

Sero-Prevalence of Brucellosis among Nomadic Herdsmen, Abattoir and Livestock Workers in Niger-Delta Region, Nigeria

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Abstract

Brucellosis is a re-emerging zoonotic disease that causes more than half a million infections to humans every year. The disease is common in most developing countries, the human mortality rate is about 2%, and the disease causes severe rheumatism, infertility in males, spontaneous abortion and also results in wastage of resources through prolonged treatment. *Brucella* organisms are also potential biological weapon which could be cheaper to produce but more devastating than chemical weapons. About 5 millilitres of blood was drawn from 228 subjects using sterile vacutainers and analyzed by using standard tube agglutination tests: (SAT³ 160) and ELISA (IgG, IgM) kits specific for *Brucella abortus* and *Brucella melitensis* antibodies. Semi structured questionnaire was administered to collect data. In the study, overall sero-prevalence was 70 (30.8%). More male participated in the study with a frequency of 24.6% of whom 7.92% falls within the age group of 20 to 30, followed by 31 - 35 years with 5.72% and 3.52% within 36 - 45 years. Least affected were those in the age groups above 46 years (2.20%). Approximately, 21.54% of the subjects had formal education either at Quranic, primary and secondary or tertiary level. Majority acquired Quranic education (9.68%), 7.48% primary and 0.88% had tertiary training. A total of 21 (9.24%) never acquired any form of education. Headache, muscle aches, malaise, chills and fatigue were the most common clinical signs and symptoms experienced by about 30% among the participants. In the distribution of *Brucella* antibodies, ELISA diagnostic kits showed high sensitivity with the prevalence rate of 18.04% (n = 41) followed by SAT 12.76% (n = 29). The sensitivity and specificity of RDPT kits were 37 % and 69%, with a positive and negative predictive value of 18% and 86% respectively. Unprocessed milk from the market and consumption of unboiled milk were associated with brucellosis. There-

fore, patients with brucellosis should be treated to prevent the devastating effect of the disease and the accompanying sequelae, public health education programs should explain modes of transmission and Brucella febrile diagnostic kit should be used at the health facilities.

Keywords

Brucella, Zoonotic, Nomadic Herdsmen, Rapid Brucella Diagnostic Kits, Nigeria

1. Introduction

Brucella is gram-negative bacteria that are pathogenic to humans and a variety of livestock animals and wildlife. The genus Brucella has six recognized species on the basis of host specificity. While all six species occur at least sporadically in the United States and sub-Sahara Africa, the greatest economic impact resulted from bovine brucellosis caused by *B. abortus*. Infection decreases reproductive efficiency, mainly by abortion. The disease has elicited Brucellosis, a zoonotic disease of public health importance; this animal disease has been eradicated in many developed countries [1]. Brucellosis remains one of the seven zoonotic diseases declared by the World Health Organization [2] but has been “neglected”. The disease has a great impact on both animal and human health as well as tremendous socio-economic impact in developing countries where people’s income relies largely on livestock breeding and dairy products [3]. Brucellosis is endemic in livestock in most countries of Africa including Nigeria [4] and is an established endemic disease among Nomadic herdsmen and cattle slaughter houses in sub-Saharan Africa and Nigeria [5] [6].

Several species of Brucella that are important to public health exist amongst which *B. abortus* and *B. melitensis* are more virulent for humans than *B. suis* and *B. canis* although serious complications can occur with any species of Brucella [7]. Humans are infected either by direct contact with blood, placenta or uterine secretions of infected animals, through breaks in the skin, by inhalation or by ingestion of unpasteurized milk and other dairy products [8]. It is known that unpasteurized milk is sold in several parts of Nigeria. Brucellosis is an occupational hazard to individuals engaged in certain professions such as abattoir workers, veterinarians, livestock farmers and herdsmen [9].

This study focuses on abattoir workers in three abattoirs and Nomadic herdsmen raising camp in Akwa Ibom State, Nigeria. This is because brucellosis is a recognized occupational hazard which is rarely diagnosed in most health facilities in the country, and is yet to be included in the Integrated Disease Surveillance and Response (IDSR) system—public health surveillance and response system for priority diseases [10] [11]. Very few studies have been done on the prevalence of brucellosis among these occupational group persons in Nigeria [12].

