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Original Research Article

Prevalence of *Brucella* antibodies in horses in Kaduna State, Nigeria

[a]Baba A.Y., [b]Saidu S.N.A., [c]Bale J.O.O., [a]Ameen S.A., [d]Kaltungo, B.Y., [d]Babashani M. & [e]Salisu U.S.

[a]Department of Veterinary Medicine, University of Ilorin, Ilorin, Kwara State, [b]Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, [c] Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Kaduna State, [d]Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Kaduna State, [e]Department of Animal Science, Federal University Dutsin-Ma, Dutsin-Ma, Katsina State, Nigeria.

*Corresponding author: baba.ya@unilorin.edu.ng, +2348030793359

ABSTRACT

This study aimed at determining the prevalence of Equine brucellosis in Kaduna State**,** Nigeria. A total of 304 sera samples were collected from horses in three Local Government Areas of the State. The samples were analysed using Rose Bengal Plate Test (RBPT) and Serum Agglutination Test – EDTA (SAT - EDTA). The overall sero-prevalences was 5.59% and 20.07% based on RBPT and SAT-EDTA respectively. The sero-prevalences by breed were 11.9% (RBPT) and 12.70% (SAT-EDTA) for Arewa, 1.69% (RBPT) and 28.81% (SAT - EDTA), for Argentine, 0.00% (RBPT) and 21.74% (SAT - EDTA), for Sudanese and 0.00% (RBPT) and 16.21% (SAT - EDTA) for Talon breed of horses. The sero-prevalences by sex were 0.84% and 29.41% for females and 8.65% and 14.05% for males. The sero-prevalences by age group were 8.33%, 8.97%, 0.99% and 2.94% for 1 - 5 years old, 6 - 10 years old, 11- 15 years old and above 15 years old respectively. Sero-prevalences by use were 11.82%, 1.34%, and 2.22% for ceremonial, polo and racing horses respectively. From the study, it was concluded that *Brucella* antibodies were circulating in the blood of the horses sampled and that there was breed, age and sex predispositions to the infection. There is the need to conduct further studies to determine the *Brucella* spp circulating among horses in the study area.

Keywords:

INTRODUCTION

Brucellosis is a highly contagious, zoonotic, and economically important bacterial disease of animals and humans worldwide (OIE, 2000). It is also one of the most important infectious causes of reproductive disorders in domestic animals (OIE, 2000). The disease is also called contagious abortion, infectious abortion, and epizootic abortion. In horses, it is called "fistulous withers" and "poll evil" (Megid *et al.*, 2010; Rust, 2012). Brucellosis is caused by members of the genus *Brucella* which is a Gram negative, facultative intracellular bacterium and can infect many animal species and man (Corbel, 1997; Young, 2000). Members of the genus are small (0.5-0.7 by 0.6-1.5µm), non-motile, encapsulated, and coccobacilli (Ryan and Ray, 2004). However, *Brucella abortus* is the species of *Brucella* documented to cause brucellosis in horses (Kaltungo *et al*., 2013). *Brucella* species can remain viable for several months in contaminated water, aborted materials, manure, wool, hay, equipment and clothing in conditions of high humidity, low temperature and if there is no exposure to sunlight (Alton and Forsyth, 1996). It has been reported by the same authors that they can, however, be destroyed by several hours of exposure to direct sunlight, surfactants such as 1% sodium hypochlorite, 70% ethanol, iodine/alcohol solutions, glutaraldehyde and formaldehyde. Brucellosis has been reported from Nigeria in ruminants, and human (Falade *et al*., 1975), in cattle (Okoh *et al*., 1978; Bertu *et al*., 2010), in pigs (Falade & Shonekan, 1981), in donkeys (Adamu & Ajogi, 1995) and in equine (Ehizibolo *et al*., 2011) (Bertu *et al*., 2010; Ehizibolo *et al*., 2011). These reports seem to indicate that brucellosis is endemic and problematic in Nigeria. There are many factors that can affect the prevalence of the disease in various species of animals. These factors include climatic conditions, vegetation type, type of animal husbandry, animal species, sex, age, and diagnostic tests applied (Bercovich & Taaijke, 1990).

