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Seroprevalence of brucella antibodies in horses in Sokoto metropolis, Nigeria

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ABSTRACT: The study was conducted to determine the prevalence of Brucella antibodies in horses reared in Sokoto metropolis using the rapid-plate test, serum agglutination test and Competitive ELISA. A total of 347 horses were sampled comprising of 329 males and 9 females within the age range of 1 to above ten years. The serum samples were harvested and subjected to Rapid Plate Test, CompELISA and Serum Agglutination Test. Out of the 347 horses sampled, 48 were found to be positive for the Rapid Plate Test and CompELISA while 41 were positive for Serum Agglutination Test. Thus, an overall prevalence of 13.83% was realized. Also, 7 horses had antibody titre of 1:40, 12 had 1:60 titre, while 29 had 1:80. The prevalence of Brucella antibodies was highest in males (14.28%) compared to females (0%). The seroprevalence to brucella antibodies was found not to be statistically significantly associated with sex of the horses as observed in this study. Horses between the age of 6 to 10 years had a higher prevalence of Brucella antibodies 24 (16.32%) as compared to those 0 to 5 years 21 (12.5%) and those above ten years 3 (9.37%). Age was also not a significant factor for brucella antibody seropositivity (p = 0.588). This study indicates that Brucellosis is prevalent in horses in Sokoto. Hence, horse attendants and livestock reared together are at risk of contracting brucella infection.

Keywords: Brucella antibodies, Brucellosis, horses, RBPT, Sokoto.

INTRODUCTION

Brucellosis is one of the most dreaded bacterial infections with considerable economic and public health burden, especially among production animals worldwide (McDermott, et al., 2013). The disease is caused by different species of Brucella organism with *Brucella abortus, Brucella melitensis* and *Brucella suis* being the most common (Nicoletti, 2010; Godfroid et al., 2011). Brucellosis causes infertility and abortion in livestock. However, the severity of infection may vary depending on the infecting strain, hosts susceptibility and immunological competence (Boschiroli et al., 2002). Brucellosis is a zoonosis and humans can suffer chronic debilitating illness affecting multiple organs, with osteoarticular diseases being one of the most common complications (Pappas et al., 2006). Transmission usually through direct contact with infected animals or bodily excretions (like milk or hygroma fluid) and also the consumption of contaminated food of animal origin. Brucellosis is an important disease mainly as it affects almost all species of animals including wildlife, thus having a wide range of hosts, which make its spread and transmission easier. While various animal species have been reported to act as reservoirs of infection, not many studies have been done on horses in Nigeria. Nonetheless, Brucella species have been isolated from horses in some parts of Nigeria, where horses are used for a variety of purpose (Ducrotoy et al., 2014).

In Nigeria, indigenous horses are associated with royalty and some special traditional festivities (Bukar et al., 2008). They are popular among institutions and private owners for entertainment, traditional and religious ceremonies, pleasure riding, and games (Ehizibolo et al., 2011). These customs and habits are driving the importation of exotic breeds with superior qualities compared to the locally available breeds (Saror, 1976). Periodically, outbreaks of active brucellosis occur in which large herds are involved and sporadic outbreaks of this disease have been reported in cattle, sheep and humans (Akinseye et al., 2016). The geographical distribution of brucellosis is continuously

changing, with new foci emerging or re-emerging (Pappas et al., 2006). Brucellosis is widely distributed in Nigeria. There is a pattern of low and high prevalence in specific areas of the country and prevalence varies between herds in the same area (Junaidu et al., 2008; Katale et al., 2013).

Sokoto State is traditionally home of durbar and polo. Most of the prominent indigenes and residents of the State mainly traditional title holders are known to keep horses for durbar, polo and at times as a source of pride. These horses are used during festivities such as Eid celebrations and as part of the hospitality accorded highly placed visitors to the State. This, therefore, poses a significant health risk to the considerable number of horse riders and their attendants.

Similarly, the type of animal husbandry practiced in this part of the country where various species of animals including horses, are herded together also contribute to the spread of brucellosis through cross-interaction (Amadou et al., 2012). Because of the preponderance of horse in the area and the recorded low productive performance, a study of this magnitude and scope will help to provide the relevant epidemiologic information on the status of the disease in Sokoto State. Therefore, this study was undertaken to determine the prevalence of equine brucellosis in Sokoto metropolis in order to understand the potential risk for human infection. Moreover, understanding the zoonotic importance of the disease and the way livestock workers relate with their animals will provide an insight into the transmission of the organism from animals to man and vice-versa.