Aims and Objectives

- The main objective of this study was to determine the sero-prevalence of Brucellosis in Akwa Ibom State, Nigeria.
- To determine the risk factors associated with human brucellosis among abattoir workers, livestock farmers and herdsmen.
- To compare the diagnostic performance of standard tube agglutination tests: (SAT¹⁶⁰) and ELISA (IgG, IgM) kits.
- To proffer the possible measures in controlling the diseases outbreak in the study area.

2. Materials and Methods

2.1. Methods and Study Sites

Three privately owned abattoirs and nomadic settlements were selected based on the highest daily slaughter of food animals and the largest population of workers one each in the three senatorial districts of Akwa Ibom State (Uyo, Ikot Ekpene and Eket) respectively (**Figure 1**). Ethical approval was granted by the Akwa Ibom State, Ministry of Health to obtain blood sample from the study population.

2.2. Study Population and Design

A cross-sectional study between January and October 2018 to determine the sero-prevalence of *Brucella abortus* and *Brucella melitensis* antibodies in sera of abattoir, livestock workers, and nomadic herdsmen, also to determine the risk factors associated with sero-positivity against brucella among this class of people in Nigeria.

2.3. Inclusion Criteria

All herdsmen and abattoir workers actively participating in the handling of the food animals, who were 18 years and above and present at the site at the time of visit were included in the study.

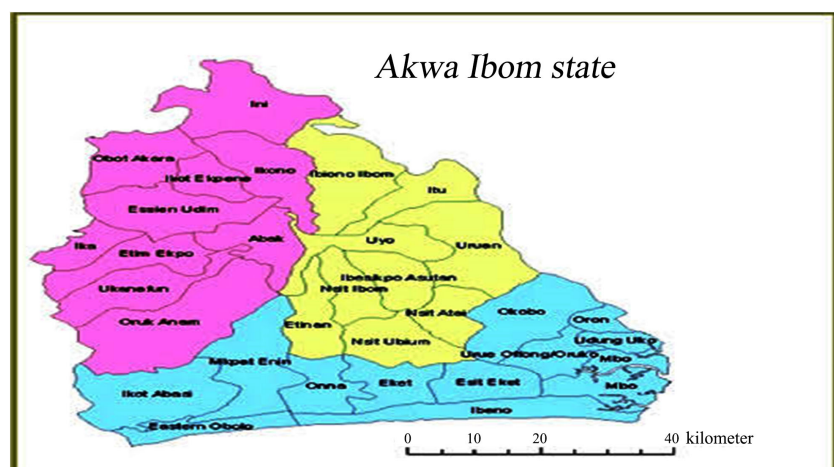


Figure 1. Map showing the three senatorial districts in Akwa Ibom State [13].

2.4. Exclusion Criteria

All meat buyers and children at the abattoir at the time of visit that are not working with the food animals were excluded from the study. A sero-positive individual was an abattoir worker or a nomadic herdsman whose on screening for the presence of *B. melitensis* or *B. abortus* antibodies had a positive brucellosis serological result by Enzyme-linked Immunosorbent Assay (ELISA) and Standard tube agglutination (SAT). A sero-negative individual was any person working in the same sites whose serum was collected at the same time with the sero-positive individuals and who on screening had a negative brucellosis serological result by ELISA and SAT.

2.5. Ethical Considerations

Approval for this study was obtained from the Akwa Ibom State Research Ethics Committee, Ministry of Health. Permission was also obtained from the management of each abattoir where the study was carried out. Informed consent was obtained from each eligible herdsman and abattoir workers before questionnaire administration and sample collection.

2.6. Sampling Method

Sample collection was done by dividing the subjects population into six groups, based on the nature of their job: butchers; meat sellers; livestock farmers/traders; abattoir cleaners; herdsman and administrative workers such as security guards, revenue officers and abattoir managers. Butchers were those responsible for slaughtering the animals. Meat sellers were those who sold the meat; Livestock workers/traders were those who raised livestock for sale at the abattoir. Herdsmen were those that move with the animals from place to place for pasture.