Horses are one of the most valuable animals in Nigeria (Musa, 2013). They are being used for ceremonial

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processions, polo and racing among others (Mshelia, 2013; Musa, 2013). They are also kept by the police and army for defense and security operations (RIM, 1992). Different breeds of horses have been reported to be kept in Kaduna State for various purposes which include polo, racing and traditional ceremonies (Garba, 2006). Horses have been used for long as beast of burden for the production of local sugar as well as the sole means of long distance journeys before the advent of modern transport facilities (Mshelia, 2013). The coming of the European expeditions saw horses being used for haulage of European goods and even merchandise (Mshelia, 2013). The domestic equine population in Nigeria is made up of 1.4 million horses and 2.5 million donkeys. (FAO, 2019).In Kaduna State, the horse population is estimated to be 2,500 (Aliyu, 2014). More than 90% of the estimated equine population is located within the semi and sub-humid zones of the country where they are used either as beasts of burden (for transport, threshing and caramel production) or in the case of horses for sports and ceremonial purposes such as durbar as well as the production of sugar (RIM, 1992; Mshelia, 2013; Musa, 2013). There is increasing use of horses for ceremonies, especially in Kaduna State during durbars where many horses are gathered. The groomers are known to be very close to these horses due to their activities in grooming them. They interchangeably borrow grooming tools from one horse to another. Therefore, horses with lesions around the polls and withers may be groomed, and without the grooming tools being properly washed and disinfected could be used on another horse (Mshelia, 2013). These therefore, could lead to spread of the disease. Polo and horse racing are both national and international programmes that involve either the movement of racing horses or horse owners in and out of the country which could be a potential means for the spread of equine brucellosis. In a situation whereby the horse owners and the horse boys are ignorant of brucellosis affecting horses, the disease could easily be spread among horses and even horse boys and their owners that actually develop the habit of close interaction with horses as reported by Mshelia (2013). Breeding programmes for horses to reduce importation is capable of introducing equine brucellosis and other diseases like African Horse Sickness and equine babesiosis (Mshelia, 2013). Diseases such as bacterial, viral, protozoan and parasitic diseases have been shown to influence the role of horses in contributing to the national economy and private horse owners. It is also known that pastoralists commonly use horses as a means of transporting the young ones during migration (Saidu *et al.*, 1991). The fact that the grooms are closely associated with horses, especially polo and racing ones through their grooming activities, can result in serious public health hazards, particularly if these groomers are not aware of the disease in horses. To what extent brucellosis causes such effect in horses seems not to be fully investigated in Kaduna State. Thus, there is therefore the need to investigate the prevalence of equine brucellosis, especially that the primary *Brucella* species in horses is *B. abortus* which has been reported to be endemic among cattle in Nigeria.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in three Local Government Areas of Kaduna State, Nigeria. Kaduna state is located in the northwest geopolitical zone of Nigeria. It lies between latitudes 6^o and 11^o north and longitudes 7^o and 44^o east, and is 1995 ft above sea level. It has distinct wet and dry seasons within the Guinea Savannah zone and part of the Sudan Savannah in Nigeria. The state shares boundaries with Katsina and Zamfara states to the north, Plateau and Bauchi states to the east, Nasarawa State and the Federal Capital Territory to the south, Niger State to the west and Kano state to the northeast, Kaduna state is made up of 23 LGAs and

