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Seroprevalence of *Brucella* antibodies among febrile human patients in Nsukka, Enugu State, Nigeria

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Abstract

Brucellosis is a zoonotic disease of major public health and economic importance. The presence of the disease in livestock within an area commonly translates to its occurrence in humans in the same environment. Although the disease is prevalent in livestock in Nigeria, data on its occurrence in humans in Nsukka, Southeast Nigeria is scarce in literature. The present study evaluated the seroprevalence of *Brucella* antibodies among febrile human patients in Nsukka, Enugu State, Nigeria. The study was a cross-sectional survey. Blood samples were collected from the 168 febrile patients that consented to participate in the study at the Bishop Shanahan Hospital Nsukka, Enugu State, Nigeria, from April to July 2023. The serum samples derived from centrifuging the blood samples were tested for *Brucella* antibodies with the Rose Bengal Test (RBT). The serum samples were further tested with the competitive enzyme-linked immunosorbent assay (cELISA) for *Brucella* antibodies as a supporting test. Results showed that out of the 168 febrile patients screened, 43 (25.60%) were positive with the RBT. However, with the cELISA test, only 9 (5.36%) of the samples were positive for *Brucella* antibodies. Based on the RBT results, 20.80% were positive for *Brucella* antibodies among febrile patients within the age group of 18 – 30 years, 43.75% among the 31 – 45 year age group and 27.27% within the age group of ≥ 45years. Further, based on the RBT test, seroprevalence of *Brucella* antibodies was 33.33% among the male participants but 24.11% among the females. Seroprevalence based on the RBT was significantly associated with age ($p = 0.029$), but not the gender ($p = 0.606$) of the participants. It is advised that doctors in human hospitals in Nsukka Nigeria should consider brucellosis in the differential diagnosis of diseases in febrile patients.

Keywords: *Brucella* antibodies; Febrile patients; Rose Bengal test; Competitive enzyme linked immunosorbent assay; Nsukka, Nigeria.

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Introduction

Brucellosis is a bacterial disease caused by the members of the genus *Brucella*. Most species of *Brucella,* though primarily host specific, also infect and cause disease in other hosts. The most common human pathogenic *Brucella* species and their primary hosts include *Brucella melitensis* commonly found in sheep and goat; *B. abortus* found in cattle; *B. suis* in found in pigs and *B. canis* found in dogs (Kurdoglu *et al.,* 2015; Bamaiyi, 2016). Being a zoonotic disease, its presence in livestock commonly translates to infection of persons in regular contact with the livestock. Transmission routes from infected animals to humans include contact with contaminated birth materials, ingestion of unpasteurized dairy products, consumption of uncooked or improperly cooked contaminated meat or meat products, laboratory acquisition as well as inhalation of aerosolized *Brucella* organisms (Hartady *et al.,* 2014). Most human infections are therefore associated with occupational exposure among veterinarians, herders, abattoir workers, laboratory workers as well as consumers of unpasteurized dairy products. There are also reports of cases of human-to-human transmission largely through coitus, maternal transfer, contact with contaminated birth materials (normal or aborted foetuses), blood transfusion, organ transplant and some undefined means (Tian *et al.,* 2019; Al-Ayyadhi *et al.,* 2023). The disease is ranked by the World Health Organization (WHO) among the world's seven most neglected diseases (Laine and Scott, 2022).

The most popular sign of brucellosis in humans is undulating fever (Mesner *et al.,* 2007). However due to multi-organ involvement, it presents a variety of clinical features including abortion, epididymo-orchitis, chronic joint pain, lymphadenitis, fatigue, spondylodiscitis (Dean *et al.,* 2012). Neurobrucellosis and endocarditis are severe complications of debility due to the disease (Zhang *et al.,* 2021). Given that the diagnosis of most diseases in Nigeria's primary health care system primarily depends on presented symptoms, such variations in the clinical presentations of the disease becomes very important. There is therefore a great deal of misdiagnosis, wrong and/or delay in treatment of brucellosis resulting in deleterious consequences. The risk of *Brucella* infection constitutes a major public health problem in Nigeria, because the disease is endemic in livestock population, and there are common practices of living in close proximity with livestock, consumption of unpasteurized dairy products, unhygienic slaughter, and also generally poor knowledge of the disease in the country.

