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Prevalence of *Brucella* antibodies in horses (*Equus Caballus*) in Jalingo, Taraba State, Nigeria

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A study was conducted to determine the prevalence of *Brucella* antibodies in Jalingo using the Rose Bengal plate test (RBPT) and the serum agglutination test (SAT). A total of 90 adult horses that were on zero grazing management system were sampled. The sampling sites were selected based on the availability of horses. The objectives of the study were to determine the prevalence of *Brucella* antibodies in horses as well as the distribution of the infection according to sex. There was no association of horses seen with other animals. The samples collected comprised of 88 males and 02 female horses from four locations in Jalingo: Barade, Sarkin dawaki, Sintali and Turaki. All the sera samples were subjected to initial screening by RBPT and positive cases were then analyzed with SAT. The overall prevalence of *Brucella* antibodies was 07 (07.8%) with all positive cases recorded from male horses for RBPT, while none was positive for SAT among the male and female horses. There was no significant difference in the prevalence of horse *Brucella* antibodies between male and female (P>0.05). Horses in Jalingo are in the four wards identified and were all sampled. Turaki ward recorded the highest prevalence of 13.3%, while Barade Ward had the lowest prevalence with 00.0%. However, there is no significant difference in the prevalence between the male and female (P>0.05). Further studies need to be conducted to determine the involvement of other species and humans.

Key words: Horse, Brucellosis, Jalingo, Rose Bengal plate test (RBPT), serum agglutination test (SAT).

INTRODUCTION

Brucellosis is an important zoonotic disease worldwide causing serious human health problems and substantial economic losses for the livestock industry (Corbel, 1997). The disease is highly contagious in domestic (camel, cattle, buffaloes, sheep and goats), wild (deer, elk, bison, zebra) and companion (horses, donkeys, mules and dogs) animals and is one of the most important and widespread zoonosis in the world (Poester et al., 2002). The disease remains an uncontrolled problem in regions with poor animal and public health standards leading to high endemic in Africa, the Mediterranean, Middle East, parts of Asia and Latin America (Capasso, 2002; Refai,

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2002). Among domesticated species, cattle, sheep, pigs and goats are most commonly affected. Infection in horses is uncommon, while cats are resistant (Benkirane, 2006). Brucellosis is generally asymptomatic in horses (Denny, 1973). Fistulous withers and poll evil are the most common clinical manifestations in the horse, and also associated with a variety of other clinical manifestations, including vertebral osteomyelitis (Collins et al.,1971; Cohn et al., 1992), abortion, infertility (Denny, 1972) and arthritis (Carrigan et al., 1987).

Prevalence of the disease in Nigeria among various animal species is reported to be 16.5% in camels (Sadiq et al., 2013), 21.3% in bovine, 11.1% in ovine and 20% in caprine (Zubairu et al., 2014). Seroprevalence rate of 11% was detected for *B. abortus* and 0% for *B. melitensis* among animal handlers, livestock keepers, butchers and middlemen (Adamu et al, 2015), while another study of the infection rate among butchers and the general public is 31.82% (Cadmus et al., 2006).

Equine infection most frequently involves *B. abortus,* but *B. suis* was isolated from horses with septic bursitis and from the internal organs of a mare with no external signs of disease (Cvetnic et al., 2005). Because of the difficulty that may be encountered in attempts to culture *B. abortus* from horses with fistulous withers, concomitant serologic testing for detection of specific antibodies was recommended. Serological surveys have indicated that many horses may be exposed to *B. abortus* without developing clinical signs of the disease (Göz et al., 2007). For serodiagnosis of horse brucellosis, many serologic tests such as Rose Bengal plate test (RBPT), serum agglutination test (SAT), complement fixation test (CFT), mercaptoethanol agglutination, Agar gel diffusion and Coombs tests have been commonly used (Hutchins and Lepherd, 1968; MacMillan, 1985).

Horses are accorded special attention due to the immense role they play in polo games, cultural festivals and security (Ehizibolo et al., 2011). *Brucella abortus* infection in horses is important not only as clinical existence but also as a source of infection to horse riders, handlers and the general public and may be significant and warrant investigation. Studies concerning brucellosis have been conducted on cattle, sheep and goats, but most have focused on cattle. However, a small number of surveys have been carried out to determine the epidemiologic role of horses and donkeys. The aim of this study was to determine the prevalence of *brucella* antibodies in horses raised in Jalingo, Taraba State, Nigeria.

MATERIALS AND METHODS

Study area

Jalingo is the capital city of Taraba state. It is also the headquarters of Jalingo Local Government. It lies between latitude 8.90°N and longitude 11.3° E. It has a good climatic condition and rich in agricultural produce with temperatures ranging between 20-40°C and mean annual rainfall of 1,500 mm per annum.

Animals

The sampling sites were selected based on the availability of horses. Therefore, virtually all the horses found within the wards were sampled. The horses were on zero grazing. The horses are primarily for ceremonial purposes and serve as a form of prestige to owners.