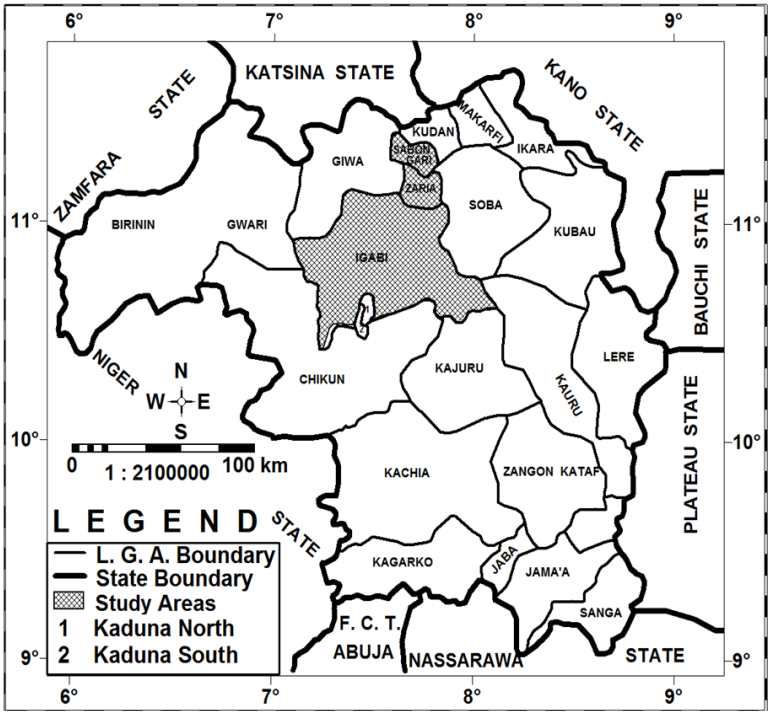

Figure 1. Kaduna State showing Study area
Source: Departmentt of Geography, Federal College of Education Zaria, 2015

occupies about 48,473.25 sq km, with a human population of over 6,006,562 people according to the 2006 census figures (KDSG, 2008).

METHODOLOGY

STUDY DESIGN

A cross- sectional study was carried out with Purposive/Judgemental sampling method. The study was conducted between February and June, 2019.

EXPERIMENTAL ANIMALS AND THEIR MANAGEMENT

Ceremonial, polo, and racing horses kept by horse owners were used in the study. Adult horses above three years of age were sampled from the three selected LGAs that were selected. Stable selection was done based on owners' willingness. The stables encountered were of two extremes viz: those with very few horses, usually between one to five, especially the traditional rulers who kept their horses for durbar and other ceremonies and those with large stables of usually twenty horses and above. All horses encountered with groomers that had a few horses were sampled while every third horse was sampled in those from large stables.

SAMPLE SIZE

The sample size was determined using the formula below as described by Michael (2005).

$$n = \frac{z^2pq}{d^2}$$

A prevalence of 14.7% as reported by Ehizibolo *et al.* (2011) was used. A significance level of 0.04 was used to increase the sample size thus increasing accuracy. A total sample size of 301 horses was arrived at for the study.

BLOOD SAMPLE COLLECTION

Blood samples were collected from horses in 60 stables for subsequent collection of serum. Five milliliters (5ml) of blood sample were aseptically collected through the jugular vein from each horse using a 10ml syringe and 21G needle after proper restraint by an assistant. The blood was then immediately transferred into a plain, EDTA free sampling bottle, according to the specification of Ahmadu Bello University Committees on Animal Use and Care.

. The samples were then appropriately labelled based on the LGA, ward, and animal species. The labelled bottles were then kept in a cooler on ice packs and transported to the Bacterial Zoonosis Laboratory in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria where they were centrifuged at 1000g for 5 minutes to allow for proper separation of serum from the clotted blood. The serum was then siphoned, using a sterile pasture pipette, into a 5 ml plastic serum tube which was appropriately labelled. All the extracted serum samples were then stored in the freezer at – 20^{o}C until used.

LABORATORY INVESTIGATION

SEROLOGY

THE ROSE BENGAL PLATE TEST (RBPT)

The stored sera were thawed to room temperature (25^{0}C) and then subjected to RBPT as described by Alton *et al* (1975) using RBPT antigen sourced from Ondersteport Biological Products Ltd, South Africa. 0.03ml of RBPT antigen and equal amount of the test serum were placed alongside on a white ceramic tile and then mixed thoroughly using an applicator. The tile was then rocked gently for 4 minutes. The sample was read as positive if any agglutination was observed and negative if no agglutination was observed.

THE SERUM AGGLUTINATION TEST WITH ETHYLENE DIAMINE TETRA ACETIC ACID (SAT-EDTA)

The test was performed using the micro-titre technique as described by Brown *et al*, (1981). The SAT antigen was obtained from Ondersteport Biological Products Ltd, South Africa.

