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Seroprevalence of brucellosis in slaughtered cattle and risk practices for its transmission among workers at the Abakaliki abattoir, Ebonyi State, Nigeria

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Abstract

Brucellosis is a zoonotic disease that affects animals and humans. Despite being common in livestock, information on the disease in slaughtered cattle in Abakaliki, Ebonyi State is scarce in available literature. This study determined the seroprevalence of brucellosis in slaughtered cattle and identified the risk practices for its transmission among abattoir workers in Abakaliki, Ebonyi State, Nigeria. The design of the study was a cross-sectional survey. Using systematic random sampling of one in twenty, one hundred (100) cattle were selected over a six-month period for the survey, made up of 69 Sokoto Gudali, 30 White Fulani and one Red Bororo; 99 adults and one young; 81 males and 19 females. Data generated were analyzed with Chi-square and p-values less than 0.05 considered significant. Results showed that the overall seroprevalence of brucellosis at the abattoir was 6%. The brucellosis seroprevalence was significantly ($p = 0.018$) associated with breed: White Fulani cattle having a significantly higher seroprevalence than the Sokoto Gudali and Red Bororo. The seroprevalence was not significantly ($p > 0.05$) associated with age or sex. The respondents engaged in practices that exposed them to *Brucella* infection: A good proportion of the abattoir workers (55%) tasted raw meat and 52% of them handled fetuses while on duty at the abattoir without wearing personal protective equipment. Use of hand gloves was found to be significantly associated ($p = 0.001$) with the level of education. Regular screening of cattle slaughtered at the abattoir for brucellosis and increasing the awareness of abattoir workers with regards to brucellosis was recommended.

Keywords: Brucellosis; Seroprevalence; Slaughtered cattle; Abakaliki abattoir, Ebonyi State, Nigeria; Risk practices; Abattoir workers.

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Introduction

Brucellosis, also known as contagious abortion or Bang's disease in bovine species, is a highly infectious zoonotic disease that causes severe morbidity and infertility (Sarma and Singh, 2022). It is a serious threat not only to animals but also to humans, and has been reported in 86 countries worldwide (Tadesse, 2016; Ogugua *et al.,* 2015). Brucellosis primarily affects cattle, sheep, pigs, goats, dogs, and humans, as well as horses, donkeys, and camels. It is associated with enormous losses in the cattle industry as well as in humans (Pal *et al.,* 2017). The disease has enormous impact on the wellness and reproductive efficiency of livestock and the in-contact persons, with resultant economic consequences.

The typical manifestations of bovine brucellosis include abortions/miscarriages, stillbirths, retained placenta or birth of weak calves, delayed calving, male sterility and a significant decrease in milk production (Mitiku and Desa, 2020; Garofolo *et al.,* 2016). *Brucella abortus*, the primary causative agent in cattle, also causes early labour in cattle and recurring fever in humans (Ali *et al.,* 2019; Christopher *et al.,* 2010)). Even if the cow does not abort when infected with Brucella, visible enlargement of the mammary gland to the navel region and vaginal haemorrhages are common (Mitiku and Desa, 2020). The observation of a pregnant cow in the ninth month, with swollen udders, could be used as an indicator of the disease's advanced stage, in which animals shed bacteria in urine, milk, and vaginal discharges (Khan and Zahoor, 2018). Fever, vesiculitis, orchitis, and epididymitis are clinical signs/lesions of the disease in bulls. In severe cases, the disease is also associated with testicular abscesses, metritis, or orchitis, all of which can lead to infertility.

