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Original Research Article

Sero-prevalence and risk factors for *Brucella melitensis* infection in goats slaughtered in Abuja metropolis, Nigeria

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ABSTRACT

Brucellosis is a disease that causes significant reproductive failure in ruminants and has been reported to be a disease of major zoonotic importance. The aim of this study was to determine the seroprevalence and risk factors for *Brucella melitensis* infection in goats slaughtered in the Abattoir section of the Livestock Market Gwagwalada Area Council, Anagada, Abuja Nigeria. A total of 240 blood samples were collected in plain tubes without anti-coagulant at point of slaughter for the harvesting of serum samples. Serum Agglutination Test with EDTA (SAT-EDTA) and Lateral Flow Assay (LFA) were used to test for exposure to *Brucella* infection. Chi square was used to test for association between catergorical variables as with level of significance set at 0.05. Overall seroprevalence rates using SAT-EDTA and LFA were 7.11% and 6.67% respectively. Based on risk factors, seroprevalence rate was higher in does (6.62% vs 6.68%), goats ≤ 1 year of age (7.45% vs 7.45%) and the Sahel breed of goats (9.80% vs 7.69%) by SAT-EDTA and LFA respectively. There was no significant difference between all variables $p > 0.05$. Highest prevalence rates were obtained in does, goats ≤ 1 year and the Sahel breed of goats. As a pathogen of public health significance to in-contact abattoir workers, it is imperative that these workers be educated on the nature of the disease and on how to minimize its risk of transmission via contacted with infected animals and their products.

. **Keywords:** *Brucella melitensis*, SAT-EDTA, LFA, Seroprevalence, Breeds of goats.

INTRODUCTION

The socio-economic benefits of small ruminant (goats and sheep) production in the livelihood of the people in developing countries with regards to nutrition, income and other substantial benefits cannot be overemphasized (Kosgey, 2004). Small ruminants constitute the largest proportion of livestock production in Nigeria with approximately 28 million goats and 23 million sheep (FAO, 2006). There are many reproductive diseases that hinder their production and reproduction in Nigeria of which brucellosis is not an exception. Brucellosis is a zoonotic disease of significant public health importance and has been reported as one of the major causes of reproductive failure in ruminant production (Neharika *et al*., 2018). The likelihood of transmission of brucellosis is high in many rural and nomadic communities in Nigeria due to the co-mingling of livestock with humans (Buhari *et al*., 2020).

Brucellosis is a bacterial disease mostly of animals caused by gram negative, facultative intracellular coccobacilli belonging to the genus *Brucella* (Young, 2000; Alton and Forsyth, 2004). Though both animals and man are susceptible to the various species of *Brucella*, *B. melitensis* has been reported to be the most pathogenic of all the species to man (OIE, 2009).

The disease affects several species of domestic, wild and marine animals (Agada *et al*., 2018). It is mostly characterized by inflammation of the genital organs and foetal membranes. Abortion, sterility, formation of localized lesions in joints and the lymphatic system are also important features of the disease (Roth *et al*., 2003; CDC, 2005; Franco *et al*., 2007).

Brucellosis is recognized as one of the neglected tropical zoonotic diseases with a global public health significance (OIE, 2018). Although the disease has been controlled/eradicated in many industrialized nations, it

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remains prevalent in parts of Asia (Sofian *et al*., 2008), South America (Dias *et al*., 2009) and Africa (Bronsvoort *et al*., 2009; Ogugua *et al*., 2015; Mubanga *et al*., 2021).

In animals, *Brucella* organisms are transmitted through sexual means, ingestion of contaminated feed and water from materials such as aborted fetuses and after birth discharges from infected animals, maternal transfer to offsprings and by artificial insemination (Corbel, 2006; Lopes *et al*., 2010). The presence of the disease in animal population invariably translates to risk of the disease in humans (Corbel *et al.*, 1989).