PREPARATION OF STOCK SOLUTION FOR SAT – EDTA

One litre of phosphate buffer saline solution containing 5mM of disodium EDTA was prepared for the modified SAT technique. The final solution contained 0.5% phenol crystals, 0.85% (W/V) sodium chloride, and 5mM disodium ethylene diaminotetraacetic acid (EDTA). The buffer also contained 31.2g sodium hydrogen orthophosphate (NaH_2PO_4) and 28.39g disodium hydrogen orthophosphate (Na_2HPO_4). Twenty-eight millilires of NaH_2PO_4 and 72nl of Na_2HPO_4 were mixed to make the required pH of 7.2. All the constituents were weighed with an electric digital balance (Mettler P 165). They were then dissolved in pre-heated measured amounts of distilled water to make – up the buffer solution (Bale, 2008).

PROCEDURE FOR SAT –EDTA

A 1:10 dilution of the concentrated SAT antigen with the prepared buffer with a pinch of 0.02% Safranin O (to provide contrast to the agglutination reaction) was made for each day's work. A 96 –well rectangular micro-titre plate (Cooke Micro – Titer System®) was set up on the work table. Labeled serum vials were placed on the work table according to positions of the well already labeled A – H and a corresponding vertical numbering of the wells were made. A representative entry of the sample details was made in the laboratory record book. Using automatic micropipette (Dragonned®), 40µl of the buffer solution was measured out into the first well and 25µl into each of the remaining micro-titre wells. This was followed by the addition of 10µl of test serum into the first micro-titre well, using a fresh disposable

Table I: Distribution of horses sampled for *brucella* antibodies in three LGAs of Kaduna State, Nigeria.

LGA	No Sampled (%)	Breed of horses Sampled			
		Arewa	Argentine	Sudan	Talon
Igabi	161 (52.96)	37 (22.98)	118 (73.29)	3 (1.86)	3 (1.86)
Sabon Gari	38 (12.50)	11 (28.95)	0 (0)	5 (13.16)	22 (57.89)
Zaria	105 (34.54)	78 (74.29)	0 (0)	15 (14.29)	12 (11.42)
Total	304 (100)	126 (41.45)	118 (38.81)	23 (7.57)	37 (12.17)

pipette plastic tip for each test sample which was later on discarded. A two-fold serial dilution was done by transferring 25µl aliquots in each well, and 25µl was discarded after the last well. 25µl of the content of the working dilution of the SAT antigen was added to each well. Finally, the contents in the micro-titre plate were gently mixed by tapping the edges of the plate. The micro-titre plates were then covered with foil to prevent evaporation of the contents in the wells and incubated for 20 hours at 37^{o}C in an incubator (Gallenkemp®, Germany) after which the results were read. The result was considered positive when there was agglutination at the bottom of the well of the micro-titre plate and negative when there was no agglutination.

DATA ANALYSIS

Data obtained from serological tests were presented in tables and analysed using SPSS version 20.0 statistical package. Descriptive statistics, Chi square and Fishers Exact tests were used to test for association between categorical variables. P <0.05 were considered significant.

RESULTS

DISTRIBUTION OF HORSES SAMPLED IN KADUNA STATE, NIGERIA

A total of 304 horses were sampled from the study area (Table 1). Out of these, 161 were from Igabi Local Government Area (LGA) while 38 and 105 were from Sabon Gari and Zaria LGAs respectively. Furthermore, 126 (41.45%) of these horses were Arewa breeds while 118 (38.81%), 23 (7.57 %) and 37 (12.17%) were Argentine, Sudan and Talon breeds of horses respectively. Among horses in Igabi LGA, 37 (22.98%) were Arewa breed while 118 (73.29%), 3 (1.86%) and 3 (1.86%) were Argentine, Sudan and Talon breeds respectively. The horses in Sabon Gari LGA included 11 (28.95%) Arewa, 5 (13.16%) Sudan and 22 (57.89%) Talon breeds. Similarly, of the 105 horses in Zaria LGA, 78 (74.29%) were of the Arewa breed while 15 (14.29%) and 12 (11.42%) were of the Sudan and Talon breeds. No Argentine horses were sampled in Sabon Gari and Zaria LGAs.