Usually, *Brucella* species are transmitted through direct contact with infected animals' placenta, foetus, foetal fluids and vaginal discharges or byproducts (e.g., milk, meat, and cheese) (Ferrero *et al.,* 2014). It can also spread vertically, according to Shoukat *et al.* (2017), by infecting newborn calves and lambs in the uterus. Brucellosis is mainly an occupational disease for those who work with infected animals or their tissues, such as farmers, shepherds, butchers, abattoir workers, veterinarians, and laboratory workers (Pereira *et al.,* 2020). In addition, health workers are at risk of infection with *Brucella* in disease-endemic areas. According to reports, approximately 12% of laboratory workers in Spain contract brucellosis while on the job (Sayin-Kutlu *et al.,* 2012; Kose *et al.,* 2014; Pereira *et al.,* 2020). Abattoir workers are at a higher risk of infection due to the greater possibility of their exposure to infected animal carcasses and viscera, as well as through cuts and wounds, infected blood and fluid that can splash into the conjunctiva (Pereira *et al.,* 2020).

There are numerous risk factors for human brucellosis, including consumption of raw milk or inadequately processed milk products, handling of foetus, placenta and hides, and contact with livestock (Acharya *et al.,* 2018). Again, abattoir employees and farmers do not always wear protective clothing and hand gloves, exposing them to infectious materials such as urine, aborted fetuses and placentas (Rodarte *et al.,* 2023**).** As such, about 2.1 million annual incidences of human brucellosis are reported globally, with Africa and Asia, having the greater risk and cases (Laine *et al.,* 2023). In Nigeria, human brucellosis is prevalent especially among occupationally exposed individuals and particularly among abattoir workers. Several researchers have reported the following prevalence of brucellosis among apparently healthy abattoir workers: 66.3% in Ibadan, South-Western Nigeria (Adesokan *et al.,* 2016), 24.1% in the Federal Capital Territory, Abuja, Nigeria (Aworh *et al*., 2017), and 43.8% in North-Central Nigeria (Ofukwu *et al*.,

undated).

Given the enormous risk of this disease spreading to abattoir workers and other at-risk occupations, appropriate mitigation measures must be implemented. Vaccination of animals against brucellosis, personal safety measures, and food safety interventions are examples of such measures (Acharya *et al.,* 2018). However, implementation of such specific measures will not be possible without proper understanding and consciousness of infection risks and statuses.

Earlier reports of sero-prevalence of brucellosis in slaughtered cattle include: 3.9% in Northern, Southern and South-western Nigeria (Ogugua *et al.,* 2015), and 5.31%, 6% and 7.8% in Ibadan, Nigeria between 2006 and 2017 (Cadmus *et al.,* 2006; 2010; Ayoola *et al*., 2017). There had also been earlier reports on the risk practices for brucellosis transmission among abattoir workers in Nigeria (Adesokan *et al.,* 2013; 2016). However, studies on slaughtered cattle as well as the risk practices that could aid the transmission of the disease among workers at the Abakaliki abattoir in Ebonyi State, Nigeria are scarce in available literature. The present study determined the seroprevalence of brucellosis in slaughtered cattle, as well as the risk practices for transmission of the disease among workers in Abakaliki abattoir, Ebonyi State, Nigeria.

Material and Methods

Study Area: This study was conducted at the Abakaliki abattoir, Abakaliki Local Government Area, Ebonyi State, Nigeria (Figure 1). Abakaliki town, which lies at the intersection of roads from Enugu, Afikpo, and Ogoja, is the largest and capital city of Ebonyi State. It is located at 6.3231°N and 8.1120°E (Elom *et al.,* 2021) and is primarily inhabited by the Igbo people. Most of the inhabitants are primarily farmers, traders, and civil servants.

Ethical Approval and Informed Consent: Approval for this study was obtained from the Ethical Committee of the Department of Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka (Reference No: VPHPM/UNN/23/202). Permission to use the abattoir for the study was obtained from the management of the Abakaliki abattoir. Informed consent was obtained from the study participants after being assured of confidentiality of the information they would supply.