Transmission of *Brucella* organisms to humans is mainly through ingestion of contaminated dairy products, direct contact with infected animals and their secretions through cuts and abrasions in the skin of wounds with infected materials and through inhalation (Sofian *et al*., 2008). It is therefore an occupational disease of livestock attendants, laboratory personnel and abattoir workers (Falade, 2002; Aworh *et al*., 2013; Traxler *et al*., 2013). In rear circumstances, human-to-human transmission through coitus, child birth, tissue transplant and blood transfusion have been reported especially with *Brucella melitensis* (Vigeant *et al*., 1995; Falade, 2002; Poulou *et al*., 2006). Abattoir workers involved in the slaughtering and processing of goats are at high risk of being infected, especially from infected uterine content and udder (European commission, 2001).

Brucellosis could result in negative economic and public health consequences ranging from culling due to infertility, decreased milk production, abortions, still births, birth of weak animals, and loss of man hours in humans protracted illness (Mai *et al*., 2012).

Several serological based researches have shown that brucellosis is endemic in livestock population across different states in Nigeria (Cadmus *et al*., 2006; Ogugua, *et al*., 2014; Agada *et al*., 2018). In some of such studies, a prevalence of 16.1% was reported in small ruminants in Plateau State (Bertu *et al*., 2010), 19.8% in small ruminants in Nasarawa state (Agada *et al*., 2018) while 8.6% was recorded in trade cattle in Ibadan (Cadmus *et al*., 2010), a few documented reports have revealed that the disease is endemic in goats in Nigeria; with prevalence of 0.86%, 14% and 25.8% reported in south-western, north-eastern and north-central Nigeria respectively (Cadmus *et al.*, 2006; Tijjani *et al*., 2009; Kaltungo *et al*., 2013).

Brucellosis continues to be a problem in Nigeria due to continuous spread because of the lack of official control policy for the disease (Ibironke *et al*., 2008), poor disease reporting (Buhari *et al*., 2020), free movement of pastoralists that rear about 95% of the livestock population (Ocholi *et al*., 2004). Constant contacts of livestock of different species during herding, at livestock market places and at watering points may give instances to the risk of infection with *Brucella* organism should any species be found infected (Kaltungo, 2012; Buhari *et al*., 2015). Spread of the disease may also occur as a result of culling and slaughter of animals in abattoirs due to brucellosis (Buhari, 2014) and those sold at various livestock market places. Similarly, the lack of enlightenment of pastoralist and small ruminant keepers on feeding, housing, vaccination, management and disease transmission and prevention may further contribute to the spread of brucellosis (Kaltungo *et al*., 2018).

Small ruminant production plays an important role in the economic improvement of "poor farmers" and contributes to poverty alleviation (Yakubu *et al*., 2011). There are many studies on brucellosis which are focused more on two of the five abattoirs in Abuja (Aworh *et al*., 2017). Currently, there is paucity of information regarding the exposure of goats to *Brucella* species in Anagada abattoir Gwagwalada, Abuja where a significant number of goats are slaughtered daily and in view of the fact that these goats are brought in from different parts of the country. In this study, we used the Serum Agglutination Test with EDTA (SAT-EDTA) followed by Lateral Flow Assay (LFA) to determine the seroprevalence of *Brucella melitensis* infection and risk factors in goats slaughtered in this study area.

MATERIALS AND METHODS

STUDY LOCATION

The study was conducted in Gwagwalada Area council Livestock market Anagada along Zuba express way, Abuja. Gwagwalada is located in the North-central geo-political zone of Nigeria. It is situated between Lat. 8° 55' and 8° 60' North, and Long. 7° 05' and 7° 11' East and has a land mass of 1,043 square kilometres. The Gwagwalada Abattoir is located at new Kutunku ward of the town, beside one of the tributary streams of river Usuma, which drains through the town (Magaji and Chup, 2012).