PREVALENCE OF *BRUCELLA* ANTIBODIES IN HORSES

Seventeen (5.59%) out of the 304 horses sampled were seropositive for *Brucella* antibodies using the RBPT while 61 (20.07%) were positive using the SAT-EDTA (Table 2). Two (1.24%) and 44 (27.33%) of the 161 horses from Igabi LGA were positive using RBPT and SAT – EDTA respectively while of the 38 horses from Sabon Gari LGA 1 (2.63%) and 5 (13.16%) were respectively positive using RBPT and SAT – EDTA. Similarly, of the 105 horses from Zaria LGA 14 (13.33%) and 12 (11.43%) were respectively positive using the RBPT and SAT – EDTA.

PREVALENCE OF *BRUCELLA* ANTIBODIES IN HORSES BY BREED IN KADUNA STATE, NIGERIA

Fifteen (11.90%) and 16 (12.70%) of the Arewa horses sampled were seropositive for Brucella antibodies using RBPT and SAT – EDTA respectively (Table 3). Similarly, 2 (1.69%) and 34 (28.81%) Argentine horses were positive using RBPT and SAT – EDTA while of the 23 Sudanese horses none was positive using RBPT while 5 (21.74%) were positive using SAT – EDTA. As for the Talon horses, 6 (16.22%) were positive for Brucella antibodies with none being positive by the RBPT.

Table II: Prevalence of Brucella antibodies in horses sampled in three LGAs of Kaduna State, Nigeria

LGA	Number of Samples tested	Number (%) Positive by test type	
		RBPT (%)	SAT-EDTA (%)
Igabi	161	2 (1.24)	44 (27.33)
Sabon Gari	38	1 (2.63)	5 (13.16)
Zaria	105	14 (13.33)	12 (11.43)
Total	304	17 (5.59)	61 (20.07)

RBPT: Fisher's exact test=16.521; df = 2; p = 0.000
SAT-EDTA: X2 = 10.533; df = 2; p = 0.005

PREVALENCE OF *BRUCELLA* ANTIBODIES BY SEX

Of the 119 female horses examined, 1 (0.84%) was positive for *Brucella* antibodies using RBPT while 35 (29.41%) were positive by the SAT-EDTA test (Table 4). Similarly, of the 185 male horses, 16 (8.65%) and 26 (14.05%) were positive

using RBPT and SAT-EDTA respectively. The sero-prevalence by LGA showed that of the 93 female horses in Igabi LGA 1 (1.08 %) and 32 (34.41%) were positive by RBPT and SAT–EDTA respectively while among the 68 male horses in the LGA 1 (1.47%) and 12 (17.64%) were respectively positive by RBPT and SAT-EDTA. Of the 12 female horses from Sabon Gari LGA, 1 (8.33%) was positive by the SAT-EDTA while none was positive by the RBPT. Similarly, of the 26 male horses from Sabon Gari LGA 1 (3.85%) and 4 (15.38%) were respectively seropositive by RBPT and SAT-EDTA. On their part, of the 14 female horses sampled in Zaria LGA, 2 (14.29%) were positive by SAT-EDTA and none was positive by RBPT while of the 91 male horses from this LGA 14 (15.38%) and 10 (10.99%) were respectively positive by RBPT and SAT-EDTA.

PREVALENCE OF *BRUCELLA* ANTIBODIES IN HORSES BY AGE

The sero-prevalence of horses by age is presented in Table 5 below. Horses between 1 and 5 years had sero-prevalence of 8.33% and 12.50% by RBPT and SAT-EDTA respectively while those between 6 and 10 years had sero-prevalence of 8.97% and 13.79% using RBPT and SAT-EDTA respectively. Similarly, horses in the 11 to 15 years old bracket had sero-prevalence of 0.99% and 27.72% using RBPT and SAT-EDTA respectively while those above 15 years had sero-prevalence of 2.94% and 35.29% respectively. As to the sero-prevalence LGA, horses in Igabi LGA of age range of between the age of 1 and 5 years had sero-prevalence of 20.00% by both RBPT and Sat-EDTA while those in Sabon Gari LGA had seropositivity of 10.00% and 20.00% by RBPT and SAT-EDTA respectively and those in Zaria LGA had sero-prevalence of 0.00% by both tests.