2.7. Justification of Sample Size

The sample size used for this study was determined based on the average number of subjects at the time of this study. Sample size was calculated using the following formula based on total population of subjects with an expected proportion of 5% and a margin of error of 3% using a 95% confidence level [14].

$$n = Z^2 \times pq / d^2 .$$

Using the above formula, where $Z = 1.96$, $P = 5\%$ or 0.05 , $q = 1 - p = 0.95$ and $d = 0.03$. A 10% non-response rate was added giving a total sample size of 223. This was the minimum sample size calculated although the actual sample size eventually used for this study was 228 for ease of allocation into groups.

2.8. Collection of Blood Sample

Five milliliters (ml) of venous blood was collected by qualified health provider under very strict hygienic conditions using sterile vacutainers and needles from the individual subject into EDTA tubes and kept in the ice pack cooler. Clear

plasma was collected in sterile vials, labeled with a subject identification code that corresponded to the study site and stored in a freezer at -20°C until analysis. Each sample was screened for *Brucella abortus* and *Brucella melitensis* antibodies using standard tube agglutination test: SAT¹⁶⁰ and ELISA (IgG, IgM) kits specific for each. The reagents in the kit were reconstituted and the test procedure was carried out according to manufacturers' instructions. Individuals were considered as positive based on a positive SAT (1:160) or ELISA result.

Briefly, serum samples and Brucella antigens were brought to room temperature ($22^{\circ}\text{C} \pm 4^{\circ}\text{C}$). Approximately 25 μl of each plasma was placed on a standard glass tube and an equal volume of antigen was added. Both were mixed thoroughly using automated shaker. The result for agglutination was read immediately after a 15-minute period. The agglutination reactions were recorded as positive (+) or negative (–) depending on whether there were agglutinations or not. Further screening of the human sera was carried out using ELISA kits (IgG and IgM) specific antibodies for both *Brucella abortus* and *Brucella melitensis* and the results were correlated with those of the SAT¹⁶⁰ (standard tube agglutination tests: SAT¹⁶⁰ test done earlier. Subjects were considered as positive based on a positive SAT¹⁶⁰ or ELISA result.

2.9. Data Collection

Data for the study were collected through interviewer-administered questionnaires by well-trained medical personnel at the time of sample collection from nomadic herdsman, abattoir and livestock workers. All nomadic herdsman, livestock and abattoir workers meeting eligibility criteria were interviewed and blood specimen was collected.

2.10. Statistical Analyses

The data were analyzed using excel 2013 and SPSS Version 23.0. Descriptive statistics, including the chi-square test, were used to assess the association between categories of subjects. $P < 0.05$ was considered statistically significant.

3. Results

Demographic Characteristics of Study Participants

A total of 228 subjects participated in the study with brucellosis seroprevalence of 30.8% ($n = 70$). More male participated in the study with a frequency of about 24.6% of whom 7.92% fall within the age group of 20 to 30, followed by 31 - 35 years (5.72%) and 36 - 45 years (3.52%) and least affected were those in the age groups above 46 years (**Table 1**). Approximately 21.54% of the subjects had attained formal education either at Quranic level, primary and secondary or tertiary. Majority (9.68%) had acquired Quranic education, 7.48% primary and 0.88% had tertiary training. Nearly 9.24% had never acquired any form of education with majority of the participants being Muslims (**Table 2**). Headache,

Table 1. Socio-demographic characteristics of Brucellosis in Akwa Ibom State, Nigeria.

Age (yrs)	No. Examined	Herdsmen	Abattoir	Livestock	No. Pos.	% Pos.
20 - 25	24	08	06	04	18	7.92
26 - 30	26	06	08	04	18	7.92
31 - 35	49	06	04	03	13	5.72
36 - 40	45	04	02	02	08	3.52
41 - 45	43	04	02	02	08	3.52
46 - above	41	03	01	01	05	2.20
Total	228	31	23	16	70	30.8

Table 2. Socio-demographic characteristics of Brucellosis in Akwa Ibom State, Nigeria.