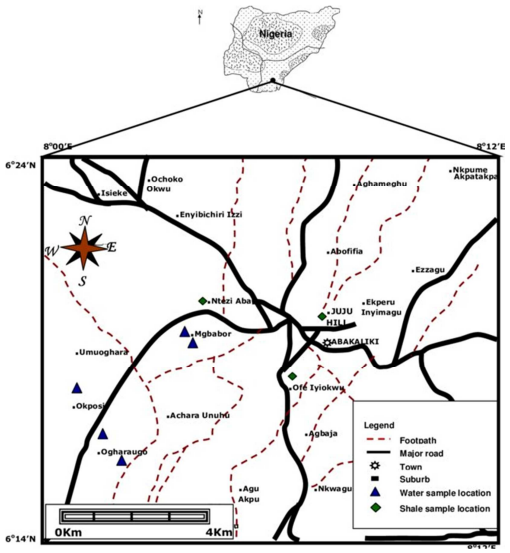

Figure 1: Map of Abakaliki town, Ebonyi State, Nigeria, where the abattoir that was studied was located. (Source: Nkpuma *et al*., 2018).

Study Design and Sample Size Determination: The study used the cross-sectional survey design. The survey was conducted on slaughtered cattle at the Abakaliki abattoir between December 2022 and April 2023. The minimum sample size of 87 was calculated using the formula: N = z2pq/d2 (Ezeh *et al.,* 2023), where n = Desired sample size, Z = Standard normal deviation (1.96), d = Degree of accuracy desired usually (0.05), p = Prevalence of brucellosis in cattle, P = 5.8%, (Cadmus *et al.,* 2006). However, for robustness 100 samples were collected.

Sampling and Sample Collection: The study entailed several visits to the abattoir over a period of 5 months, spanning December 2022 to April 2023. During each visit, slaughter cattle were chosen using the systematic random sampling technique by sampling one out of every twenty cattle slaughtered.

About 5 ml of blood was collected from each cattle at slaughter into a plain test tube, which was placed in a slanted position to clot. Each sample was appropriately labeled, and the sex, age and breed documented. After the blood sample collection, the samples were transported in an ice pack to the Teaching and Research Laboratory of the Faculty of Agriculture, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State, Nigeria and the sera decanted. The serum was stored at - 20 °C until it was tested for *Brucella* antibodies.

Serological Test: The serological test was conducted in the Teaching and Research Laboratory of the Department of Animal Science, Faculty of Agriculture, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State. The serum samples were subjected to the Rose Bengal test (RBT) as described by Ogugua *et al.,* (2015). The RBT antigen, consisting of standardized *B. abortus* antigen (controls), was sourced from the Animal and Plant Health Agency, Surrey, UK. Briefly, equal volumes (30 µL) of antigen and test serum were thoroughly mixed on a plate using a stick applicator, and the plate was rocked for 4 minutes. The appearance of agglutination within the 4 minutes was regarded as positive for Brucella, and its absence was considered negative.

The Questionnaire Survey/Interview Schedule: A semi-structured and pre-tested questionnaire/interview schedule was used to obtain data on the demographical characteristics and risk practices for the disease transmission among the abattoir workers. The interview schedule was translated into the local dialect for those who were not fluent in English and administered to participants by the interviewer after oral informed consent was obtained. Participants included butchers and meat processors at the abattoir and meat sellers.

Data Analysis: Chi-square statistics was used to test for associations between the seroprevalence of brucellosis and factors such as sex, age and breed of the cattle, as well as association between the socio-demographic characteristics of the respondents and the risk practices for *Brucella* transmission. All statistical analysis was conducted using SPSS version 25, and p-values less than 0.05 were considered significant.

Results

Demographic characteristics of the cattle sampled: A total of one hundred (100) cattle were screened for Brucella antibodies during the study. The 100 cattle were made up of 69 Sokoto Gudali, 30 White Fulani and one Red Bororo (Table 1). Ninety nine percent of the cattle screened were adults (> 2 years of age), and 81 were males and 19 were females (Table 1).