Horses of 6 to 10 age range in Igabi LGA had 1.89% and 18.87% Seropositivity by RBPT and SAT-EDTA respectively while those of the same age bracket in Sabon Gari LGA had 0.00% and 14.29% by RBPT and SAT-EDTRA respectively. Similarly, horses of this age bracket in Zaria LGA had 16.90% and 9.86% sero-prevalence by RBPT and SAT-EDTAS respectively. As to the horses in the 11 to 15 years old bracket, horses in Igabi LGA had 0.00% and 31.51% seropositivity by RBPT and SAT-EDTA respectively while those in Sabon Gari LGA had 0.00% for both tests. The corresponding figures for horses in Zaria LGA for this age bracket are 4.76% and 23.81% respectively. The sero-prevalence of Brucella antibodies for horses above 5 years in Igabi LGA were 0.00% and 36.67% by RBPT and SAT-EDTA while the corresponding figures for the same age bracket for horses from Sabon Gari LGA were 0.00% respectively for the two tests. As for the figures for horses in Zaria LGA, a sero-prevalence of 25% by ach of the two tests was determined.

PREVALENCE OF *BRUCELLA* ANTIBODIES IN HORSES BY ACTIVITY

Of the 304 horses sampled 110 of the horses were ceremonial while 149 and 45 of them were respectively Polo and Racing horses (Table 6). Thirteen each (4.28 %) of the 110 ceremonial horses were respectively seropositive by RBPT and SAT-DTA while 3 (0.99%) and 39 (12.83%) of the Polo horses were respectively positive by RBPT and SAT-EDTA and 1(0.33%) along with 11 (3.62%) of the 45 racing horses were positive by RBPT and SAT-EDTA respectively.

Sero-prevalence by use and Local Government Area indicated that of the 22 ceremonial horses from Igabi LGA, 3 (13.66%) were seropositive by SAT-EDTA and none of them was positive by RBPT (Table 4.6). Similarly, of the 122 Polo horses from this LGA, 2 (1.64%) and 36 (29.51%) were seropositive by RBPT and SAT-EDTA while of the 17 racing horses from the LGA 7 (41.18%) were positive by SAT-EDTA and none was positive by RBPT.

As for the horses in Sabon Gari LGA, no ceremonial horse was tested for Brucella antibodies. However, of the 18 Polo horses in this LGA, 1 (5.56%) and 3 (16.67%) were positive for Brucella antibodies while 2 (10.00%) of the 20 racing horses were positive by SAT-EDTA and none was positive by RBPT (Table 6). Of the 88 ceremonial horses from Zaria LGA, 13 (14.77%) and 10 (11.36%) were respectively positive for *Brucella* antibodies using RBPT and SAT-EDTA while of the 9 Polo horses none was positive by both tests and of the 8 racing horses 1 (12.50%) and 2 (25.00%) were positive by RBPT and SAT-EDTA respectively.

DISCUSSION

From the study, Arewa, Argentine, Sudanese and Talon horses were found to be kept in the study area by horse owners among who are traditional rulers, polo and race horse owners. This indicated the presence of exotic breeds of horses in the area and is quite understandable as Zaria is a traditional home for horse racing and polo activities (Musa, 2013). The presence of polo and racing horses has further shown that polo and racing are still popular in the study area as reported by Mshelia (2013) and Musa (2013).

The study has shown that the overall sero-prevalence of *Brucella* antibodies in horses in the study area was 5.59% by RBPT and 20.07% by SAT-EDTA. This is low compared with the sero-prevalence study carried out by Ehizibolo*et al*. (2011) in Jos Plateau State, where they obtained a sero-prevalence 6.1% using RBPT. From the study, SAT-EDTA 20.07%) has been shown to be more sensitive than RBPT (5.59%) as it identified more horses with the antibodies.

