic variables and the positivity for *Brucella* genus by qPCR in humans.

| Variable | qPCR+ | qPCR− | $X^2$ | *p*-value |
|---|---|---|---|---|
| Sex | | | | |
| Bull | 4 | 44 | 0.66 | 0.415 |
| Cow | 13 | 89 | | |
| Location | | | | |
| Ifelodun | 9 | 51 | 1.67 | 0.433 |
| Ilorin East | 2 | 29 | | |
| Moro | 6 | 53 | | |

**Table 5**. Test of association between socio-demographic variables and the positivity for *Brucella* genus by qPCR in cows.

| Variable | qPCR+ | qPCR− | $X^2$ | *p*-value |
|---|---|---|---|---|
| Sex | | | | |
| Buck | 1 | 12 | 0.891 | 0.345 |
| Doe | 7 | 32 | | |
| Location | | | | |
| Ifelodun | 7 | 13 | 11.412 | 0.003 |
| Ilorin East | 0 | 17 | | |
| Moro | 1 | 14 | | |

**Table 6**. Test of association between socio-demographic variables and the positivity for *Brucella* genus by qPCR in goats.

As there was 100% concordance between RBPT and qPCR in clinical samples, we advocate for routine clinical screening of pyretic patients for brucellosis. The 5.8% brucellosis prevalence detected in pyretic patients in this study was higher than the prevalence previously reported in Sokoto (0.7%)[18], and Borno (3.8%)[19] but lower than those previously reported in Kwara state (22.2%)[7], and the national pooled prevalence of 17.6%[6]. The prevalence in humans is also within the range of 0–55.8% reported by Qureshi et al.[20] and Djangwani et al.[10] in the sub-Saharan African region, highlighting the significant presence of brucellosis infection in this area. These differences in prevalence could be due to the differences in disease burden across the country and sampling strategy. Our findings highlight the substantial health burden of brucellosis and the potential for misdiagnosis as malaria or other febrile illnesses[21]. The burden of brucellosis in humans could be attributed to risk factors such as

close contact through occupational exposure[22], the consumption of raw dairy products[23–25], and religious beliefs that denounce practices that include the test and slaughter of positive livestock[26,27].

Among the 150 cattle samples screened, the RBPT revealed a prevalence of 18% which was higher than the national pooled prevalence of 12.2% in cows[6] and the regional pooled prevalence of 8.6% from the 3213 cattle screened in the North Central region between 2001 and 2021[6]. This prevalence was also higher than the 9.6% reported in cattle slaughtered at the Ilorin Metropolitan abattoir in 2020[7] and the 14.2% reported in samples collected from cattle farms in 2017[8]. According to a recent global brucellosis review, the prevalence of brucellosis in cattle in Sub-Saharan Africa ranged from 0.2–43.8%[20]. The RBPT also revealed a prevalence of 25% in goats which was higher than the North Central pooled prevalence of 19.2%, the national pooled prevalence of 10.2%[6], and the range of 0.0–20.0% in goats[20].

Generally, controlling brucellosis in humans involves controlling the disease in its reservoir animal hosts. However, small-scale livestock keepers have been known to avoid diagnostics for lack of compensation and fear of consequences (e.g., the slaughter of their entire herd) if a single animal were to test positive for brucellosis[2,22]. Typically, brucellosis treatment is discouraged, and the affected animals are usually culled[2] because of the carrier status, the emergence of antibiotic-resistant strains, and the need for continued treatment to avoid recurrence. The chronic nature of brucellosis, combined with the capacity of *Brucella* to reside within host cells and sequester at difficult-to-reach sites, can contribute to treatment relapse[20]. The relapse rate in uncomplicated cases is estimated to be 5–15%. The cause of these relapses is unclear because of the emergence of antimicrobial resistance (AMR) or the inability to eradicate germs at the infection sites. However, studies on *Brucella* in endemic middle-income countries have generally shown that bacteria remain susceptible to doxycycline and rifampicin, which are commonly used antibiotics for brucellosis treatment[20,28,29].

The qPCR assay revealed 100% concordance in human clinical samples but significant discordance in the samples from cattle and goats. The qPCR revealed a prevalence of 11.3% in cattle (as against 18% by RBPT) and 15.3% in goats (as against 25% by RBPT). The discordance in the diagnostic or analytical sensitivity could be attributed to the proven lack of serological assay to detect ongoing infections with the potential for detectable antibodies to persist for a long. Since pyretic clinical samples were collected, antibodies may coincide with the presence of an ongoing infection. Despite the discordant diagnostic sensitivity between these two highly reliable tests, the diagnostic specificity was 100% as there were no false negative samples detected. This qPCR was previously reported to be a highly sensitive and specific diagnostic tool for brucellosis with a low CT value[12].