SAMPLE SIZE DETERMINATION

The sample size for this research was determined using the formula, $n = \frac{Z^2\ P(1-P)}{d^2}$ (Thrusfield, 2005), Where;

n= sample size

z= confidence interval

p= expected true proportion

d= desired absolute precision

The sample size for goats was determined using a prevalence rate of 19.6% from slaughtered goats screened in Abuja (Aworh *et al*., 2017) with an error margin of 5%. Hence, the calculated minimum sample size (n) was 242.

SAMPLING TECHNIQUE AND SAMPLE COLLECTION

Sampling was done by random selection without replacement from goats presented to the abattoir for slaughter.

About 3ml of whole blood sample was collected from each goat through the jugular vein at the point of slaughter using plain sample tubes without anticoagulant. Each tube was labelled using codes that corresponds to individual sample with relevant information such as the age, breed and sex, each of the goats sampled were aged by their dentition (Hassan & Hassan, 2005).

The tubes were kept in a slanting position and left for 12 hours at room temperature to allow for clothing and serum separation. Serum from each sample was harvested using Pasteur pipettes after which it was transferred into 2ml serum vials which were labelled accordingly. All samples were transported in a cool man's box to the Bacteria zoonosis Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and stored at -20^{0}C until assayed (Alhaji *et al*., 2013).

LABORATORY ANALYSIS

The stored sera samples were removed from the freezer and allowed to thaw at room temperature before commencement of analysis.

SERUM AGGLUTINATION TEST WITH ETHYLEDIAMINOTERAACETIC ACID (EDTA)

Serum agglutination test was performed according to the method described by Brown *et al*., (1981). The stored sera samples were thawed before commencement of the experiment. Phenol saline solution was prepared with 0.5 g phenol crystals, 0.85 g sodium chloride and 1.86g of disodium EDTA all dissolved in 100 mls of warm distilled water. For each day work, sample processing 1:10 dilution of concentrated SAT antigen was used. A 96-well "U- shaped" microtiter plate was set up on the working bench and labelled. Test serum vials were placed on the bench to correspond to the positions of each microtitre wells.

Using a micropipette, 40µl of phenol saline was dispensed into the first well in a row of a microtiter plate while 25µl was dispensed into each of the remaining wells. Thereafter, 10µl of the test serum was added into the first well using a new disposable pipette tip for each test sample. A two-fold serial dilution was carried out by transferring 25µl aliquots from the first well up to the fifth well. After the last well, 25µl of the aliquot was discarded.

The content of the working dilution of the SAT antigen was mixed gently and 25 µl was transferred to each well after which the contents of the microtiter plate were mixed by gently tapping the edges of the plate for 20 seconds. A foil paper was then used to cover the microtiter plate to prevent evaporation of its contents. The plates were incubated at 37^{O}C for 20 hours in an incubator (Gallenkemp®, Germany) after which the results were read.

A mat of cells covering the bottom of the well surrounded by slightly opaque diluent indicated a positive reaction while a large bottom of particles in the centre of the well (button-like), encircled by clear diluents was indicative of negative reaction. All wells with agglutination titres of 1:40 (equivalent to 50 i.u.) or greater were considered positive.

LATERAL FLOW ASSAY

Lateral Flow Assay Technique was performed according to the manufacturer's instruction using kits obtained from BioNote Inc. Korea. The contents of the test kit were removed from the foil pouch and placed on a flat, dry surface. Using the capillary tube provided in the kit, 20µL of the serum sample was dropped in the sample hole marked "T" on the test device after which 4 drops of the assay diluent was dropped in the same hole. The test results were read after 20 minutes by visual inspection for staining of the test and control lines. Tests were scored negative when staining was observed only on the control line and scored positive when staining was observed on both the test and control lines.

DATA ANALYSIS

Data were analyzed using IBM SPSS version 20.0 (2011). Chi-square test was used to test for association between categorical variables. Statistical significance was set at a value of $p \leq 0.05$. The overall data generated were presented as tables.