Education	No. Examined	Herdsmen	Abattoir	Livestock	No. Pos.	% Pos.
Tertiary	21	00	01	01	02	0.88
Secondary	48	02	04	02	08	3.52
Primary	52	08	07	02	17	7.48
Quranic	63	12	05	05	22	9.68
None	44	09	06	06	21	9.24
Total	228	31	23	16	70	30.8

muscle aches, malaise, chills and fatigue were the most common clinical signs and symptoms experienced by about 30% among the participants (**Figure 2**). In the distribution of Brucella antibodies ELISA diagnostic kits showed high sensitivity with the prevalence rate of 18.04% ($n = 41$) followed by SAT (12.76%) (**Table 5**). Workers within the ages of 20 to 30 showed significant at bivariate analysis dues to long years of occupational exposure to the animal (**Table 1**).

4. Discussion

This research highlights the sero-prevalence (30.8%) of brucellosis among Herdsmen, Abattoir and Livestock workers in an urban set up of Akwa Ibom State, Nigeria (see **Table 3**). This is in agreement with the reported by Pappas *et al.* [5] with 35% sero-prevalence of brucella bacteria in Tunisia and 34.2% in Iraq [1]. The study in Iraq mainly looked for the disease among febrile patients who were principally abattoir workers and herdsmen. This could be explained probably by the high incidence and unstandardized control protocols of the disease in livestock as well as inability to appropriately manage brucellosis in humans which is contrary to practices in developed countries. Among the various categories of the subjects that were screened, nomadic herdsmen had the highest sero-positivity rate of 31 (13.6%) followed by abattoirs workers 23 (10.1%) (see **Table 1**). Similar findings have been documented from studies done in South West Nigeria, Tanzania and Egypt [14] [15] [16], suggesting that they are more at risk probably because of their close contacts with blood and tissues of infected animals. In this study, brucellosis infection was present across all the genders and educational status, which corresponds with study by Mantur *et al.* [4].

In addition, males appeared to be more at risk of infection with brucellosis 26.4% (**Table 4**). However, it should be noted that cattle raising and butchering is a male-dominated activity and this may have accounted for this finding.

Workers with tertiary education working in the abattoir seem to be less at risk of infection. This category of workers will usually have become managers with many apprentices working under them and are rarely responsible for the direct rearing and slaughtering but usually serve in supervisory or advisory capacities [17].

In the present study, the prevalence rate of brucellosis was 30.8% and standard tube agglutination test was positive in 12.76% (29/228) at a titre level of 1:160 while ELISA rapid test was 18.04% (41/228) of the subjects (see **Table 5**). The finding of the present study indicates a low specificity for SAT¹⁶⁰. Similar results have been reported in other studies from endemic areas, where there may be high levels of specific and cross reacting antibodies [18].

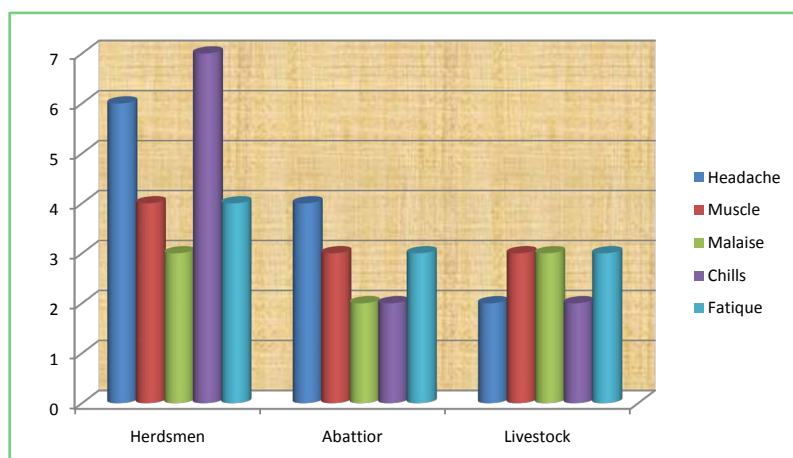


Figure 2. Distribution of clinical signs and symptoms of brucellosis subjects in niger-delta region, Nigeria (N = 70).