As an endemic, under-reported, and neglected zoonoses, the surveillance of *Brucella* spp. could be improved using one health approach and a combination of RBPT and qPCR assay. The evidence of exposure to brucellosis is worrisome because, aside from its negative effect on reproductive performance such as abortion, stillbirth, and reduced productivity of livestock, it is a persistent and highly transmissible pathogen to humans[16,30]. In addition, the disease is associated with significant economic losses[3,31]. One key intervention needed to reduce the burden of brucellosis in Nigeria is vaccination which was reported to be cost-effective for brucellosis control instead of the test-and-slaughter approach[32]. In addition, Nigeria must work to improve animal identification systems including animal tagging and movement control. Some countries have achieved eradication through a combination of efficient diagnosis, vaccination, and test-and-slaughter[33]. Hence, mass animal vaccinations using the *B. abortus* S19 or the RB51 vaccine should be instituted across brucellosis hotspots across the country[33].

As our analysis revealed that none of the sociodemographic variables is associated with the occurrence of brucellosis in humans or cattle, it implies that any public health intervention should be channeled towards all age groups, gender, and level of education across the state with a focus on reducing identified risk behaviours. Generally, more emphasis should be placed on public awareness on reducing the consumption of raw milk particularly in persons that were observed to be drinking raw milk directly from the udder of the cow, since *B. abortus* has been isolated from raw and sour milk of Fulani cattle in Nigeria[22]. While there was no statistically significant association between the different sampling points in humans and cattle, there were significant variations between the burden of brucellosis in each of the three LGAs with more cases in Ifelodun than the other two LGAs. This was in agreement with the report of Ogugua et al.[23] who reported that herd location significantly impacted the occurrence of brucellosis in Southwestern Nigeria.

Since infected ruminants can shed *Brucella* spp. and other zoonotic pathogens, the Codex Alimentarius recommends the pasteurization of raw milk[34]. It is, therefore, essential to educate farmers on the need for pasteurization at the appropriate temperature as well as post-pasteurization handling of dairy products to reduce the burden of this disease. In addition, the various initiatives aimed at improving the dairy value chain in Nigeria must intensify screening for zoonotic milk-borne pathogens such as *Brucella* spp. Furthermore, other important endemic zoonotic pathogens such as *Coxiella burnetii*, *Mycobacterium bovis* (zoonotic tuberculosis), *Salmonella* spp., *Listeria* spp., *E. coli*, *Campylobacter* spp., and *Clostridium* spp. could be transmitted through the consumption of unpasteurized milk.

One of the main strengths of this study is its uniqueness and novelty in the study area. It is the first published work that utilized both RBPT and qPCR for the detection of *Brucella* spp. in Nigeria in pyretic patients and domestic animals in their proximity. Despite this, the following limitations may affect its widespread replication in Nigeria: sampling bias, small sample size, and the study's participants were limited to specific LGAs of the state, so the observed awareness and practices may not be representative of the entire state.

This study established that brucellosis should be considered as a differential diagnosis in pyretic patients in Kwara State. In addition, its high prevalence in cattle and goats could be a source of infection for humans as the bacteria is shed in milk which is consumed unpasteurized in certain parts of Nigeria. We advocate for mass screening for brucellosis, public enlightenment with emphasis on the identified risk factors, and mass vaccination of domestic animals. More molecular studies are needed to further study the genotypic relatedness of human and animal genotypes.

## Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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## Author contributions
NE and BY-O conceptualized the study. BY-O and NRC did the sample collection. NE, BY-O, NRC, IAO, and AIA did the molecular assays. NE, BY-O, and AIA wrote the draft manuscript. All authors read and approved of the final manuscript.

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## Declarations

## Competing interests
The authors declare no competing interests.

## Animal ethics approval
In addition to the institutional approval granted by the University of Ilorin ethical review committee, we obtained ethical approval from the Kwara State Ministry of Agriculture and Rural Development. The animal sampling was conducted in accordance with the ARRIVE guidelines. Individualized informed consent was obtained from each animal owner prior to blood sampling. In certain cases, the consent was obtained from the head of the settlement.

## Additional information
**Correspondence** and requests for materials should be addressed to N.E. or A.I.A.-M.