The finding of positive horses (17 (5.59) and 61 (20.07) for *Brucella* antibodies in this study has indicated that the organism is circulating among horses in the study area. This

Table III: Prevalence *of Brucella* antibodies in horses by breed sampled in three LGAs of Kaduna State, Nigeria.

LGA	Horse Breeds											
	Arewa			Argentine			Sudan			Talon		
	No of Sample	RBPT	SAT-EDTA	No of Sample	RBPT	SAT-EDTA	No of Sample	RBPT	SAT-EDTA	No of Sample	RBPT	SAT-EDTA
Igabi	37	0 (0.00)	6 (16.22)	118	2 (1.69)	34 (28.81)	3	0 (0.00)	2 (66.67)	3	0 (0.00)	1 (33.33)
Sabon Gari	11	1 (9.09)	1 (9.09)	0	0 (0.00)	0 (0.00)	5	0 (0.00)	0 (0.00)	22	0 (0.00)	4 (18.18)
Zaria	78	14 (17.95)	9 (11.54)	0	0 (0.00)	0 (0.00)	15	0 (0.00)	3 (20.00)	12	0 (0.00)	1 (8.33)
Total	126	15 (11.90)	16 (12.70)	118	2 (1.69)	34 (28.81)	23	0 (0.00)	5 (21.74)	37	0 (0.00)	6 (16.21)

RBPT: Fisher's exact test=10.805; df = 3; p = 0.007; SAT-EDTA: $X^2 = 11.445$; df = 3; p = 0.010

Table IV: Prevalence of *Brucella* antibodies by Sex of horses sampled in three LGAs of Kaduna State, Nigeria.

LGA	Sex					
	No Sampled	Female		No Sampled	Male	
		No Positive (%)			No Positive (%)	
		RBPT	SAT-EDTA		RBPT	SAT-EDTA
Igabi	93	1 (1.08)	32 (34.41)	68	1 (1.47)	12 (17.64)
Sabon Gari	12	0 (0.00)	1 (8.33)	26	1 (3.85)	4 (15.38)
Zaria	14	0 (0.00)	2 (14.29)	91	14 (15.38)	10 (10.99)
Total	119	1 (0.84)	35 (29.41)	185	16 (8.65)	26 (14.05)

Table V. Prevalence of *Brucella* antibodies by age of horses sampled in three LGAs of Kaduna State, Nigeria

Age	LGAs										
	Igabi			Sabon Gari			Zaria			Total	
	No Sampled	RBPT	SAT-EDTA	No of Sample	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	RBPT	SAT-EDTA
1 – 5 years	5	1 (20.00)	1 (20.00)	10	1 (10.00)	2 (20.00)	9	0 (0.00)	0 (0.00)	2 (8.33)	3 (12.50)
6 – 10 years	53	1 (1.89)	10 (18.87)	21	0 (0.00)	3 (14.29)	71	12 (16.90)	7 (9.86)	13 (8.97)	20 (13.79)
11 – 15 years	73	0 (0.00)	23 /(31.51)	7	0 (0.00)	0 (0.00)	21	1 (4.76)	5 (23.81)	1(0.99)	28 (27.72)
> 15 years	30	0 (0.00)	11 (36.67)	0	0 (0.00)	0 (0.00)	4	1 (25.00)	1 (25.00)	1 (2.94)	12 (35.29)
Total	161	2 (1.24)	45 (27.95)	38	1 (2.63)	5 (13.16)	105	14 (13.33)	13 (12.38)	17 (5.59)	63 (20.72)
Chi-square		2.837*	3.508*		-	1.769*		7.256*	3.240*		
p-value		0.347	0.283		-	0.538		0.034	0.247		

finding is also significant as it brings challenges to polo an racing tournaments in the study area as it could lead to the spread of the infection to other horses from distant areas that might have come to the study area for tournament. It could also mean that, should horses from the study area go out for tournaments in other areas, the infection could also spread that way.

The fact that the seropositivity was highest in the Arewa breed (16 (12.70)) of horses was not surprising since they were found more in traditional owners' stables who use the horses more for durbar, weekend riding by youths and sugar milling during the production of local sugar ('Mazarkwaila') as reported by Mshelia (2013) and Musa (2013). The near zero seropositivity for the Sudanese and Talon breeds of horses could be accounted for possibly by their owners better management practices since this study has demonstrated that owners of such horses made sure the horses had minimal access to veterinary medical care.