MATERIALS AND METHODS

Study area

Sokoto State is located between longitudes 11° 30′ to 13° 50′ East and latitude 4° to 6′ North (Alkali et al., 2015). It is bordered to the north by the Niger Republic, Zamfara State to the east while Kebbi State borders most of the southern and western parts of the state. The state has a land area of 26,648.48 square kilometres. In terms of vegetation, the State falls within the savanna zone. Annual rainfall starts late (June) and ends early (October) with mean falls ranging from 500 to 1,300 mm. Livestock and Horse rearing is the principal agricultural activity of the inhabitants second to crop cultivation; and the State is the second-largest cattle producing state in Nigeria after Borno (Shittu et al., 2008).

Sample size

The sample size was determined using the formula below:

$X = Z^2 \times p\,(1-p)/e^2$ (Thrusfield, 2005)

Where: X= sample size, Z = 1.96, E = margin of error at 95% confidence interval and p = expected prevalence (16% as reported by Ardo and Abubakar (2016)).

However, the samples were increased to 347 in order to increase the chances of detection.

Sample collection

A cross-sectional approach using a purposive sampling technique to identify all the available stables within the Sokoto metropolis was employed to draw samples from horses. However, only stables whose owners consented were included in the study and random sampling was used to select horses within each of the chosen stables. As the blood samples were collected from the horses, the age (using dentition) and sex of the horses were recorded. A total of 347 horses were sampled comprising of 329 males and 9 females within the age range of 1 to above ten years.

Serological screening

Standardised Brucella antigens and competitive ELISA Kit were purchased from Germaine Technologies Inc. 415 Sisterdale Road, Borevne, Texas. The kit is a 96 well ELISA kit for the qualitative detection of Brucella IgG antibodies.

Rapid slide agglutination test

Rapid Slide Agglutination Test was performed using the Serum Agglutination Test *Brucella abortus* antigen following the manufacturer's instructions. The Serum Agglutination Test *Brucella abortus* antigen suspension was adequately mixed by gently swirling before use to ensure uniform suspension. A drop (0.03 ml) of the positive control serum was placed on a glass slide, and one drop (0.03 ml) of the negative control serum was placed on another spot on the glass slide. A drop of (0.03 ml) antigen was then placed side by side of the test sera using dropper pipette. The antigen and serum were then mixed thoroughly. The slide was gently rocked for 4 minutes and, after that, the slide was examined. The formation of greenish granules (agglutination) was recorded as positive.

Serum agglutination (SAT) estimation of antibody titre

The antibody titres for the positive samples were estimated using the tube agglutination test. The antigen was prepared in the laboratory and standardised for agglutination units. A response was considered positive in the SAT when 50% agglutination at a serum dilution of 1:40, equal to 60 IU/ml, was achieved (Alton et al., 1988). The sera were subjected to a 5-tube agglutination test using a standardised *Brucella abortus* antigen. A five-fold dilution of the serum was first prepared. Five (5) test tubes labelled A to E were arranged in each row in a test tube

rack. Half ml of phenol saline (diluents) was placed in the last four tubes (B, C, D, and E) using a pipette. About 0.8 ml of phenol saline was placed in the first tube (tube A), then 0.2 ml of the test serum was pipetted into tube A and mixed thoroughly without frothing, from which 0.5 ml of this mixture in test tube A was transferred to the next tube (tube B) using a pipette. This process was carried out up to the last test tube (tube E), and 0.5 ml of the mixture from tube E was discarded. Thus, 0.5 ml volume was left in each of the 5 tubes.

Similarly, equal volume (0.5 ml) of the diluted *Brucella abortus* antigen was added to each of the five test-serum dilution tubes using a dropper pipette. The tubes were covered and the content mixed by handshaking the test tube rack on the bench. The tubes were then incubated at 37^0C for 20 to 24 hours. The degree of agglutination (formation of granules) and clearance was read under a black background. The titre of each serum was determined; titre of 1:40 (50 IU/ml) and above was taken as diagnostic for *Brucella abortus* infection (Alton et al., 1988). A similar procedure was followed for the positive control serum. The test was interpreted by assessing the degree of agglutination. Any sample with clear background has 100% agglutination, samples with slightly turbid background has 75% agglutination, while those with moderately turbid and very turbid background has 50% and 25% agglutination respectively. When the sample is homogeneous, it indicates that there was no agglutination.

Competitive enzyme-linked immunosorbent assay for Brucellosis diagnosis

The competitive Elisa kit was obtained from Veterinary Laboratory Agency Weybridge, United Kingdom. Preparation and reconstitution of the kit reagents was done according to the manufacturer's instruction. These include diluting buffer, washing solution, stopping solution, conjugate and control sera. The test procedure was conducted based on the instruction of the kit manufacturers.

Statistical analysis

Data generated in the study was subjected to chi-square test, to determine any association between Brucella infection and the sex and age. A p-value of less than 0.05 was considered significant.