Prevalence of brucellosis in cattle slaughtered at Abakaliki abattoir as detected by the RBT: Six out of the 100 serum samples subjected to RBT were positive (6% seroprevalence). Five out of the six cattle serum samples that were positive were obtained from White Fulani cattle (16.7%), while the remaining one was from Sokoto Gudali breed (1.4%) [Table 2]. Seroprevalence was significantly ($p = 0.018$) associated with breed; White Fulani cattle having a significantly higher seroprevalence (Table 2). Four out of the 81 males (4.9%) and two out of the 19 females (10.5%) were seropositive, and there was no significant association ($p = 0.852$) between seroprevalence and sex (Table 2). Six out of the 99 adult cattle were seropositive, while the only one young cattle surveyed was seronegative; there was no significant

association (p = 0.064) between seroprevalence and age (Table 2).

Demographic characteristics of the abattoir workers surveyed: The abattoir workers who responded to the questionnaire/interview schedule were made up of 35 adults (> 25 years of age) and 65 young adults (15 – 25 years of age), 82 males and 18 females (Table 3). 48% of the respondents were single, while 52% were married, and majority of them (48%) had only secondary school education (Table 3). A large percentage of them (65%) have more than 5 years' experience of work at the abattoir, with 26% having 1 – 2 years of experience in the work at the abattoir and only 9% having 2 – 5 years of experience (Table 3).

Table 1: Demographic characteristics of cattle sampled at Abakaliki abattoir, Ebonyi State, Nigeria.

Variables	Characteristics	Frequency	Percentage
Breed	White Fulani	30	30%
	Sokoto Gudali	69	69%
	Red Bororo	1	1%
Age (years)	Young Adult (< 2 years)	1	1%
	Adult (> 2 years)	99	99%
Sex	Male	81	81%
	Female	19	19%

Table 2: Seroprevalence of brucellosis among cattle slaughtered in Abakaliki abattoir, Ebonyi State, Nigeria, in relation to sex, age and breed.

Variable	Characteristics	Samples positive for RBT (%)	Samples negative for RBT (%)	χ^2	P-value
Breed	White Fulani	5 (16.7)	25 (83.3)	8.649	0.018*
	Sokoto Gudali	1 (1.4)	68 (98.6)		
	Red Bororo	0 (0.0)	1 (100)		
Sex	Male	4 (4.9)	77 (95.1)	0.852	0.319
	Female	2 (10.5)	17 (89.5)		
Age	Young Adults (< 2 years)	0 (0.0)	1 (100)	0.064	1.000
	Adults (> 2 years)	6 (6.1)	93 (93.9)		

Table 3: Demographic characteristics of abattoir workers interviewed at Abakaliki abattoir, Ebonyi State, Nigeria.

Variables	Characteristics	Number	Percentage
Age (years)	Young Adults (15 – 25)	65	65
	Adults (> 25)	35	35
Sex	Male	82	82
	Female	18	18
Marital status	Single	48	48
	Married	52	52
	Separated	0	0
	Widowed	0	0
Level of Education	None	23	23
	Primary (1)	17	17
	Secondary (2)	48	48
	Tertiary (3)	12	12
Years of experience	1 – 2 years	26	26
	> 2 – 5 years	09	09
	> 5 years	65	65