Several cases of the brucellosis have been documented in different species of animals and humans in several parts of the Nigeria (Ducrotoy *et al.,* 2014). In the South East, a prevalence of 27.7% was reported in dogs in Anambra and Enugu States (Anyaoha *et al.,* 2020); 0.6% prevalence has been reported in pigs in South East Nigeria (Onunkwo *et al.,* 2011), and 34.7% prevalence has been reported in indigenous cattle breeds in Nsukka (Ogugua and Onunkwo*,* 2023). Further, 3% prevalence has been reported in slaughter horses in Enugu State (Njoga *et al.,* 2018), and 2.5% and 4.1% in cattle and goats in South East (SE) Nigeria, respectively (Ekere *et al.,* 2018). Meta-analyses of brucellosis in Nigeria by Akinyemi *et al*. (2022) showed a slightly higher prevalence of brucellosis in humans (17.6%) than animals (13.3%). In addition, a prevalence of 24.1% was recorded among slaughterhouse workers in Abuja (Aworh *et al.,* 2013). Although there are reports of the disease in animal populations in South East, Nigeria, studies on the prevalence of the disease in humans are scarce in available literature. The present study screened the serum of consenting febrile patients in Bishop Shanahan Hospital, Nsukka, South East, Nigeria for *Brucella* antibodies.

Materials and Methods

Study Area: This study was conducted in Nsukka, Enugu State, Nigeria (Figure 1). Nsukka has a population of 111,017 (World Population Review, 2023). The Bishop Shanahan Hospital Nsukka, used for the study, is a primary health care and referral hospital, and the biggest in the town. Nsukka town hosts the University of Nigeria, Nsukka. The major abattoir in the town is the Ikpa abattoir. Although there are small ruminants kept within many households, presence of piggeries and poultries; most cattle consumed in the area are sourced from northern parts of the country.

Study Design, Study Population, Sampling and Sample Size Determination: The study adopted the cross-sectional survey design. The concept behind the study was explained to potential participants and only individuals that consented were recruited into the study. Blood samples were collected from consenting febrile patients on Tuesdays and Thursdays from April to July, 2023. Only consenting febrile patients of 18 years and above that presented at Bishop Shanahan hospital Nsukka during the period were included. Persons below 18 years of age or with diminished ability to willingly consent, and those that did not consent were excluded from the study.

The sample size was calculated using the formula, n = 1.96^2*P*exp (1–*P*exp)/d^2 as earlier cited (Agada *et al.,* 2018) based on hospital prevalence of 7.6% in fever patients in Nigeria (Ofukwu *et al.,* 2007). A total of 108 samples from the hospital patients were calculated. However, a total of 168 samples were collected to improve robustness.

Ethical Clearance: Ethical approval for this study was obtained from the Health Ethics Committee of Anambra State Ministry of Health with approval number MH/AWK/M/321/344.

Collection and Handling of Samples: Due consent was sought from the potential participants following standard practices. Collection of samples was done by the phlebotomists in the Diagnostic Laboratory of the hospital. About 3 ml of blood was collected using plain sample bottles, with the sex and age of the patients noted. The blood samples were kept in a slanted position, allowed to clot and transported in coolers containing ice packs to the Department of Veterinary Public Health and Preventive Medicine, University of Nigeria Nsukka Laboratory where they were centrifuged at 3000g for 10 minutes. The sera were decanted into serum bottles and stored at -20 °C until they were assayed by the Rose Bengal test and competitive enzyme linked immunosorbent assay.

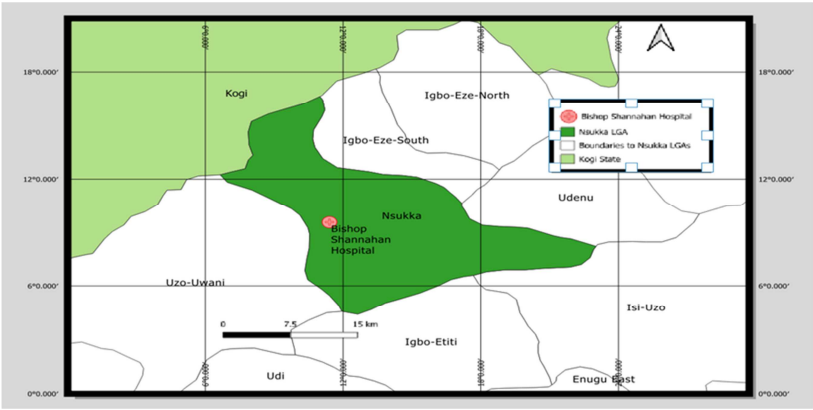

Figure 1. Map of Nsukka Local Government Area, Enugu State, Nigeria, showing the study area and location.

Rose Bengal Test (RBT): The RBT was conducted using the modified RBT method (Ogugua and Onunkwo, 2023). Briefly, a 10 µl drop of *Brucella abortus* antigen (Animal and Plant Health Agency [APHA], New Haw, Addlestone, Surrey, KT15 3NB) was mixed with 30 µl drop of the test serum on a white tile with a stick applicator and rocked for 4 minutes. Occurrence of agglutination within 4 minutes was considered Positive for Brucella antibodies, and the lack of agglutination as Negative.

