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Original research

Clinico-serological studies of *Brucella* infection of horses in Kano metropolis, Kano State, Nigeria

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ABSTRACT

This study aimed at determining the clinico-serological status of brucellosis among horses in Kano, Kano State Nigeria. Four Local Government Areas (LGAs) were selected from Kano metropolis based on convenience and owner's consent. A total of 328 horses, comprising ceremonial, polo and racing breeds, were used for the study. Clinical signs of brucellosis and vital parameters of the horses were documented. Blood samples were collected and the sera were subjected to modified Rose Bengal Plate Test (mRBPT) for detection of *Brucella* antibody. Positive samples were further screened using Serum Agglutination Test with EDTA (SAT-EDTA). The 2-Mercaptoethonol Test (2-MET) was used to determine the forms (acute or chronic) of *Brucella* infection. A total of 79 (24.09 %) horses had seropositivity for *Brucella* infection using mRBPT. The result was not statistically significant (P = 0.07). Of these 79 horses, 27 (34.18 %) were having mild infection while 12 (25.19%) others were severe. However, based on forms of brucellosis, it was inferred that out of the 39 horses that were positive for SAT-EDTA, 17 (43.59%) had acute form while 22 (56.41%) had chronic infection. From the study, it was evident that horses in Kano Metropolis had *Brucella* infection and this has far reaching public health implications. One of the limitations of this study was gaining free access to horse stables and insufficient funds. There is therefore, need to educate horse owners, grooms and the general public on the zoonotic implications of brucellosis. Regular surveillance should be conducted to establish the true prevalence of equine brucellosis in Nigeria.

KeyWords: *Brucella*, clinico-serological study, horses, Kano Metropolis

INTRODUCTION

Horse keeping in Nigeria has been an age long tradition (Baba *et al*., 2021). With the coming of European expeditors, polo and racing became popular (White, 2012; Mshelia, 2013). Horses were used as a means of transport and haulage of goods. The European expeditors used them for traversing the land, especially in Northern Nigeria during the colonial days (Mshelia, 2013). In the process, there were traditional healers who could examine sick horses and offer treatment through the use of herbs (Abdullahi *et al*. 2017). Horses were and are still being used for extraction of juice from sugar cane in the process of producing granulated brown sugar (Baba *et al*., 2021). Some of the diseases which were successfully managed by traditional healers included helminthosis and colic (Edeh, 2018).

Horses are used by various parastatals in Nigeria which include the Police, Immigration, Customs Service, as well as ceremonial engagements, leisure riding, durbar and a couple of others (Useh *et al*., 2005; Bukar *et al*., 2007). The close contact with horses puts humans at risk of contracting zoonoses. It is for this reason that this study was under taken to determine the clinic-serological status of brucellosis in horses in Kano Metropolis, Kano state, Nigeria. Due to the popularity of horses in the area and particularly high human population density in the study area, if the organisms abound in the area, the risks could be high. Earlier reports of *Brucella* infection in equine in Nigeria include those of Bale and Kwanashie (1984), Useh *et al*. (2005), Ehizibolo *et al*. (2011), Tijjani *et al*. (2017), Ardo *et al*. (2018), Njoga *et al*. (2018) and Baba *et al*. (2021).

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Other reasons for the study include the close contact of horses with their owners and grooms. Baba *et al*. (2022), in a survey of *Brucella* infection in horses in Kaduna State, a State that shares common border with Kano State, demonstrated that *Brucella* antibodies were detected in the horses. Also, grooms handling these horses had fair knowledge of the disease, brucellosis in horses, though their attitude and practices did not reflect such knowledge. There has also been frequent importation of horses for the purposes of polo and racing into the State and Nigeria in general. However, there has not been any report of clinical brucellosis in horses in Nigeria. Similarly, poor veterinary services along with inadequate laboratory backup could lead to the missing of clinical cases of the disease, if present (Baba *et al*., 2021).