RESULTS

The results of the sero-prevalence of brucellosis in goats based on sex, age and breed using Serum Agglutination Test with EDTA (SAT-EDTA) is presented in Table I. A total of 239 serum samples was tested using Serum Agglutination Test with EDTA (SAT-EDTA), of which 17 was positive with an overall seroprevalence rate of 7.11%

With respect to sex, the sero-prevalence rate was higher in the does 5(8.62%) compared to the bucks 12 (6.62%). There was no statistical difference between the sexes ($p > 0.05$) (Table I).

Based on age groups, the highest sero-prevalence rate 2(7.45%) was recorded in goats ≤ 1 year of age. This was followed closely by goats older than 1 year to 3 years of age 5 (7.04%) while 0 (0.00%) was recorded for goats above the age of 3 years. There was no statistically difference ($p > 0.05$) between sexes (Table I).

Finally, based on breeds, the highest sero-prevalence rate 5(9.80%) was obtained in the Sahel breed of goats while 12(7.19%) was obtained in the Kano brown breed of goats. None of the West African Dwarf breed of goats was positive

0(0.00%). There was no statistical difference in the seroprevalence rates between the breeds of goats ($p > 0.05$) (Table I).

From a total of 240 serum samples tested using the Lateral Flow Assay, 16 was positive with an overall prevalence of 6.67% (Table II).

Table I: Sero-prevalence of brucellosis in goats slaughtered in Abuja, Nigeria based on sex, age and breed using Serum Agglutination Test with EDTA (SAT-EDTA). WAD=West African Dwarf

Variables	Level	No. Sampled	No. positive (%)	Chi-square value (χ^2)	P-value
Sex	Bucks	181	12 (6.62)	0.264	0.608
	Does	58	5 (8.62)		
Age	≤1-year	161	12 (7.45)	0.800	0.977
	>1year-3years	71	5 (7.04)		
	>3-years	7	0 (0.00)		
Breeds	Kano	167	12 (7.19)	2.168	0.338
	brown	51	5 (9.80)		
	Sahel	21	0 (0.00)		
Total	WAD	239	17 (7.11%)		

Based on sex, a higher sero-prevalence rate 3(6.68%) was recorded in does while in the bucks, the sero-prevalence rate 13(6.63%) was lower. There was no statistically significant difference ($p > 0.05$) between sexes (Table2).

Based on age groups, different levels of sero-prevalence rates were observed with the highest rate 12(7.45%) in goats ≤ 1 year of age, followed by 4(5.56%) in goats above one year to 3 years of age and 0(0.00%) for goats above 3 years of age. There was no statistical difference in the seropositivity between the age groups ($P > 0.05$) (Table II).

Based on breeds, the highest sero-prevalence rate 4(7.69%) was recorded in the Sahel breed of goats. This was followed by 11(6.59%) recorded in the Kano brown breed while the least sero-prevalence rate 1(4.67%) was recorded in the West African Dwarf breed of goats. There was also no statistically difference between prevalence rates among the breeds ($P > 0.05$) (Table II).

Table II: **Sero-prevalence of Brucellosis in goats slaughtered in Abuja, Nigeria based on sex, age and breed using Lateral Flow Assay**

Key: WAD = West African Dwarf

Variable	Level	No. Tested	No. positive (%)	Chi-square value (χ^2)	P-value
Sex	Bucks	181	12 (6.63)	0.315	0.575
	Does	59	4 (6.68)		
Age	≤1year	161	12 (7.45)	1.553	0.907
	>1year-3years	72	4 (5.56)		
	>3years	7	0 (0.00)		
Breed	Kano				
	brown	167	11 (6.59)	0.212	0.899
	Sahel	52	4 (7.69)		
	WAD	21	1 (4.76)		
Total		240	16(6.67%)		

