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Sero-Prevalence of Brucella Antibodies in Goats in Giwa Local Government Area of Kaduna State, Nigeria

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Abstract: *Brucellosis is a zoonotic disease that results in great economic losses to farmers due to abortion and infertility leading to low productivity. To determine the prevalence of Brucella antibodies, 280 serum samples were collected from goats in six districts in Giwa Local Government Area (LGA) of Kaduna State. Rose Bengal Plate Test (RBPT) and Competitive ELISA (cELISA) were used to detect Brucella antibodies in the samples. Out of the 280 sera tested, 23 (8.2%) and 7 (2.5%) were positive using RBPT and cELISA respectively. Female goats had more (8.3%) antibodies to Brucella species than the males (8.0%), whereas, the males had more (4.0%) antibodies to Brucella melitensis than the females (2.2%). The West African Dwarf and Kano Brown Breeds had the highest positive reactors to Brucella antibodies than Red Sokoto breed using the RBPT, however, Brucella melitensis' antibodies were higher (6.3%) in the Kano Brown as compared to the other breeds. Goats above 36 months of age showed higher rate of Brucella antibodies by RBPT and cELISA than the other age groups. There were no statistical associations (P>0.05) between breed, sex and age of the goats sampled and prevalence of Brucella antibodies. This study has shown that goats in Giwa LGA had antibodies to Brucella and this is of public health importance.*

Keywords:*Antibodies, Brucella, Giwa LGA Kaduna State, Goats,Sero-prevalence.*

I. Introduction

Zoonotic infections account for over two thirds of all human infectious diseases worldwide and one infectious disease which particularly impedes international trade is brucellosis [1, 2]. Brucellosis is a bacterial disease caused by members of the Brucella genus that can infect humans but primarily infects livestock. Brucella organisms are facultative, small, gram negative, non-motile, non-spore forming, coccobacilli bacteria [3, 4]. Brucella has several species but the most important ones and their preferred natural host includes: B. abortus(cattle), B. melitensis(goats and sheep), B. suis(pigs), B. canis (dogs), B. ovis(sheep), B. neotomae (desert wood rat), B. ceti and B. pinnipedialis(isolated from cetaceans and pinnipeds respectively) [5,6,7].

Caprinebrucellosis is a highly contagious and zoonotic disease of public health importance that is caused by B. melitensisandB. abortuswith clinical signs such as abortion, infertility, birth of weaklings, retention of the placenta, dead offsprings and increased calving interval resulting in economic losses to the animal industry especially ruminants and decrease in the farmer's income [8]. Once an animal is infected with Brucella organism, it may persist for the whole life of the affected animal [9, 10, 11].

Caprine brucellosis is endemic in Nigeria [12, 13, 14, 15, 16, 17] and it is important not only as a hindrance to increased production but also as a zoonosis that affects ruminant production systems in most parts of the world [18,15]. World-wide, most cases of human brucellosis are caused by B. melitensis which is considered the most invasive and pathogenic species of the genus [19]. Although the disease has been eradicated in most industrialized nations, its occurrence is still on the increase especially in developing countries such as Nigeria, where it remains a serious zoonotic disease [20, 21, 22, 23].

In Nigeria, goat production plays an important role in the economic improvement of poor farmers and contributes to poverty alleviation, but if not reared hygienically poses a threat to human health as it may serve as a route for transmission of zoonotic diseases such as brucellosis [24]. This research is pertinent as it could help provide important information that will ensure safety and better yield in goat production and control of brucellosis and other zoonotic diseases.

II. Materials and Methods

2.1 Study Location

This study was carried out in Giwa Local Government Area (LGA) of Kaduna State. Giwa LGA (Fig. 1) consists of eleven (11) districts with many villages. The study area has a landmass of about 3,350 square meters with a human population of about 280,427 [25] and located 30km north-west of Zaria. It lies between latitudes 11°30′N and longitudes 7°45′E. The vegetation is of the northern guinea savannah type [26]. The climate in the area is characterized by the occurrence of a wet season between June and September and a dry season from October to early May. The dry season is further divided into a cool-dry season which is referred to as harmattan period from November to February and hot-dry season from March to early May. The major ethnic

groups are Hausa and Fulani. Giwa LGA has a rich soil suitable for the cultivation of a wide range of crops, with the produce and the crop residues serving as a rich source of food to their livestock especially goats, thereby engaging most of the inhabitants in farming activities as their primary occupation.