Table 3. Percentage distribution of *Brucellosis* among Herdsmen, Abattoir and Livestock workers in Akwa Ibom State, (N = 228).

Senatorial	Sample No.	Herdsmen	Livestock	Abattoir	No. Pos.	% Pos.
Uyo	76	10	05	08	23	10.1
Ik Ekpene	76	11	06	08	25	11.0
Eket	76	10	05	07	22	09.7
TOTAL	228	31	16	23	70	30.8

Table 4. Socio-demographic characteristics of Brucellosis in Akwa Ibom State, Nigeria.

Gender	No. Examined	Herdsmen	Abattoir	Livestock	No. Pos.	% Pos.
Male	166	31	16	13	60	26.4
Female	62	00	07	03	10	04.4
Total	228	31	23	16	70	30.8

Table 5. Comparative distribution of *Brucella* Antibodies using SAT 160 or ELISA diagnostic kits among Herdsmen, Abattoir and Livestock workers in Akwa Ibom State.

Analysis	No. Examined	Sources	Total pos.	% pos. Herdsmen	Abattoir	Livestock
SAT (1:160)	228	12	10	07	29	12.76
ELISA	228	19	13	09	41	18.04

Enzyme-linked immunosorbent assay ELISA is based on detection of IgG and IgM antibodies which appear in detectable titres as early as the second day of illness. In many circumstances, especially among partially treated cases presenting to health facilities, combining SAT¹⁶⁰ and ELISA may reduce the diagnostic difficulty in brucellosis. The ELISA offers an additional advantage among serologic diagnostic tests for brucellosis in that the test strips do not require an ELISA reader for evaluation. Also, only minimal operator training is required. Nevertheless, the higher cost of the test in comparison with the SAT¹⁶⁰, as well as cold-storage requirements for test reagents, are additional impediments in using this test in developing countries.

In the present study, we conclude that ELISA is a practical alternative to SAT¹⁶⁰ in the diagnosis of *Brucella* infection on account of its increased sensitivity, early detection of cases, and ease of procedure with minimal infrastructure and availability of results on the same day. However, a larger prospective study would be required to fully evaluate the usefulness of this test in countries endemic to *Brucella* disease.

Clinical presentation of the illness among the study participants was similar although fatigue, chills and headache were the most clinical symptoms experienced by *Brucella* positive cases. Indiscriminate clinical signs are a common phenomenon that has been reported from other studies [15].

5. Conclusions

The sero-prevalence of human brucellosis among abattoir workers in Akwa Ibom State was high, with Nomadic herdsmen having the highest sero-positivity. Rearing of animals and handling aborted fetuses couple with occupational exposure of over 6 years were important factors associated with sero-positivity for brucellosis. Health education is at the risks that herdsmen and abattoir workers face from contracting zoonoses and the consistent use of personal protective equipment by Nomadic and abattoir workers will go a very long way in preventing infection in the population at risk. Based on the findings of this study, the following recommendations were made to the relevant authorities. Government at all levels should organize public enlightenment campaigns aimed at highlighting the importance of zoonoses including brucellosis among Nomadic herdsmen, abattoir and livestock workers who should also be encouraged to go for brucellosis screening every 3 years. Abattoir workers should be discouraged from eating raw meat and educated on adherence to safe animal-product handling practices. A continuous sero-survey should be considered at regular intervals as a monitoring tool for the prevalence of human brucellosis in Nigeria.

The screening was carried out using human IgG and IgM ELISA kits specific for *B. melitensis* and the results were correlated with those of the SAT (1:160) test. The reagents in the kit were reconstituted and the test procedure was carried out according to manufacturers' instructions. Individuals were considered as positive based on a positive SAT (1:160) or ELISA result.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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