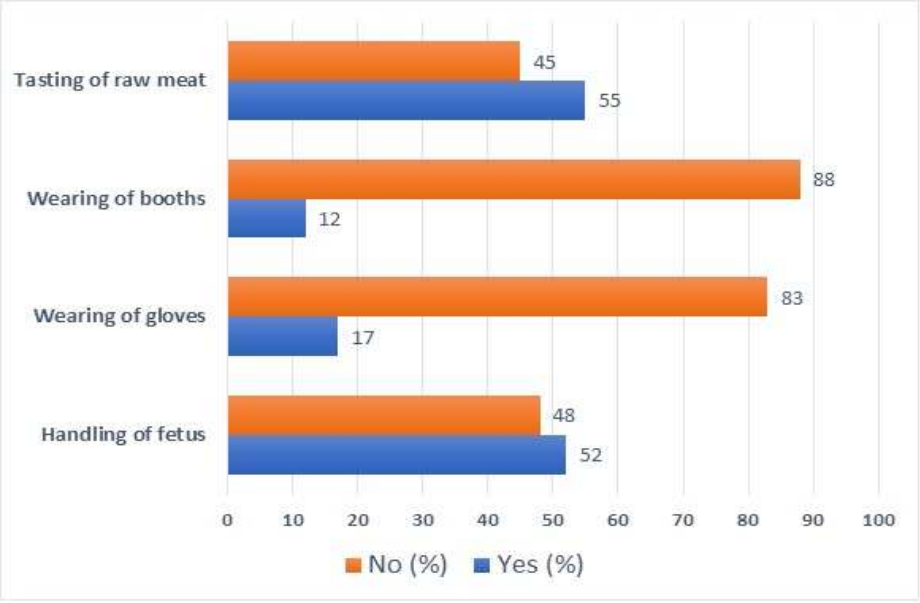

Figure 2. Practices that expose to *Brucella* infection among workers at Abakaliki abattoir, Ebonyi State, Nigeria.

Risk practices associated with transmission of brucellosis among workers at the Abakaliki abattoir: Most of the respondents (55%) admitted tasting raw meat, while 52% of the respondents admitted handling foetuses without protective clothing (Figure 2). Only 12% of the respondents admitted wearing booths while at work at the abattoir, while also only 17% admitted wearing hand gloves while at work at the abattoir (Figure 2). Among all risk practices considered, the wearing of hand gloves was significantly associated (p = 0.001) with respondents' level of education (Tables 4 and 5).

Table 4: Bivariate analysis of wearing of gloves and booths by abattoir workers who responded to the questionnaire/interview schedule at Abakiliki abattoir, Ebonyi State Nigeria.

Parameters	Variables	Wearing of hand gloves			Wearing of booths		
		Yes n (%)	No n (%)	P-value	Yes n (%)	No n (%)	P-value
Age (years)	Young Adult (15 – 25)	11 (16.9%)	54 (83.1%)	1.000	5 (7.7)	60 (92.3%)	0.105
	Adult > 25	6 (17.1%)	29 (82.9%)		7 (20)	28 (80%)	
Level of Education	None	2 (8.7%)	21 (91.3%)	0.001*	1 (4.3%)	22 (95.7%)	0.597
	Primary	2 (11.8%)	15 (88.2%)		3 (17.6%)	14 (82.4%)	
	Secondary	6 (12.5%)	42 (87.5%)		6 (12.5%)	42 (87.5%)	
	Tertiary	7 (58.3%)	5 (41.7%)		2 (16.7%)	10 (83.3%)	
Gender	Male	12 (14.6%)	70 (85.4%)	0.296	9 (11%)	73 (89%)	0.688
	Female	5 (27.8%)	13 (72.2%)		3 (16.7%)	15 (83.3%)	
Years of Experience	1 – 2 years	5 (19.2%)	21(80.8)	0.766	3 (11.5%)	23 (88.5%)	0.591
	2 – 5 years	2 (22.2%)	7 (77.8%)		0 (0%)	9 (100%)	
	> 5 years	10 (15.4%)	55 (84.6%)		9 (13.8%)	56 (86.2%)	

Table 5. Bivariate analysis of harvesting of foetuses without wearing protective clothing and tasting of raw meat by abattoir workers who responded to the questionnaire/interview schedule at Abakiliki abattoir, Ebonyi State Nigeria.