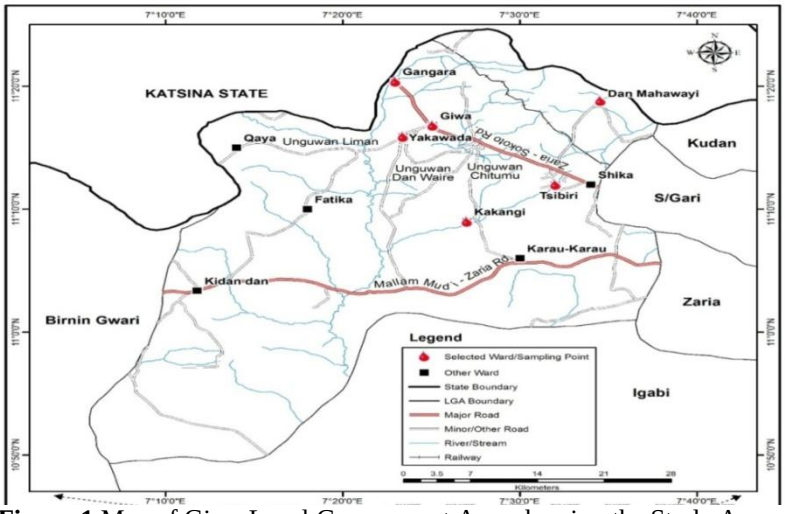

Figure 1. Map of Giwa Local Government Area showing the Study Areas.
Source: Modified from the Administrative Map of Giwa LGA.

2.2 Study Design and Sampling Procedure

A cross sectional study was conducted for a period of 2 months, from August to September, 2014 within Giwa LGA of Kaduna State. Venous blood samples were obtained from goats of both sexes in six (6) districts, namely; Giwa, Yakawada, Tsibiri, Dan Mahawayi, Kakangi and Gangara within Giwa LGA. A simple random sampling technique was used to select households in each district and the goats were sampled from households in each of the districts by employing the systematic random sampling method, whereby a maximum of 3 and 5 goats were sampled from households having ≤15 goats and >15 goats in a flock respectively for collection of blood samples. A total of 280 serum samples from goats were screened for Brucella antibodies in this study.

2.3 Collection of Blood

Five millilitres (5ml) of blood was aseptically obtain from jugular vein of each selected goat using a 10ml sterile syringe into a plain 10ml sample bottle containing no anticoagulant. Each sample was labelled accordingly. The samples were then transported on ice packs in a Cole-man box to the laboratory and centrifuged at 3,000 revolutions for five minutes to obtain clear sera. The sera obtained were stored at -20^{0}C until required for analysis.

2.4 Laboratory Test

The sera were subjected to the Rose Bengal Plate Test (RBPT) using the standardized Rose Bengal Plate antigen and the Competitve ELISA. The antigen and test kit were obtained from Veterinary Laboratory Agency (VLA), Surrey, United Kingdom.

2.4.1 Rose Bengal Plate Test

The test was carried out as described by [27]. The antigen and the test sera were brought to room temperature before the commencement of the test. Using a clean micropipette, 0.03ml of the serum sample was placed on a white tile and an equal volume of antigen was placed near the serum's spot. Both were mixed thoroughly using a sterile applicator stick. The mixture was then rocked manually for 4 minutes before examination. The presence of agglutination (pinkish granules) in the reaction was recorded as positive, while samples that appeared clear without agglutination were recorded as negative.

2.4.2 Competitive Enzyme-Linked Immunosorbent Assay (cELISA)

The cELISA generally uses 96-wells low protein binding capacity polystyrene microplates which includes the conjugate, positive and negative controls.

