

## Survey on Bovine Brucellosis in Sokoto Metropolitan Abattoir, Nigeria

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**Abstract:** Bovine brucellosis is a highly contagious disease of cattle transmissible to humans. The disease is of economic and public health importance as infected cattle can shed and spread the bacteria to susceptible animals and humans in close contact or via consumption of unpasteurized milk. Molecular detection of the IS711 gene segment in *Brucella abortus* was employed to examine lymph nodes of cattle slaughtered at Sokoto metropolitan abattoir. The overall prevalence of the infection was 28.24% and it was seen to have a statistically significant association with sex ( $p < 0.001$ ) and age ( $p = 0.025$ ). The study revealed presence of *Brucella abortus* infection in cattle slaughtered at the abattoir which presents great risk to abattoir workers and other meat handlers. There is the need to develop a monitoring programme for animal brucellosis in the state. Individuals in close contact with animals such as livestock farmers and abattoir workers should be enlightened on the zoonotic risk of *Brucella* pathogen.

**Key words:** Survey, bovine, brucellosis, abattoir, Sokoto, Nigeria

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### INTRODUCTION

Brucellosis is an economically important disease of animals particularly in developing countries of Asia, Africa and Latin America. The disease is caused by *Brucella*, a bacteria genus comprising 11 species but most important are *Brucella abortus*, *Brucella melitensis*, *Brucella ovis*, *Brucellae suis* and *Brucella canis*. *Brucella abortus* is one of the highly contagious species associated bovine brucellosis. The disease is characterized by late term abortions, weak calves, still births, infertility, placentitis, epididymitis and orchitis with excretion of the organisms in uterine discharges and milk (England *et al.*, 2004). Bovine brucellosis is associated with huge economic losses in the livestock industry owing to its high morbidity and abortion storm. *Brucella abortus* is a zoonotic pathogen transmissible to humans via consumption of contaminated animal products and contact with infected animals. Human brucellosis is an acute febrile illness characterized by intermittent fever, anorexia, insomnia, profuse sweating, chills, arthralgia, headache, hepatomegaly, splenomegaly, weight loss and endocarditis (Young, 1995; Uddin *et al.*, 1998; Pappas *et al.*, 2006). The disease remains an occupational hazard to veterinarians, livestock farmers, abattoir workers and laboratory personnel who are in contact with animals (Pappas *et al.*, 2005). Over 500,000 cases of human brucellosis are reported globally, however, the disease is poorly understood in sub-Saharan Africa with prevalence rates ranging from 10.2-25.7% (Mangen *et al.*, 2002). In Sokoto Nigeria, a seroprevalence of 32.2% was

reported in cattle at prisons farm (Junaidu *et al.*, 2011). However, there was no study on molecular detection of *Brucella abortus* in cattle despite its considerable zoonotic risk. Thus, this study was aimed at using polymerase chain reaction to identify brucellosis infection in cattle slaughtered at Sokoto metropolitan abattoir.

### MATERIALS AND METHODS

**Study area:** The study was conducted in Sokoto metropolitan, the capital of Sokoto State, Nigeria. The state is located at latitude 13° N and between longitudes 40° 8' E and 60° 54' E in the extreme North Western part of Nigeria, covering an area of approximately 56,000 km<sup>2</sup> (Blench, 1999). The state shares border with Niger Republic to the North, Kebbi State to the South and Zamfara State to the East. Based on the 2006 National population census, the state has a projected population of about 4,244,399 (Anonymous, 2006). The state is ranked second in the livestock population in Nigeria with an average of 3 million cattle, 4 million goats, 3.85 million sheep, 0.8 million camels and a million poultry (Anonymous, 2008).

**Study design and sample collection:** A cross-sectional study was carried out between June to September 2018. Cattle slaughtered in Sokoto metropolitan abattoir were randomly sampled. The breed, sex and age of each animal were determined before slaughter. At post mortem, the inguinal lymph nodes were carefully excised into a sterilized sample container and labelled properly. A total

Table 1: Oligonucleotide sequences for PCR amplification of IS711 region in *Brucella abortus*

Primer name	Primer sequence 5' to 3'	Expected band size (bp)
<i>Brucella abortus</i>	F-GAC-GAA-CGG-AAT-TTT-TCC-AAT-CCC	498
IS711	R-TGC-CGA-TCA-CTT-AAG-GGC-CTT-CAT	

170 samples were collected and transported in an ice chest to the Central Research Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto.