DISCUSSION

This study established the presence of anti-*Brucella* antibodies in goats sampled from Anagada abattoir in Gwagwalada area council livestock market, Abuja. The sero-prevalence rate of 7.11% obtained was found to be higher using the SAT-EDTA. This recorded prevalence is quite lower than 19.6% reported by Aworh *et al*., 2017 in goats slaughtered in abattoirs in Abuja. This difference in the prevalence rates in both studies may be attributed to the types of test being used in both researches. Aworh *et al*., 2017 used the Rose Bengal Plate Test (RBPT) which is mainly a screening test with very high sensitivity but with less specificity than the SAT-EDTA being used in the present study. Some other researchers like Buhari *et al*., (2020) recorded a comparable sero-prevalence rates of 6.37% and 8.90% in a seroprevalence study of *Brucella* infection in small ruminants from two institutional farms and a slaughter slab in Zaria, Nigeria. However, Ogugua *et al*., (2014) conducted a similar study in goats in Nigeria and reported variable sero-prevalence rates ranging from 17.30% in Benue state to 2.05%, 0.60% and 0.00% in Borno, Oyo and Sokoto states respectively.

The difference is the sero-prevalence rates reported may also be attributed to differences in the study location, serological tests used and also the numbers of animals screened. This agrees with Lopes *et al*., (2010) who reported that study location was known to affect the sero-prevalence of *Brucella* infection as it is more endemic in some locations compared to others. Also, the serological tests used may affect the sero-prevalence rate of diseases due to sensitivity and specificity difference or whether the test is a screening test or a confirmatory test (Godfroid *et al*., 2002).

The sero-prevalence rate on age bases was higher in younger goats of ≤ 1 year of age. This agrees with Buhari *et al*., (2020) who recorded a

higher sero-prevalence in animals of ≤1 year of age when compared to their older counterparts using SAT-EDTA. However, this report is contrary to those of several other researchers like, Cadmus *et al*., (2010), Junaidu *et al*. (2011) and Kaltungo *et al*. (2013)

who reported that age does not affect the sero-prevalence of brucellosis, with the older animals being more seropositive than the younger ones. The younger animals may have acquired the infection in-utero as reported by Grillo *et al.*, (1997) that the transmission of brucellosis could be in-utero when their dams are infected. This may explain the persistent infection in their offspring who may remain latent carriers of the infection. This difference may also be linked to the fact that more of the goats that were sampled were ≤ 1year of age as they were more of this age group presented for slaughter at the sampling abattoir.

This study also revealed a higher sero-prevalence in does than in bucks using both the SAT-EDTA and LFA. This agrees with the findings of Bertu *et al*., (2010), Kaltungo *et al*., (2013) and Zubairu *et al*., (2014). Generally, higher sero-prevalence in females may be linked to the fact that more females are allowed in the flock to attain sexual maturity and as such are kept for much longer periods for breeding purpose which allows for more exposure potentials. Conversely, the males are kept in the flock for shorter periods, as they are sold out or slaughtered for different occasions. In addition, the reproductive tract of the female animals (especially the uterus) possesses a sugar alcohol erythritol for which *Brucella* species have affinity which may further explain these findings (Cutler *et al*., 2005).

There was higher sero-prevalence rate (9.80% vs 7.69%) in the Sahel breed of goats using both SAT and LFA respectively. This finding is contrary to that of Buhari *et al*., (2020) who reported the highest seroprevalence rate (8.33%) in WAD breed of goats in Zaria, Kaduna State and Aworh *et al*., (2017) who recorded a the highest sero-prevalence rate in Sokoto red breed of goats in Abuja using m-RBPT, SAT-EDTA and RBPT, c-ELISA respectively. This difference may be linked to the difference in study location and the number of a particular breed tested in the different studies.

CONCLUSION

The overall sero-prevalence rates of *Brucella* infection were found to be highest in does, goats ≤ 1 year of age and Sahel breed of goats. High risk groups like the veterinarians (meat inspectors), butchers, livestock handlers and other stake holders in abattoirs should be educated on the nature of the disease and on how to minimize its risk of transmission through slaughter and consumption of infected animals and their products.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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