Kit contents:Microplates pre-coated with B. melitensis LPS antigen, concentrated conjugate, controls (Negative and Positive serum), chromogen substrate, diluting buffer, wash solution and stopping solution.

Reagent Preparation: The reagents were reconstituted according to the manufacturers' instructions.

Test Procedure**:** The diluting buffer was warmed to room temperature (23 ± 3^0C) in a water bath, while the conjugate concentrate (BM40) was mixed to working strength in the diluting buffer. The microplate was prepared by adding 20 µl of each test serum per well while two columns (11 and 12) were used as control wells. Twenty microlitre (20 µl) of the negative control was added to wells A11, A12, B11, B12, C11 and C12, while 20 µl of the positive control was added to wells F11, F12, G11, G12, H11 and H12. The remaining wells in columns 11 and 12 (i.e D11, D12, E11 and E12) had no sera added to them, thereby acting as conjugate control.

One hundred microliters (100µl) of the prepared conjugate solution was immediately dispensed into all wells, giving a final serum dilution of 1:6. The plate was then vigorously shaken for 2 minutes in order to mix the serum and conjugate solutions. The plate was then covered with a lid and incubated at room temperature (21 ± 6^0C) for 30 minutes and shaken with the hand after 10minutes intervals for 1 hour. The contents of the plate were discarded by agitating the plate. The plates were then rinsed 5 times with a washing solution and dried by tapping on absorbent paper towel.

The substrate and chromogen solution were prepared immediately by dissolving one tablet of urea H_2O_2 in 12ml of distilled water; OPD tablet was also added and mixed thoroughly. 100µl of the solution was then added to each well. The plate was covered and incubated at room temperature for 15 minutes. 100µl of stopper solution was added to all wells to slow the reaction. Absorbent paper towel was used to dry the plate and the plate was read using the microplate reader at 450nm. Visual examination of the plate was also used to determine whether a sample is positive or negative.

Result Interpretation:The resulting colouration was interpreted by visual reading whereby the appearance of white colouration indicates the presence of Brucella antibodies whereas yellowish colouration indicates that there are no Brucella antibodies in the test sample. Sixty percent (60%) of the mean of the optical density (OD) of the 4 conjugate control wells was calculated. Any test sample giving an OD equal to or below this value was regarded as being positive.

3.7 Statistical Analysis

The data obtained were analyzed using Statistical Package for Social Sciences (SPSS) to carry out descriptive analysis for interpretation. Chi-square (χ^2) and Fisher's exact tests were used where appropriate to test association between categorical variables. P-value less than 0.05 ($P<0.05$) was considered statistically significant.

III. Result

3.1 Sero-Prevalence of Brucella Antibodies in Goats in Giwa LGA by RBPT and cELISA based on Districts

Out of the 280 serum samples evaluated for Brucella antibodies using RBPT and cELISA, a total of 23 (8.2 %) and 7 (2.5%) tested positive respectively. Four (1.4%) samples in Gangara and 5 (1.8%) in Tsibiri districts where 43 serum samples were obtained in each of the districts tested positive to Brucella antibodies by RBPT, while using the cELISA, a prevalence of 2 (0.7%) was obtained in each of the two districts. In Giwa district where 45 serum samples were collected, 6 (2.1%) and 1 (0.4%) tested positive by RBPT and cELISA respectively, while out of the 56 serum samples that was tested in Yakawada district by RBPT and cELISA, 5 (1.8%) and 1 (0.4%) positive results were obtained. In Dan Mahawayi district, 1 (0.4%) serum sample tested positive by RBPT out of the 41 serum samples and there were no positive reactors by cELISA. In Kakangi district where 52 samples were tested, 2 (0.7%) tested positive to Brucella antibodies by RBPT, while 1 (0.4 %) serum sample tested positive by cELISA. There was no statistical association ($P>0.05$) between Brucella antibodies and the districts sampled (Table 3.1).