Parameters	Variables	Harvesting of foetus			Tasting of raw meat		
		Yes n (%)	No n (%)	P-value	Yes n (%)	No n (%)	P-value
Age (years)	Young Adult (15 – 25)	35 (53.8%)	30 (46.2%)	0.677	37 (56.9%)	28 (43.1%)	0.675
	Adult (> 25)	17 (48.6%)	18 (51.4%)		18 (51.4%)	17 (48.6%)	
Level of Education	None	12 (52.2%)	11 (47.8%)	0.186	13 (56.5%)	10 (43.5%)	0.981
	Primary	5 (29.4%)	12 (70.6%)		9 (52.9%)	8 (47.1%)	
	Secondary	29 (60.4%)	19 (39.6%)		27 (56.3%)	21 (43.8%)	
	Tertiary	6 (50%)	6 (50%)		6 (50%)	6 (50%)	
Gender	Male	42 (51.2%)	40 (48.8%)	0.799	48 (58.5%)	34 (41.5%)	0.190
	Female	10 (55.6%)	8 (44.4%)		7 (38.9%)	11 (61.1%)	
Years of Experience	1 – 2 years	14 (53.8%)	12 (46.2%)	0.243	14 (53.8%)	12 (46.2%)	0.385
	2 – 5 years	7 (77.8%)	2 (22.2%)		7 (77.8%)	2 (22.2%)	
	> 5 years	31 (47.7%)	34 (52.3%)		34 (52.3%)	31 (47.7%)	

Discussion

The finding in this study of a high frequency of occurrence and slaughter of Sokoto Gudali (69%) and White Fulani (30%) cattle breeds during the survey is in agreement with earlier reports that Sokoto Gudali is one of the most common cattle breeds in Nigeria (Ogugua *et al*., 2015), and that White Fulani cattle is one of the most numerous and widespread of all Nigerian cattle breeds (Dandare *et al*., 2014). This study also found that bulls outnumbered the cows in the cattle slaughtered at the abattoir; this observation can be attributed to the fact that cows are usually maintained for breeding (Rodero-Serrano *et al*., 2013), and are only culled when their reproductive performance declines due to ageing, poor reproductive performance or low milk production (Ogugua *et al*., 2015). Also, 99% of the cattle presented for slaughter were adults; this can be attributed to the fact that adult cattle are more commonly presented for slaughter when compared to the young ones. The young ones (< 2 years) are presented only

when they are sick or greatly traumatized.

The brucellosis seroprevalence of 6.0% recorded in this study for slaughter cattle sampled at Abakiliki abattoir is similar to the 6% reported by Cadmus *et al*. (2010) in southwestern Nigeria, the 6.1% reported by Ishoola and Ogundipe (2001) at Ibadan Nigeria, the 7.8% reported by Ayoola *et al*. (2017), and the 5.3% reported by Cadmus *et al*. (2006) in Ibadan, Southwest, Nigeria. However, it is higher than the 3.9% reported by Ogugua *et al*. (2015) in slaughtered cattle in three different parts of Nigeria. It is thought that the relatively minor differences in the seroprevalence of brucellosis in slaughter cattle could be attributed to sample size, animal sources, management practices in the farm sources and sampling methods. It is important to note that the majority of cattle slaughtered in most abattoir facilities in the southern parts of Nigeria are sourced from different parts of Northern Nigeria (Akinyemi *et al.,* 2022; Gimba *et al*., 2020), as well as neighboring countries sharing boundaries with Northern Nigeria (Ogugua *et al.,* 2015).

The finding in the present study that the seroprevalence of brucellosis is significantly associated with the breed of cattle sampled is in agreement with the reports of some other researchers who studied the disease in cattle in other parts of the country (Cadmus *et al.,* 2013; Junaidu *et al.,* 2011), but in contrast to the reports of Ogugua *et al.* (2015) and Cadmus *et al.* (2010). It has been posited that brucellosis seroprevalence varies across breed on account of genetic polymorphisms. These polymorphisms have been linked to cattle breeds that have been shown to be resistant or tolerant of *Brucella* infection via antibody response (Ogugua and Onunkwo, 2023; Quéméré et al., 2020).