**DNA extraction:** Modified boiling method of DNA extraction as earlier described was adopted (Cao *et al.*, 2003). Briefly, about 25 g of each tissue sample (lymph nodes) was transferred into a sterilized ceramic mortar and 1 mL of sterile nuclease free water was added. The sample was homogenized using a sterilized pestle and 600 µL of the homogenates was transferred into a 1.5 mL microfuge tube. The tube was vortexed for 15 sec and boiled at 95°C in a water bath for 30 min. The tube was centrifuged at 14,000 rpm for 10 min. Chloroform (600 µL) was added to the mixture, vortexed for 15 sec and centrifuged at 14,000 rpm for 10 min. The supernatant was transferred to a new 1.5 mL microfuge tube and 250 µL of isopropanol was added and incubated at -20°C overnight. The tubes were centrifuged at 14,000 rpm for 10 min and the supernatant was discarded. Without disturbing the pellets, 250 µL of 70% ethanol was added and centrifuged at 14000 rpm for 10 min to wash the pellets. The tubes were air dried in an oven at 55°. The pellets were resuspend in 50 µL of molecular grade water.

**Polymerase chain reaction:** A 25 µL reaction was prepared containing 12.5 µL MasterMix 2x (BioLabs®), 6.5 µL nuclease free water, 5 µL of DNA template and 0.5 µL of each primer Table 1 amplifying the IS711 region of *Brucella abortus*. The reaction was subjected to 35 cycles of amplification (1.15 min at 95°C, 2 mins at 55.5°C, 2 min 2 at 72°C) followed by 5 mins of final extension at 72°C (Bricker and Halling, 1994). Sterile nuclease free water was used as negative control and *Brucella abortus* strain 19 was used as positive control.

**Agarose gel electrophoresis:** Agarose gel (1.0%) was prepared by suspending 1 g of agarose powder in 100 mL of 1X Tris-Borate-EDTA (TBE) buffer. The mixture was heated in a microwave oven until completely dissolved. Ethidium bromide (2.5 µL) was carefully added into the liquid agarose before pouring into a gel caster set and allowed to solidify at room temperature. The caster was rightly placed into an electrophoresis tank and flooded with 1XTBE buffer to the maximum level before carefully removing the comb. The PCR products (5 µL each) were mixed with 2 µL Gel Loading Dye Blue 6X (BioLabs Inc. New England) and loaded into the wells. The 4 µL of 100 bp ladder (BioLabs Inc. New England) was also loaded into one of the wells before connecting the tank to a

power pack and plugged to the mains supply. The products were electro phoresed at 85 V for 50 mins and immediately viewed using a Gel Doc™ (BioRad). The gel image was captured and labelled accordingly.

**Data analyses:** The results obtained were presented in narratives and tables. Chi square ( $\chi^2$ ) statistics and fisher's exact test were used to determine possible association between positive samples and variables such as sex, age and breed of the animals. A significant level of 5% and 95% confidence interval were used for the statistical analyses.

## RESULTS AND DISCUSSION

A total of 170 cattle were sampled comprising 20% (34) being Sokoto Gudali breed and 80% (136) white fulani breed. Over half (60%) of the cattle were cows and the remaining 40% were bulls. Most of the animals sampled were adults over a year old (78.2%) and the 21.8% were young animals <1 year old. The overall prevalence of *Brucella abortus* was 28.24% (48/170). The prevalence rates among Sokoto Gudali and White Fulani were 9/24 (26.60%) and 39/136 (28.68%), respectively. Cows had a higher prevalence rate of 40.20% (41/102) while the prevalence among bulls was 13.23% (9/68). The infection was detected more in adult cattle than the young with prevalence rates of 32.33% (43/133) and 13.51% (5/37), respectively. In this study, there was a statistically significant association between detection of *Brucella abortus* and cows ( $p < 0.001$ ; or = 4.4; 95% CI: 1.969-9.859) as well as adult cattle older than 2 years ( $p = 0.025$ , OR: 3.1, 95% CI: 1.114-8.397). However, there was a non-significant association between the infection and breed of cattle ( $p = 0.761$ ) Table 2.