Table 3.1 Sero-Prevalence of Brucella Antibodies in Goats in Giwa LGA by RBPT and cELISA based on Districts

Districts	No. of Sera Tested	RBPT	cELISA	*F-test	P-value
		No. Positive (%)	No. Positive (%)		
Giwa	45	6 (2.1)	1 (0.4)		
Gangara	43	4 (1.4)	2 (0.7)	**5.535	**0.337
Yakawada	56	5 (1.8)	1 (0.4)	***2.893	***0.754
Dan Mahawayi	41	1 (0.4)	0	df=5	
Kakangi	52	2 (0.7)	1 (0.4)		
Tsibiri	43	5 (1.8)	2 (0.7)		
Total	280	23 (8.2)	7 (2.5)		

*Fisher's exact test

**RBPT

***cELISA

3.2. Sero-Prevalence of Brucella Antibodies in Goats in Giwa LGA by RBPT and cELISA based on Breed, Age and Sex Distribution

Of the 256 Red Sokoto breed sampled, 20 (7.8%) were positive to Brucella antibodies by RBPT, whereas 6 (2.3%) were positive by cELISA. One (12.5%) out of the 8 breed representing the West African Dwarf was positive to RBPT while none (0%) was positive by cELISA. Sixteen Kano Brown breed were tested, of which 2 (12.5%) and 1 (6.3%) were positive by RBPT and cELISA respectively.

Twenty-four goats aged less than 12 months showed no positive reaction when tested using both RBPT and cELISA. Six (5.4%) and 2 (1.8%) out of 111 goats within the age range of 12-36 months were positive by RBPT and cELISA. Seventeen (11.7%) and 5 (3.4%) out of 145 goats aged above 36 months tested positive by RBPT and cELISA respectively.

Four (8.0%) out of the 50 male goats tested were positive to Brucella antibodies while 19 (8.3%) out of 230 females tested had antibodies to Brucella as tested by RBPT. Two males (4.0%) and 5 (2.2%) females were positive by cELISA. There was no statistical association between the different breeds, age and sex and the prevalence of Brucella antibodies (P>0.05) (Table 3.2).

IV. Discussion

This study has shown that antibodies to Brucella were detected in goats in Giwa Local Government Area (LGA) of Kaduna State. The relatively high prevalence of Brucella antibodies in goats from Giwa LGA could be attributed to the fact that these goats are housed together and are allowed to graze freely, thereby coming in contact with other animals and environmental contaminants that could be harbouringBrucella organisms. Brucella is known to withstand dry environmental conditions, particularly in the presence of extraneous organic material and will remain viable in dust and soil, thereby infecting any susceptible host available [28, 29, 30]. These farmers also introduced new goats into the flock without quarantining or vaccinating them, and these new animals could be carriers of infectious organisms including Brucella and possibly introduce them into the animal population. In Nigeria, there are no quarantine or vaccination programmes against Brucella infection at present and this could be a factor promoting the spread of this infection from one flock to another [12].

The sero-prevalence of Brucella antibodies in goats by Rose Bengal Plate Test (RBPT) is low as compared to what was reported by [13], who recorded a prevalence of 10.1% in goats from Plateau State and [31,14] who got a prevalence of 30.76% and 22.93% in goats sampled from Sokoto prison farm and Sokoto metropolis respectively, but higher than that reported by [32] (2.8%) [12] (0.86%), [33] (4.75%) and [15] (8.8%) in goats in Maiduguri, Ibadan, Bauchi and the arid zone of Nigeria respectively.

Table 3.2 Sero-Prevalence of Brucella Antibodies in Goats in Giwa LGA by RBPT and cELISA based on Breed, Age and Sex Distribution

Variables	No. of Sera Tested	RBPT		cELISA		*F-test	P-value
		No. Positive	Specific rate (%)	No. Positive	Specific rate (%)		
Breed							
Red Sokoto	256	20	7.8	6	2.3	**2.047	0.285
West African Dwarf	8	1	12.5	0	-	***2.051	0.440
Kano Brown	16	2	12.5	1	6.3	df=2	
Age							
<12 months	24	0	-	0	-	**5.066	0.66
12-36 months	111	6	5.4	2	1.8	***0.722	0.842
>36 months	145	17	11.7	5	3.4	df=2	
Sex							
Male	50	4	8.0	2	4.0		**1.0
Female	230	19	8.3	5	2.2	df=1	***0.612

*Fisher's exact test

**RBPT

***cELISA

The prevalence of Brucella melitensis in this study was lower as compared to what was reported by [15, 31] in the arid zone of Nigeria and Sokoto prison farm but higher to that reported by [17] in Kaduna.The differences observed in this research as compared with other studies may be due to the variation in the sensitivity and specificity of the type of test used to detect Brucella antibodies and also the management system employed by farmers in the flock.