Although not at a statistically significant level, the relatively higher seroprevalence recorded in cows relative to bulls in the present study is similar to what was reported by Cadmus *et al.* (2013) and Ogugua *et al.* (2015). The higher prevalence in cows may be due to the fact that cows are normally kept for a longer period in the herds, resulting in a greater likelihood of exposure to infection with *Brucella,* especially in endemic areas (Sabra *et al.,* 2021). Furthermore, female cattle are usually culled when their reproductive performance is poor, and this is among the clinical signs of brucellosis in cows (Khurana *et al.,* 2021). On the other hand, highly productive females are retained for a long time in the herds, and high parity has been recorded as being associated with brucellosis (Abera *et al.,* 2019). In multiple pregnancies, the stress associated with pregnancy as well as calving is known to depress immunity in female animals (Merlot *et al.,* 2013). The immune depression increases the chances of infection with *Brucella* given the endemicity of brucellosis in areas where the animals are sourced and the fact that management systems in these areas are extensive (Moriyón *et al.,* 2020). Pastoralism has been associated with the transmission and maintenance of brucellosis in and between herds (Njenga *et al.,* 2020).

It was worrisome to note that 55% of the abattoir workers (respondents) in this study admitted that they eat raw meat. This is far higher than the 22% reported by Hambolu *et al.* (2013) and the 29.7% reported by Adesokan *et al.* (2016). Consumption of contaminated raw meat or meat products increases the risk of zoonotic transmission (Madzingira *et al.,* 2023). As reported by Madzingira *et al.* (2023) and Adesokan *et al.* (2016), raw meat consumers are more likely to suffer from brucellosis.

Harvesting and handling fetuses without wearing protective clothing was also common among respondents (52%). In infected animals, uterine discharges are known to contain enormous quantities of the *Brucella* organisms (Pal *et al.,* 2020), and contact with such discharges is associated with cases of

brucellosis (Tulu, 2022). In cattle, *Brucella* organisms have a special affinity for the female reproductive tract and foetal tissues because of the presence of erythritol, a sugar that contributes to the multiplication of *Brucella* organisms (Yin *et al.,* 2023). Other researchers have also reported the harvesting and handling of fetuses among abattoir workers in the country (Aworh *et al.,* 2013; Njoga *et al.,* 2023). Handling of fetuses without personal protective equipment (PPE), such as gloves and face masks, may facilitate the zoonotic transmission of brucellosis among abattoir workers. Foetuses harvested from abattoirs are known to contribute to brucellosis transmission. A study by Cadmus *et al.* (2011) found that dogs fed with fetuses of abattoir origin were more likely to have brucellosis than those that were not fed with foetuses.

The frequency of use of hand gloves among respondents was found to be significantly associated (p = 0.001) with level of education, with educated workers being more likely to use them. When compared to uneducated populations, educated populations have demonstrated higher levels of awareness about *Brucella* infection (Onono *et al.,* 2019). This observation may explain why educated workers used gloves to protect themselves from the disease in this study. This finding is also consistent with that of Alhaji and Baiwa (2015), who found that respondents' educational status was significantly associated with preventive practices among abattoir workers in North-Central Nigeria.

Limitations of the study: Only the Rose Bengal plate test was used to diagnose the disease in this study. However, in areas where routine vaccination is not practiced, such as Nigeria, the RBT is ideal for brucellosis screening (Ducrotoy, 2014); it has been used as the only diagnostic test in cattle in three geographical regions of the country (northern, southern, and south-western Nigeria) (Akinseye *et al.,* 2016) and also in south-eastern Nigeria (Njoga *et al.,* 2018). In addition, isolation, which is the only method of confirming the disease, was not done in this study. However, several studies have used only serology to screen for the disease in livestock populations (Deb *et al.,* 2023; Bifo *et al.,* 2020; Ali *et al.,* 2013).

Conclusions and Recommendations: This study showed that the seroprevalence of brucellosis in cattle slaughtered at Abakiliki abattoir is 6%, and that the seroprevalence was significantly higher in White Fulani cattle when compared to Sokoto Gudali and Red Bororo breeds. The use of hand gloves among the abattoir workers was significantly associated with the worker's level of education.

It was recommended that abattoir workers should be educated on brucellosis and its zoonotic nature and be dissuaded from consuming raw meat. Protective clothing, hand gloves, and face masks should be made available free of costs or supplied at a subsidized rate to abattoir workers to forestall their contacting and transmitting the disease.

Conflict of interest

The authors declare no conflict of interest.

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