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Kano Metropolis, Kano State, Nigeria. Kano State is situated between latitudes 13^0N and 11^0N and longitudes 8^0W and 10^0E (Fig 1). Kano State is made up of forty-four Local Government Areas and has a land area of 20,760sq kilometers with a population of 9,383,682 (NPC, 2006). The domestic equine population in Nigeria is made up of 340,000 horses and 940,000 donkeys of which 2,500 horses are found in Kano State (RIM, 1992; Anon, 1994; Musa, 2013). Most of the horses in Kano State are concentrated in the Kano Metropolis (Musa, 2013).

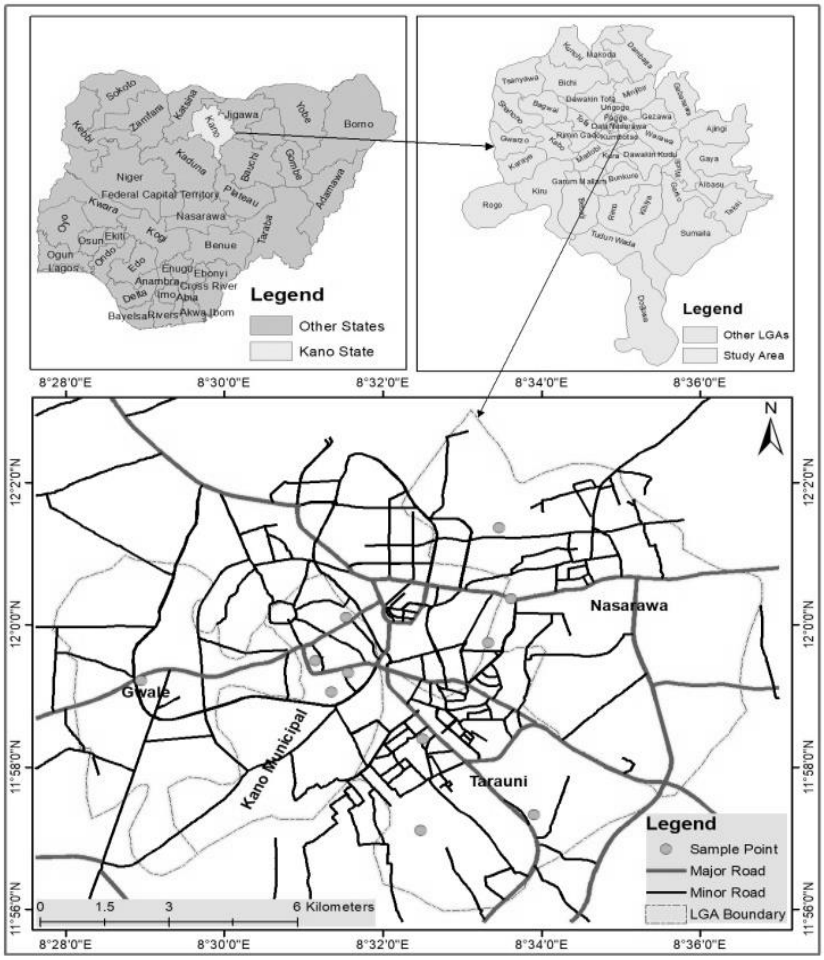

Figure 1: Map of Nigeria highlighting Kano State and the Local Government Areas sampled in the metropolis.

The study was conducted according to the specification of the Ahmadu Bello University Committees on Animal Use and Care (ABUCAUC/2018/029).

STUDY DESIGN AND SAMPLE SIZE

The research consisted of a cross-sectional study involving horses in Kano metropolis. A purposeful sampling was used to select the study area as Kano Metropolis which is made up of eight Local Government Areas namely Municipal, Gwale, Dala, Tarauni, Kumbotso, Nasarawa, Fagge and Ungogo LGAs. Furthermore, four out of the eight LGAs were selected based on convenience and agreement by the horse owners and cooperation by the grooms for the study.

Horses used in the study included ceremonial, polo and racing horses to enable us access the role of each of these horse types in brucellosis, if present in them. The sample size used in the study was derived from the formula by Thrusfield (2005). Thus:

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

Where:

n - Minimum sample size

z - Appropriate value for the standard normal deviation set at 95% confidence interval (1.96).

p - Prevalence (20.05% as reported by Baba, 2016).

q - Complementary probability, 1 – p

d - Level of significance 5% (0.05).