Competitive Enzyme-linked Immunosorbent Assay (cELISA): The cELISA kit was also sourced from APHA. The kit contained the cELISA plate which was coated with the lipopolysaccharide (LPS) of *B. melitensis* M16. Reagents in the kit included control sera, diluting buffer, conjugate (monoclonal antibody and horse raddish peroxidase), wash solution, chromogen and stop solution (sodium hydroxide). The test was performed according to the manufacturer's instructions. Briefly, 20 µl of each test serum was added per well, with wells in columns 11 and 12 left reserved for controls. 20 µl of Negative Control was added to wells A11, A12, B11, B12, C11, and C12. Also, 20µl of Positive Control serum was added to wells F11, F12, G11, G12, H11 and H12. The remaining D11, D12, E11 and E12 wells had no serum added and were the conjugate controls where there was no competition. 100µl of the prepared conjugate solution was added to all wells. The plate was initially shaken for 30 seconds followed by 10 seconds hand-shakings every 10 minutes for a period of 1 hour. The contents of the plate was poured out and rinsed 5 times with washing solution and dried by tapping on an absorbent paper. Immediately before use, the substrate and chromogen solutions were prepared by dissolving one tablet of urea H_2O_2 in 12 ml of distilled water. The chromogen (OPD) tablet was then added, mixed thoroughly and 100 µl of the solution dispensed into all wells. The plate was left at room temperature for 15 minutes and 100 µl of Stop solution finally added to all wells and the microplate read with a microplate/ELISA reader (Intertek Multiscan M11®) at the optical density (OD) of 450 nm. A positive/negative cut off was calculated as 60% of the mean of the OD of the conjugate control wells (as specified by the manufacturer). Samples in wells with OD equal to or less than the cut-off point were scored Positive, while those above were Negative.

Results

Most of the febrile patients that participated in the survey (74.40%) were between 18 and 30 years of age (Table 1). Patients between 31 and 45 years of age constituted 19.05% of the participants surveyed, while those above 45 years were 6.55% of the total (Table 1). Also, most of the febrile patients that participated in the survey were females (83.93%), while the rest (16.07%) were males (Table 1).

Based on the RBT, the prevalence of Brucella antibodies among the febrile patients was 25.60% (43 out of 168). The age-based distribution of the prevalence was 20.80% among those that were between 18 and 30 years of age, 43.75% among the age group of 31 to 45, while the prevalence among persons above 45 years was 27.27% (Table 2). There was no significant association ($p = 0.315$) between prevalence based of *Brucella* antibodies and sex based on the RBT (Table 3).

With the cELISA test, a prevalence of 5.36% (9 out of 168) was recorded. Age based distribution of prevalence among the cELISA positive patients was 4.80% among those of age group 18 to 30 years, while those between 31 and 45 years was 9.38% (3/32), but none of the patients above 45 years was found positive (Table 4). The sex specific prevalence based on the cELISA test showed 7.41% among the males (2/27) and 4.96% (7/141) among the females (Table 5).

Table 1. Age and sex-based distribution of the 168 febrile patients that were screened for *Brucella* antibodies at Bishop Shanahan Hospital, Nsukka, Nigeria.

Variables	Category	Number of patients in the category	Percentage of patients in the category
Age (years)	18 - 30	125	74.40
	31 - 45	32	19.05
	≥ 45	11	6.55
Sex	Male	27	16.07
	Female	141	83.93

Table 2: Age-based distribution of the prevalence of *Brucella* antibodies in febrile patients at Bishop Shanahan Hospital, Nsukka Nigeria, based on the Rose Bengal test.

Variable	Category	Number Positive, with % in bracket	Number Negative, with % in bracket	P-value
Age (years)	18 – 30 years	26 (20.80%)	99 (79.20%)	0.029
	31 – 45 years	14 (43.75%)	18 (56.25%)	
	≥ 45 years	3 (27.27%)	8 (72.73%)	

Table 3: Sex-based distribution of the prevalence of *Brucella* antibodies in febrile patients at Bishop Shanahan Hospital, Nsukka Nigeria, based on the Rose Bengal test.

Variable	Category	Number Positive, with % in bracket	Number Negative, with % in bracket	Chi-square	P-value
Sex	Male	9 (33.33%)	18 (66.67%)	0.01	0.315
	Female	34 (24.11%)	107 (75.89%)		

Table 4: Age-based distribution of the prevalence of *Brucella* antibodies in febrile patients at Bishop Shanahan Hospital, Nsukka Nigeria, based on the competitive enzyme linked immunosorbent assay (cELISA) test.