RESULTS

RBPT, SAT and competitive ELISA

Out of the 347 horses sampled, 48 were found to be positive by the Rapid Plate Test and CompELISA while 41 were positive by Serum Agglutination Test. This indicate that both the RBPT and ELISA compare favourably with superior sensitivity to the tube agglutination test. Thus, an overall prevalence of 13.83% was recorded. The antibody titer in the horses revealed that 7 samples had brucella antibody titer of 1:40, while 29 had 1:80 titer, with 12 showing a titre of 1:60. Out of the total samples analysed, 338 were males, while 9 were females (Table 2). All the 48 positive samples were male. However, no significant association existed between Brucella infection and sex (Table 1).

The age predisposition revealed that 117 horses belonged to the 0 to 5 year's category, while 168 horses were in the age group of 5 to 10 years, and 32 in the above ten years. Out of this number, 21 were positive in the age range 0 to 5. However, the association between Brucella infection and age was not statistically significant (X^2 = 0.5877, Table 2).

DISCUSSION

Brucellosis is a zoonosis with global distribution. The three common species associated with clinical infection in horses are *B. abortus, B. suis,* and to a lesser extent, *B. canis* (Karthik et al., 2016). *Brucella abortus* infection in horses is not only important as a clinical entity, but also as a potential source of infection in human and other susceptible livestock. There is strong evidence that cattle are the primary source of *Brucella abortus* infection in horses (Denny, 1973). Unfortunately, mixed husbandry system, where different livestock species are reared together is the common practice in Sokoto, Nigeria. This may lead to increased human exposure especially because the popular mode of transmission being via direct contact with infected animals or consumption of unpasteurized milk (Addis, 2015; Junaidu et al., 2008; Karthik et al., 2016).

In this investigation, the results indicated that out of the 347 horses screened 48 were found to be positive with an overall prevalence of 13.83% based on the RBPT and ELISA. The 13.83% prevalence obtained in this study is lower than 16 and 22.7% reported in Taraba (Ardo et al., 2016) and Plateau states (Ehizibolo et al., 2011), respectively, but higher than 6.0% reported in Kaduna State (Ehizibolo et al., 2011), both in northern Nigeria. The idea behind using the three tests (i.e RBPT, ELISA and Tube Agglutination Test) was to identify the best suitable test for the diagnosis of equine brucellosis in the study area. This is because earlier reports have indicated that an agreement between two tests varies depending on the prevalence (Manishimwe et al., 2015). Sokoto, like Taraba and Plateau States, practice multispecies housing and mixed grazing, which has been reported to increase the spread and transmission of the infectious agent (Adamu et al., 2016; Jajere et al., 2016). Mixed grazing can also expose horses to infection with Brucella through ingestion of byproducts of infected sheep, goats, dogs, cattle and pigs.

Table 1. Sex distribution of Brucella infection in horses based on the competitive ELISA.

Sex	Brucella infected	Non-infected	Total
Male	48 (14.28%)	290 (85.80%)	338
Female	0 (0%)	9 (2.66)	9
Total	48 (13.83%)	299 (88.46%)	347

p = 0.4661.

Table 2. Age distribution of Brucellala infection in horses based on the competitive ELISA.

Age group	Brucella infected	Non-infected	Total
0-5	21 (14.28%)	126	147
6-10	24 (14.28%)	144	168
Above 10 yrs	3 (9.37%)	29	32
Total	48	299	347

$X^{2} = 0.5877$; p= 0.7454.

With respect to age predisposition, the results indicate that the prevalence was higher in those within the 5 to 10 years of age group. However, this difference is not statistically significant. This observation conforms with the study of Nasinyama et al (2014) where they reported that there was no association between age and seropositivity to brucella antibodies among cattle keepers in Uganda. Furthermore, while 48 of the male horses were positive (14.28%), none of the females was positive. Worthy of note is the disproportionate distribution of the samples concerning sex where male horses predominate. This is so because most of the inhabitants keeping horses do so for traditional festivities like durbar, sports and racing in which case male horses are more desired (Fenner et al., 2019). Finally, the rapid plate test RBPT and the competitive ELISA were found to show superior sensitivity in detecting the *Brucella abortus* antibodies in circulation, though not statistically significant. According to the results of this investigation, RBPT and competitive ELISA are the preferred test assays for the serological diagnosis of brucellosis.

Conclusion

In conclusion, it is imperative to note that, while some of the horses sampled exhibited clinical signs indicative of ill health, the vast majority were healthy. Ironically, some showed high levels of circulating *Brucella abortus* antibodies, which could mean they may be incubating the disease and hence can also serve as a potential source of infection to other horses and susceptible animals reared together. Moreover, since the control and prevention efforts in Nigeria are mainly on cattle, efforts should be made to include other susceptible livestock including horses. In doing so, the potential risk of transmission to humans, especially caretakers, riders and veterinarians will be significantly curtailed.

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CONFLICTS OF INTEREST

The authors wish to declare that no conflict of interest exists between them.

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