Sample size (horses) = $\frac{1.96^2 \text{ x } (0.2005 \text{ x } 1 - 0.2005)}{(0.05)^2}$

n = 246.

However, a total of 328 blood samples for serum were collected with the intention of increasing the sample size to accommodate attrition.

SAMPLE COLLECTION AND PRESERVATION

The clinical signs suggestive of being those of brucellosis were determined by first taking the body vital parameters like body temperature, respiratory rate and pulse. Areas of the poll and withers were similarly examined for evidence of

openings and discharges as characterized for poll evil (septic supra-atlantal bursitis) and fistulous withers (septic supraspinatous bursitis) respectively (Saidu *et al*., 2017). Where lesions were observed closer look was made and the sample of the lesions were collected for characterization and biopsy was taking for laboratory examination using microbiology and histopathology standards. For determination of status and forms of *Brucella* infections, blood samples were collected via the jugular vein using 18G needles and 10ml syringes after each horse was restrained by an assistant. Each of the collected blood samples was decanted into anticoagulant- free sample bottles, labeled with the horse number, and location. The sample bottles were then kept in a slanting position in a Coleman box in which ice packs were added and transported to the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. Other information on the samples like sex, age, location and use were recorded in a log book. In the laboratory, the blood samples were centrifuged at 1000g for 5 minutes to allow for proper separation of serum from the clotted blood. Sera were pipetted into a 5 mL plastic sterile serum tubes. which was labeled appropriately in line with the original number of the blood sample. A new pipette tip was used for each serum sample. All the extracted serum samples were then stored in the freezer at –20^{o}C until further use.

SEROLOGICAL ANALYSIS

The serum samples were first screened for *Brucella* infection using mRBPT as described by Bale (1980) and modified by Bertu (2014). The stored sera were thawed to room temperature (25^{0}C) and then subjected to mRBPT 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 a clean tooth pick which was discarded after use. The tile was rocked gently using both hands for 4 minutes (rotation period). The sample was classified positive if any agglutination was observed and negative if no agglutination was observed. Serum samples that were positive were further screened using Serum Agglutination Test (SAT-EDTA) as described by Brown *et al*. (1981). Phenol saline with EDTA buffer solution containing 5g phenol crystals, 8.5g sodium chloride, and 1.8612g disodium EDTA, dissolved in 100ml of warm distilled water was prepared. 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 microtitre 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 (Plate 1). 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 microtitre wells. This was followed by the addition of 10µl of test serum into the first microtitre well, using a fresh disposable 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. The content of the working dilution of the SAT antigen was mixed gently by tapping the container and 25µl added to each well. Finally, the contents in the microtitre plate were gently mixed by tapping the edges of the plate for 20seconds. The mcirotitre plates were then covered 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 a red dot surrounded by a circular zone at the bottom of the well of the microtitre plate and negative when there was no circular zone that was surrounded by a dot. All samples that were positive at 1:40 serial dilutions were regarded as having mild infection while those with 1:80 dilutions and above as having severe infection. The RBPT and SAT antigens were obtained from the Onderstepoort Biological Products Ltd, South Africa.

As for the determination of the forms (acute and chronic) of *Brucella* infections the Mercaptoethonol Test (2-MET) as described by Bale (2008) was used. A 0.2 molar solution of 2-ME was made by diluting the stock solution 1:5 with distilled water before use. A 96 well "U bottom" microtitre plate was set up on the bench. Labelled serum vials were placed on the bench to correspond to the positions of the wells already labelled. A representative entry of the sample details was made in the laboratory record book. Using automatic micropipette, 10 µl of the serum were dispensed into the first well using a fresh disposable pipette tip for each sample. This was followed by the addition of 40 µl of 0.85% saline, and 50 µl of 0.2 molar stock solution into the serum to make a dilution of 1:10. Fifty (50 µl) of the 0.85% saline were dispensed in the remaining microtitre wells. The microtitre plate was then incubated at 37^{0}C for 1 hour to ensure reactivity. A twofold serial dilution was done by transferring 50 µl aliquots from the first well up to the fifth well after which 50 µl of the aliquot were discarded from the last well. This was followed by the addition of 50 µl of *B. abortus* SAT-EDTA antigen. The microtitre plates were then covered with a foil paper to prevent evaporation of the contents in the wells and the plates incubated for 20 hours at 37^{0}C in an incubator after which the results were read.