The prevalence of Brucella antibodies in goats varied from one District to another in Giwa LGA, with Giwa district having the highest detection rate of antibodies by RBPT. This could be attributed to the fact that Giwa district hosts the largest goat market in the LGA. These goats are kept in close contact to one another within a poorly hygienic pen and also with other species of animals, feeding collectively. This practice may enhance the spread of infectious agents amongst these goats including Brucella organisms, resulting in infection and re-infection within the flock, including other animal population in the area. However, the prevalence of antibodies to Brucella melitensis was high in Gangara and Tsibiri districts as recorded by cELISA. The possible source of infection could be from the place where these goats were bought or from environmental contaminants.

In this study, the prevalence of Brucella antibodies varied among the male and female goats. Using RBPT, the female goats had a slightly higher prevalence than the males. However, the males had a higher prevalence of B. melitensisthan the females who had more antibodies to other Brucella species other than B. melitensis. The presence of erythritol, a sugar which favours the growth of Brucella in the reproductive organs of susceptible animals could be a factor making these goats prone to Brucella infection. This sugar is secreted in large quantity in goats of reproductive age especially females during pregnancy, leading to rapid multiplication of Brucella in the placenta and uterus, causing placentitis and metritis, which could result in abortion. The higher prevalence in female than male goats obtained in this study by RBPT is similar with the findings of [13, 14, 17] in goats in Plateau, Sokoto metropolis and Kaduna respectively. [15] recorded a higher prevalence of B. melitensis in male goats (8.5%) than the females (3.8%) and this is similar to what was obtained in this study with the use of cELISA. Interestingly, earlier studies have shown that females are more susceptible to Brucella infection than the males [34, 35, 36].

The result showed a higher prevalence of Brucella antibodies in the West African Dwarf and Kano Brown breeds, as compared to Red Sokoto breed by RBPT, while antibodies to Brucella melitensiswere more in the Kano Brown breed as obtained by the cELISA. This could be due to the fact that although these breeds of goats had antibodies to Brucella, the Kano Brown tend to have higher rate of antibodies to Brucella melitensis than the other species of Brucella. This result is in contrast with the findings of [14] who reported a higher prevalence in the Red Sokoto as compared to the sahelians and cross breed of goats in Sokoto metropolis. However, [37] and [16] reported that Brucella infection is not breed specific.

Goats above 36 months of age showed higher prevalence of Brucella antibodies than the younger ones as demonstrated by both the RBPT and cELISA. This could be attributed to the fact that younger goats (kids) are conferred with maternal immunity and therefore, are protected against infectious agents at the early stage of their lives. Studies have shown that Brucella infection is usually high in sexually matured animals than the younger ones because animals within this age range are actively involved in breeding and this could predispose them to Brucella infection which may have serious economic implications in terms of loss through reproductive wastages [38, 39, 17] The findings in this research is similar to what was reported by [13] and [16]. Although, [14]reported the highest prevalence of Brucella antibodies in goats within the age range of 13-24 months in Sokoto metropolis.

V. Conclusion

This study has established the existence of Brucella antibodies in goats in Giwa LGA. Antibodies to Brucella melitensis was also detected more in the West African Dwarf than the other breeds of goats. The male goats tested had higher rate of Brucella melitensis antibodies and older goats in the flock tend to have more antibodies than the younger ones. This is of public health importance due to close contact between these goats and man.

Therefore, Public health education/campaign should be embarked upon in order to enlighten the farmers more on the zoonotic implication of the disease and government should enact policies that can help to curb this disease from animal populations through vaccination programs and active surveillance system to monitor animal movements especially in the study area.

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