Bovine brucellosis is one of the neglected endemic diseases in Nigeria characterized with high morbidity and zoonotic risk. The prevalence of the disease in this study (28.24%) is lower than that by Junaidu *et al.* (2011) who reported a seroprevalence of 32.20% in Sokoto prisons farm. Although, the studies were both conducted in the same state, the study design and screening technique used may be responsible for the varying prevalence rates. Junaidu *et al.* (2011) performed a herd based study which has been characterized with high risk of disease transmission as a result of prolonged close contact, high stocking density and high birth index (Ducrottoy *et al.*, 2014). Furthermore, serological assays such as Rose Bengal Plate Test (RBPT), Serum Tube Agglutination Test (SAT) and Competitive ELISA (CELISA) are designed to detect antibodies against *Brucella* pathogen

Table 2: Univariable analysis between *Brucella abortus* infection and some disease determinants

Variables/Levels	Prevalence (%)	$\chi^2$ values	p-values	OR	95% CI
<b>Sex</b>					
Female	40.20 (41/102)	14.285	<0.001	4.4	1.969-9.859
Male	13.23 (9/68)				
<b>Age</b>					
Adult (>2 years)	32.33 (43/133)	5.058	0.025	3.1	1.114-8.397
Young ( $\leq$ 2 years)	13.51 (5/37)				
<b>Breed</b>					
Sokoto Gudali	26.60 (9/24)	0.092	0.761	0.9	0.375-2.048
White Fulani	28.68 (39/136)				

OR: Odds ratio, CI: Confidence interval

irrespective of being an active or passive infection. Thus, these tests have the tendency to produce higher prevalence rates than molecular detection which is designed to detect the pathogen in an active infection (Godfroid *et al.*, 2010; Gurbilek *et al.*, 2017). Statistical analysis revealed a significant association of bovine brucellosis with sex ( $p < 0.001$ ) as cows are 4.4 times more likely to be positive than bulls. The finding is similar to a study in Pakistan where cows were seen to have a significantly higher prevalence than bulls (Ali *et al.*, 2017). Some other studies have shown bull to be at higher risk of infection than cows (Awah-Ndukum *et al.*, 2018). In view of the contagiousness of the disease irrespective of sex both cows and bulls have equal susceptibility to the infection. Similarly, detection of *B. abortus* in this study was seen to be significantly associated with age ( $p = 0.025$ ) as cattle older than 2 years are 3 times more likely to be infected than younger ones. The finding concurs with earlier studies in Nigeria, Cameroun and South Sudan where bovine brucellosis was reported to be a disease of sexually matured animals, hence, the reason for higher infection rates among adults than the young ones (Mai *et al.*, 2012; Ayoola *et al.*, 2017; Awah-Ndukum *et al.*, 2018; Madut *et al.*, 2018). On the other hand, the disease was observed not to be significantly associated with breed of cows ( $p = 0.761$ ) which agrees with the reports by Mai *et al.* (2012) and Awah-Ndukum *et al.* (2018). However, numerous studies have shown some level of association between bovine brucellosis and different cattle breeds. Junaidu *et al.* (2011) reported higher prevalence in Sokoto Gudali breed, Cadmus *et al.* (2008) described more positive reactors in Red Bororo breed while Esuruoso (1974) reported Ndama breed to be more susceptible. Genetic variation amongst breeds of animals have been seen to play role in conferring resistance to brucellosis (Barthel *et al.*, 2001), however, the research field is yet to be explored using Nigerian breeds of cattle.

### CONCLUSION

Cattle slaughtered at Sokoto metropolitan abattoir have been shown to be positive for *Brucella abortus* which is significantly associated with age and sex of the

animals. The disease is zoonotic and therefore, poses an important health threat to the public, especially, livestock farmers and abattoir workers who are in close contact with animals. There is the need to develop a monitoring and surveillance programme for animal brucellosis in the study area. Individuals in close contact with animals as well as the general public should be enlightened on the zoonotic risk of brucellosis.

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