Unchanged or increased titre from the SAT-EDTA after 2-mercaptoethanol (2-ME) treatment is indicative of presence of IgG which was indicative of a chronic infection. However, decreases in the titre after addition of 2-ME is indicative of IgM which was indicative of an acute infection. The serum samples that were positive at 1:80 by SAT-EDTA were used for the determination of the forms of *Brucella* infections.

STATISTICAL ANALYSIS

The data from the study were presented as tables and thereafter analyzed using SPSS version 17.0 (2009). Fisher's exact test and Pearson Chi-Square were used to test for agreement between various tests such as; mRBPT, SAT-EDTA and 2-ME. P <0.05 was considered significant.

Table II: Seroprevalence of *Brucella* infection using RBPT and SAT-EDTA of horses sampled in four LGAs of Kano Metropolis, Kano State, Nigeria

LGA	Number of Samples tested	Number (%) Positive by test type	
		mRBPT (%)	SAT-EDTA (%)
Kano Municipal	92	28 (30.43)	17 (60.71)
Gwale	37	8 (21.62)	4 (50.00)
Nasarawa	129	28 (21.71)	13 (46.43)
Tarauni	70	15 (21.43)	5 (33.33)
Total	**328**	**79 (24.09)**	**39 (11.89)**

mRBPT: Pearson Chi-Square= 2.821[a], P= 0.420, SAT-EDTA: Pearson Chi-Square= 5.763[a], P= 0.124, LGA (Local Government Area)

RESULTS

DETERMINATION OF CLINICAL SIGNS OF *BRUCELLA* INFECTION IN SAMPLED HORSES

The body temperature, respiratory and pulse rates of the horses under the study are presented in Table 1. Of the 328 horses sampled 324 (98.78%) were seen to have normal body temperature (36.6 ± 0.4^0C) while the remaining 4 (1.22%) had temperatures above normal (39.07^0C). Furthermore, of the 328 horses, 272 had mean normal respiratory rate of 10.6 beats/min while 56 of them had abnormal respiratory rates of 15.2 beats/min. As for pulse rate, 325 horses had normal mean pulse rates of 32.75 cycles /min and 3 had above normal pulse rates of 37.66 cycles/min.

None of the 328 horses under the study exhibited any signs that could be ascribed as being poll evil or fistulous withers as the supra-atlantal and supraspinatous bursae indicated normal anatomical values. However, there was a horse that exhibited swelling at the right olecranon bursa area which the groom handling the horse suspected it to be brucellosis and he used firing and local herbs to treat it (plate 1).

Table 1: Vital parameters of horses sampled at Kano Metropolis, Kano State, Nigeria

Vital parameters	No. Sampled	No. with Normal parameter	SEM	No. with Abnormal parameter	SEM
Body Temperature (^{0}C)	328	324	0.02393	4	0.16520
Respiratory rate(cycles/min)	328	272	0.05419	56	1.76242
Pulse rate (beats/min)	328	325	0.06223	3	0.33333

However, 3 (0.91%) horses with swollen olecranon bursa were identified to which the owners claimed the swellings to be those due to *Brucella* infection and even had the habit of firing them as a means of treatment.

STATUS OF *BRUCELLA* INFECTION (MILD AND SEVERE)

A total of 79 (24.09%) out of the 328 horses sampled were seropositive for *Brucella* infection using mRBPT (Table 2). There was no significant difference LGA (mRBPT: Pearson Chi-Square= 2.821[a], df= 3, P= 0.420; SAT-EDTA: Pearson Chi-Square= 5.763[a], df= 3, P= 0.124) in the seropositivity of horses by LGA. These serum samples were therefore used in the determination of the status of infection using SAT-EDTA. A total of 79 serum samples tested using SAT-EDTA indicated 27 (34.18%) of the horses having mild infection with *Brucella* infection while 12 (25.19%) others had severe infection (Table 3). A consideration of the status of infection by sex indicated that 11 (68.75%) out of the 16 female horses had mild infection while 5 (31.25%) others had severe infection. Of the 23 male horses, 16 (69.56%) had mild infection while 7 (30.44%) others had severe *Brucella* infection (Table 4). There was significant difference (Fisher's Exact Test=6.732; P=0.030) in the status of *Brucella* infection by sex