From this study, the sero-prevalence in males was higher than that in female horses. This is contrary to the findings of Kaltungo (2013) and Buhari (2014) who respectively reported sero-prevalences of 12.05 % and 14 % respectively in female ruminants which is higher than those reported in male animals in Katsina and Kaduna States respectively. The higher sero-prevalence in males as obtained in this study could be due to the fact that, unlike in small ruminants and cattle as reported by Kaltungo (2013) and Buhari (2014) respectively, more male horses are found among horse owners in the study area. Furthermore, many horse owners are in the habit of using geldings in preference to female horses.

The study has also demonstrated that seropositivity to *Brucella* antibodies in horses increased with age. This is in agreement with the findings of Aulakh *et al.* (2008), Abubakar *et al.* (2010), Kaltungo (2013) and Buhari (2014) who reported higher positivity in sexually matured small ruminants and cattle respectively in Kaduna State.

The seropositivity as seen in 1 to 5 years old horses in this study could be as a result of contaminated feeds during grazing as the respondents in this study reported grazing their horses along with other species of animals and even grazed the horses where there has been a history of animals having aborted. Furthermore, positivity could be through milk consumption during their young age from mares that are seropositive to *Brucella* antibodies in this study. Areas on that can be advanced for the low seropositivity in this age class is that these horses were not sexually mature yet as to acquire the infection sexually through mating with other infected horses.

The fact that ceremonial horses were more seropositive for *Brucella* antibodies could be due to the less attention to veterinary care by the owners while the owners of polo and racing horses had more premium for it as they considered their activities as investments that required returns. Furthermore, the participation of ceremonial horses at weekend horse riding by youths and for marriage outing by grooms and their friends and well-wishers could account for the extra sources of exposure for the *Brucella* organisms for this group of horses in the area.

CONCLUSIONS

The study has determined that the sero-prevalence of *Brucella* antibodies in the study area was 5.59% using the RBPT and 20.07% using the SAT-EDTA. The sero-prevalence of *Brucella* antibodies by breed was highest in the Arewa breed with a sero-prevalence of 11.90% followed by the Argentine breed with 1.69% and least by the Sudanese and Talon Breeds with 0.00% sero-prevalence, respectively. The sero-prevalence of *Brucella* antibodies in horses was higher in males with a sero-prevalence of 8.65% than in

Table VI: Prevalence of *Brucella* antibodies in horses by activity in three LGAs of Kaduna State, Nigeria

LGA	USES								
	Ceremonial			Polo			Racing		
	No of Sample	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA	No Sampled	RBPT	SAT-EDTA
Igabi	22	0 (0.00)	3 (13.66)	122	2 (1.64)	36 (29.51)	17	0 (0.00)	7 (41.18)
Sabon Gari	0	0 (0.00)	0 (0.00)	18	1 (5.56)	3 (16.67)	20	0 (0.00)	2 (10.00)
Zaria	88	13 (14.77)	10 (11.36)	9	0 (0.00)	0 (0.00)	8	1 (12.50)	2 (25.00)
Total	110	13 (4.28)	13 (4.28)	149	3 (0.99)	39 (12.83)	45	1 (0.33)	11 (3.62)
Chi-square		0.763	2.147		-	0.368		0.612	2.275
p-value		0.833	0.412		-	0.653		1.000	0.324

*Fisher's exact test

female who had a sero-prevalence of 0.84% using the RBPT. The sero-prevalence of *Brucella* antibodies was seen to increase with age among all the horses with horses within the 6 to 10-year-old bracket having the highest sero-prevalence of 8.89% followed by those in the 1- 5 year old bracket and least by those in the 11 -15 year old bracket using the RBPT. The sero-prevalence of *Brucella* antibodies was highest in ceremonial horses (4.28%) followed by Polo (0.99%) and least in racing horses (0.33%) as used by the RBPT. The Serum Agglutination Test (SAT-EDTA) was more sensitive than RBPT as it identified more horses' positive than RBPT.

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