Variable	Category	Number Positive, with % in bracket	Number Negative, with % in bracket	Chi-square	P-value
Age (years)	18 – 30 years	6 (4.80%)	119 (95.20%)	1.72	0.424
	31 – 45 years	3 (9.38%)	29 (90.63%)		
	≥ 45 years	0 (0%)	11 (100%)		

Table 5: Sex-based distribution of the prevalence of *Brucella* antibodies in febrile patients at Bishop Shanahan Hospital, Nsukka Nigeria, based on the competitive enzyme linked immunosorbent assay (cELISA) test.

Variable	Category	Number Positive, with % in bracket	Number Negative, with % in bracket	Chi-square	P-value
Sex	Male	2 (7.41%)	25 (92.49%)	0.26	0.606
	Female	7 (4.96%)	134 (95.04%)		

Discussion

The present study found *Brucella* antibodies to be prevalent among the febrile patients screened, based on both RBT and cELISA. The prevalence of 25.6% recorded in the present study based on the RBT is comparable to 24.1% recorded at Abuja (Aworh *et al.,* 2013), lower than 33.5% in Bauchi (Igawe *et al.,* 2020), but higher than the average national level of 17.6% in Southern Nigeria reported by Akinyemi *et al.* (2022) from 2001 to 2021, 9.6% in Ilorin Kwara State (Bamidele *et al.,* 2020), and 10% in Nasarawa State (Agada *et al.,* 2018). The high prevalence recorded in the present study may be attributed to the fact that the research was done among febrile patients. Fever is one of the cardinal signs of brucellosis in humans. The occurrence of *Brucella* antibodies in humans in the study area underscores the fact that brucellosis should form an important differential consideration in febrile diseases diagnosis in the study area, especially when we consider the fact that brucellosis prevalence in human population is a reflection of the status of the disease in the livestock population in the area.

Being a zoonotic disease, the primary source of the disease for humans is most probably the livestock population. Recent studies in the Nsukka area showed that the disease is prevalent in livestock – slaughter cattle, sheep and goat, and in herds of indigenous cattle breeds (Ogugua and Onunkwo, 2023; Ezeh *et al.,* 2024; Ogugua *et al.,* 2024). In earlier studies, it was reported that some individuals in the study area engaged in risk behaviors that could result to brucellosis transmission, including consumption of raw meat (Ogugua *et al.,* 2024); living in close contact with livestock (Ogugua and Onunkwo, 2023), and handling of animals without wearing protective gears (Njoga *et al.,* 2018).

The present study found that the prevalence of *Brucella* antibodies in the febrile patients screened was associated with age, with the highest prevalence occurring in the 31 to 45 years age group. However, an earlier report in Turkey (Geyik *et al*., 2002) showed that brucellosis affects persons of all age groups, while Al Hamad *et al*. (2022) recorded brucellosis cases more in older persons (81 years old), and brucellosis occurrence was reported to be highest among the young people in Maiduguri, Nigeria (Igawe *et al.,* 2020). This observation of the age group of between 31 and 45 years having the highest prevalence may be because the age group is usually most active, and in Nigeria where knowledge of the disease is poor and practices that expose to brucellosis is common, this age group are more likely to be exposed to the disease.

The prevalence obtained based on the RBT in the present study was much higher than that obtained using the cELISA test. This concurs with findings in some earlier studies (Mai *et al*., 2012; Cadmus *et al*., 2013), but contrasts with the finding of Agada *et al*. (2017) who reported higher prevalence with the cELISA than the RBT. The ability of serological tests to

detect particular immunoglobulins differ. The immunoglobulins found in early infections are the IgM and IgG1 (Ismail *et al*., 2002; Agada *et al*., 2017) unlike in recurrent and chronic infections where the IgG2, IgG3 and IgA hold sway. Earlier reports show that the RBT being an agglutination reaction is better suited in detecting acute cases (IgM and IgG1) while the cELISA does better in detecting persistent infections (IgG2, IgG3 and IgA) and has been used to monitor chronic and relapse cases in humans (Smits *et al*., 2003). Furthermore, the RBT has been reported to be highly sensitive and can detect non-specific antibodies due to infections with *Yersinia enterocolytica* 0:9, *Salmonella urbana*, *Escherichia coli 0:*157 and *Francisela tularensis* (Chenais *et al*., 2012; Agada *et al*., 2017).

Conclusion: This study found a 25.6% prevalence of *Brucella* antibodies in febrile patients at Bishop Shanahan Hospital Nsukka, Nigeria using the RBT, and 5.36% prevalence based on the cELISA. The seroprevalence of Brucella antibodies in the patients was significantly associated with age. We propose and suggest that more comprehensive studies should be conducted among individuals at higher risk of *Brucella* infection with a view to determining the specific *Brucella* species responsible for human *Brucella* infection in the study area. There should also be enlightenment campaigns and One Health approach towards the control of human brucellosis in Nsukka area.

Conflict of interest

The authors declare no conflict of interest.

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