Considering status of *Brucella* infection by age, of the 20 horses in the 1 to 5years old bracket, 8 (40.00%) had mild infection while 2 (10.00%) others had severe infection. Similarly, of the 51 horses in the 6 to 10years old bracket, 15 (29.60%) had mild infection with *Brucella* species while 10 (19.60) others had severe infection. Of the 3 horses in the age group equal to or greater than 15 years, only

one (100.00%) had mild infection and none of them had severe infection (Table 5). The status of *Brucella* infection as to mild or severe by age was statistically significant (Fisher's Exact Test= 9.464; p = 0.018).

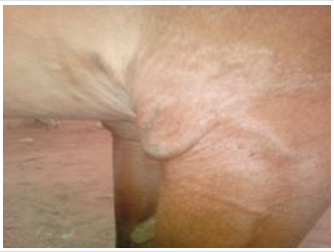

Plate 1: Swollen elbow in the right fore limb (arrow) at Polo ground, Nasarawa, Kano State suspected by groom to be brucellosis

Table 3: Status of mild and severe *Brucella* infections among horses by Local Government Area using SAT-EDTA in Kano Metropolis, Kano state. I.U; International Unit, LGA; Local Government Area

LGA	No. of sera Tested	No. Mild infections (%) (1:40 = 67 I.U)	No. Severe infections (%) (≥ 1:80 ≥ 134 I.U)
Kano Municipal	28	11 (39.29)	6 (21.43)
Gwale	8	3 (37.50)	1 (12.50)
Nasarawa	28	10 (35.71)	3 (10.71)
Tarauni	15	3 (20.00)	2 (13.33)
Total	79	27 (34.18)	12 (15.19)

Pearson Chi-Square= 0.787[a]; p= 0.853

FORMS OF *BRUCELLA* INFECTION (ACUTE AND CHRONIC)

From the study, 39 (16 females and 23 males) horses that were positive for brucellosis were further used to determine the forms of infection. Of these 39 horses, 17 (43.59%) had acute form of *Brucella* infection while the remaining 22 (56.41%) had chronic infection (Table 6). Furthermore, of the 16 female horses, 8 (50.00%) had acute and chronic infections respectively (Table 7). Similarly, of the 23 male horses 9 (39.13%) had acute infections while the remaining 14 (60.87%) had chronic infections (Table 7). The form of infection by sex was statistically not different (Fisher's Exact Test=5.732; p=0.072).

With regard to form of infection by age the sera of 9 horses within 1 to 5 years old 3 (33.33%) had acute infection with brucellosis while the remaining 6 (66.67%) had chronic infections. Similarly, 11 (44.00) horses out of the 25 horses within ages 6 to 10 years old bracket indicated acute infection while the remaining 14 (56.00%) showed chronic infection. Considering the sera of horses in the 11 to 15years old age bracket, 4 sera were tested of which 3 (75.00%) showed acute infection and the remaining 1 (25.00%) showed chronic infection. Only one horse in the above 15 years old age bracket was tested for form of infection and it indicated having chronic infection (Table 8). The form (acute or chronic) of infection in the horses was statistically significant by age (Fisher's Exact Test = 6.464; p=0.028).

Table 4: Status of *Brucella* infection using SAT-EDTA in horses sampled in four LGAs of Kano Metropolis by sex

LGA	Sex					
	Female			Male		
	No. of sera tested	No. Mild infections (%) (1:40 = 67 I.U)	No. Severe infections (≥ 1:80 = ≥ 134 I.U)	No. of sera Tested	No. of Mild infections (%) (1:40 = 67 I.U)	No. of Severe infections (≥ 1:80 = ≥ 134 I.U
Kano municipal	0	0 (0.00)	0 (0.00)	17	11 (64.71)	6 (35.29)
Gwale	4	3 (75.00)	1 (25.00)	0	0 (0.00)	0 (0.00)
Nasarawa	8	6 (75.00)	2 (25.00)	5	4 (80.00)	1 (20.00)
Tarauni	4	2 (50.00)	2 (50.00)	1	1 (100.00)	0 (0.00)
Total	16	11 (68.75)	5 (31.25)	23	16 (69.56)	7 (30.44)

Fisher's Exact Test=6.732; P=0.030, LGA (Local Government Area). I.U (International Unit)

Table 5: Status of *Brucella* infection by age using SAT-EDTA in horses sampled in four LGAs of Kano Metropolis

LGAs	1-5 Years old			6-10 Years old			11-15 Years old			>15 Years old		
	No. sampled	Mild (No. positive (%)	Severe (No. positive (%)	No. of sample	Mild (No. positive (%)	Severe (No. positive (%)	No. of sample	Mild (No. positive (%)	Severe (No. positive (%)	No. of sample	Mild (No. positive (%)	Severe (No. positive (%)
KMC	4	3 (75.00)	0 (0.00)	23	8 (34.78)	6 (26.09)	1	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)
Gwale	1	1	1 (100.00)	7	2 (28.57)	1 (14.29)	0	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)
Nasarawa	6	1 (16.67)	0 (0.00)	17	5 (25.41)	2 (11.76)	4	3 (75.00)	1 (25.00)	1	1 (100.00)	0 (0.00)
Tarauni	9	3 (33.33)	1 (11.11)	4	0 (0.00)	1 (25.00)	0	0 (0.00)	0 (0.00)	2	0 (0.00)	0 (0.00)
Total	20	8 (40.00)	2 10.00)	51	15 (29.60)	10 (19.60)	5	3 (60.00)	1 (20.00)	3	1 (33.33)	0 (0.00)

Table 6: Forms of *Brucella* infection in sampled horses from selected Local Government areas of Kano state, Nigeria

LGA	No. tested	No. acute infection (%)	No. chronic infection (%)
Kano Municipal Council	17	4 (23.53)	13 (76.47)
Gwale	4	3 (75.00)	1 (25.00)
Nasarawa	13	8 (61.54)	5 (38.46)
Tarauni	2	2 (40.00)	3 (60.00)
Total	**39**	**17 (43.59)**	**22 (56.41)**

Pearson Chi-Square, P=0.020, LGA (Local Government Area)

Table 7: Forms (acute/chronic) of *Brucella* positive horses using 2-Mercaptoethanol Test by sex in four LGAs of Kano Metropolis

LGA	Sex					
	Female			Male		
	No. of sera Tested	No. of Acute infections	No. of Chronic infections	No. of sera Tested	No. of Acute infections	No. of Chronic infections
Kano Municipal	0	0 (0.00)	0 (0.00)	17	4 (23.53)	13 (76.47)
Gwale	4	3 (75.00)	1 (25.00)	0	0 (0.00)	0 (0.00)
Nasarawa	8	4 (50.00)	4 (50.00)	5	4 (80.00)	1 (20.00)
Tarauni	4	1 (25.00)	3 (75.00)	1	1 (100.00)	0 (0.00)
Total	16	8 (50.00)	8 (50.00)	23	9 (39.13)	14 (60.87)

DISCUSSION

From the study it was evident that there were no frank clinical signs of brucellosis in all the horses under the study even though horses were identified to have mild to severe and acute to chronic forms of the infection. . Lack of frank clinical signs could possibly be due to the absence of other organisms like *Streptococcus zooepidemicus*, *Streptococcus equi*, and *Staphylococcus* spp according to Cohen *et al.* (1992) and Hawkins and Fessler (2000) who reported that these organisms were commonly seen in lesions diagnosed for poll evil and fistulous withers. The OIE (2019) reported that clinical signs of brucellosis in horses are characterized by lesions at the poll and withers and pregnant mares might abort. Similarly, Kumaragurubaran *et al*. (2016) reported that equine brucellosis lesions are usually seen in joints, especially those at the poll and withers. They further reported that diagnosis of equine brucellosis is usually based on serological tests like those with RBPT and SAT even though they observed that isolation of the organisms is the gold standard. Thus, the lesions seen at the olecranon bursa in this study could be due to *Brucella* species and this could also indicate that the grooms had a fair knowledge and treatment of the disease. The absence of clinical signs due to *Brucella* infection as observed in this study has similarly been reported by other workers even despite the fact that serologically the horses had demonstrating antibodies (Kaltungo *et al*. (2019); Buhari *et al*. (2020) and Baba *et al*. 2021) In an experimental study, MacMillan *et al.* (1982) could not produce clinical signs due to *Brucella abortus* infection even two months following infecting the horses. However, they were able to demonstrate antibodies to the organism by using RBPT, SAT, complement fixation test, Coombs antiglobulin, 2-mercaptoethanol, and agar gel immunodiffusion tests. Furthermore, they reported that the horses had mild pyraexia just as is also seen in this study.

The study has indicated that 34.18% and 15.17% of the horses showed mild and severe *Brucella* infections by using serological tests (mRBPT and SAT-EDTA). This means that these horses were harbouring the organisms even though not presenting clinical signs of the disease. This has great risk implications as brucellosis is zoonotic. This means that the horse owners and grooms were at risk of contracting the disease. It also means that the grooms, through their habitual exchange of grooming tools with their colleagues could spread the disease to other horses. Not only that, these horses could pass on the infection to other animals as Kaltungo *et al*. (2019) and Buhari *et al*. (2020) and Baba *et al*. (2022) reported in sheep and goats, cattle and horses respectively commonly meeting among themselves at grazing and watering points.

The fact remains that these positive horses were definite risks for the society, especially that Njoga *et al*. (2018) and Baba *et al*. (2021) reported that horses are processed for meat in certain parts of Nigeria. The danger is even greater as, in Nigeria, there are youths who take pleasure at weekend horse riding along with horse processions during weddings. The poor diagnostic facilities at both human and veterinary laboratories will also add to the risk of diseases languishing in the society. Furthermore, many human cases are diagnosed by clinical signs only and patients treated as such. Another dilemma is the low number of both human and veterinary surgeons that routinely handle cases at clinics. Thus, cases of brucellosis could be diagnosed as either malaria or typhoid since there is no laboratory backup.

Table 8: Forms of *Brucella* infection by age using SAT-EDTA in horses sampled in four LGAs of Kano Metropolis

Fisher's Exact Test = 6.464; p=0.028, LGA (Local Government Area)

LGAs	1-5 Years old			6-10 Years old			11-15 Years old			>15 Years old		
	No. of sample	Acute (No. positive (%)	Chronic (No. positive (%)	No. sample	Acute (No. positive (%)	Chronic (No. positive (%)	No. of sample	Acute (No. positive (%)	Chronic (No. positive (%)	No. of sample	Acute (No. positive (%)	Chronic (No. positive (%)
KMC	3	1 (33.33)	2 (66.67)	14	3 (21.43	11 (78.53)	0	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)
Gwale	1	0 (0.00)	1 (100.0)	3	3 (100.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)
Nasarawa	1	1 (100.0)	0 (0.00)	7	4 (57.14)	3 (42.86)	4	3 (75.0)	1 (23.0)	1	0 (0.00)	1 (100.00)
Tarauni	4	1 (25.00)	3 (75.00)	1	1 (100.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)
Total	9	3 (33.33)	6 (66.67)	25	11 (40.00)	14 (56.00)	4	3 (75.0)	1 (25.0)	1	0 (0.00)	1 (100.00)

Where such patients do not recover, they would be left with no alternative but to resort to traditional medicine with attendant consequences.

From the study, it was evident that horses in Kano Metropolis, Kano State, Nigeria were infected with *Brucella* species and this has far reaching public health implications. The study has similarly shown that these horses had mild to severe and acute to chronic infections. There is therefore the need to educate the horse owners and grooms on the disease, brucellosis, as well as its implications along with advising the public. Veterinary authorities should look more closely in surveillance against the disease. Among the limitations of the study were insufficient funds and difficulties in gaining access to the horse stables.

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