Samples collection

All the horses found within the wards were sampled. The horses were on zero grazing. The horses are primarily for ceremonial purposes and serve as a form of prestige to owners. Verbal approval was sought from the District head (Galadima) of Jalingo who gave us one of his aides to assist us reach the horse owners.

Five milliliters of blood samples were collected by venipuncture via the jugular vein using syringe and needle. Information about sex, age and location of the equines sampled were recorded. All the samples collected were placed in sterile blood sample bottles and allowed to stand for 30 min before centrifuging. The sera were separated, placed in ice packs and transported to the microbiology laboratory, College of Agriculture, Jalingo and stored at -20°C till needed for analysis.

Serological tests

Rose Bengal plate test (RBPT)

The procedure described by Nielsen and Dunkan (1990) was followed. The antigen used was from Veterinary Laboratories Agency (VLA, UK). The serum samples earlier stored in the refrigerator were removed and left at room temperature for 30 min. 30 µl (0.03 ml) of the serum samples were dispensed onto the plate and 30 µl (0.03 ml) of RBPT antigen were dropped alongside the sera. Using applicator stick the antigen and the sera were mixed together and examined for agglutination.

Serum agglutination test

1 ml of SAT antigen was diluted with 9 ml of prepared phenol saline buffer. 0.8 ml of the phenol saline solution was dispensed in the first tube and 0.5 ml was dispensed into the remaining four tubes (Morgan et al., 1978). 0.2 ml of the test serum were dispensed into the first tube, the second and up to the fifth tube and mixed properly. 0.5 ml of the mixture was transferred serially from the first to the last tube and 0.5 ml was discarded from the last tube. This resulted in dilutions of 1:5, 1:10, 1:20 and so on. 0.5 ml of the diluted SAT antigen (1:9) was added to each tube, mixed properly and gave a final dilution of 1:10, 1:20, 1:40, 1:80 and 1:160. The tubes were covered with aluminum foil and incubated at 37°C in a water bath for 24 h and the result was read.

Sample analysis

Simple percentages and Chi square were used to analyze the data by subjecting it to SPSS-16 statistical software.

RESULTS

In this study, 07 (07.8%) of the 90 sampled horses were

Table 1. Prevalence of *Brucella* antibodies in Horses in Jalingo.

Location	No. examined	RBPT		SAT		X^2
		No. +ve (%)	No. -ve (%)	No. +ve (%)	No. -ve (%)	
Barade	15	00(00.0)	15(100.0)	00(00.0)	15(100.0)	3.53
S/Dawaki	18	02(11.1)	16(88.9)	00(00.0)	18(100.0)	
Sintali	27	01(03.7)	26(96.3)	00(00.0)	27(100.0)	
Turaki	30	04(13.3)	26(86.7)	00(00.0)	30(100.0)	
Total	90	07(07.8)	83(92.2)	00(00.0)	90(100.0)	

*The figure in parenthesis is percentages (%) of the number positive or negative, No. +ve = Positive, No. -ve = negative, % +ve = percentage positive, % -ve = percentage positive, RBPT = Rose Bengal plate test. PV= 0.3260 X^2= 3.53 df= 3 a=0.05.

Table 2. Sex and specific prevalence of *Brucella* antibodies in horses.

Sex	No. examined	RBPT		SAT		X^2
		No. +ve (%)	No. -ve (%)	No. +ve (%)	No. -ve (%)	
Male	88	07(07.9)	81(92.1)	00(00.0)	88(100.0)	3.53
Female	02	00(00.0)	02(100.0)	00(00.0)	02(100.0)	
Total	90	07(07.8)	83(92.2)	00(00.0)	90(100.0)	

*The figure in parenthesis is percentages (%) of the number positive or negative, No. +ve = Positive, No. -ve = negative, % +ve = percentage positive, % -ve = percentage positive, RBPT = Rose Bengal plate test. PV= 1,000 X2= df= 1 a=0.05.

positive by RBPT. Turaki ward recorded 04 (13.3%) prevalence and had the highest seroprevalence (Table 1). This was followed by Sarkin Dawaki with 02 (11.1%), Sintali 01 (03.7), while Barade recorded no positive case. All the RBPT positive cases that were subjected to SAT recorded no positive case. They had titers between 1/10 and 1/20 values and others showed no titers at all. There was no significant difference among the wards (p>0.05).

This study also indicated that male horses were the ones that were positive for *Brucella* antibodies with 07 (07.9%) of the 88 male horses' samples screened with RBPT, with no positive case observed in the 02 female horses screened (Table 2). All the RBPT positive cases showed no titer that was equal to or greater than 1:40 when tested with SAT. However, there was no significant difference in the prevalence between the male and female ones in Jalingo (p>0.05).

DISCUSSION

The overall prevalence of 07.8% by RBPT and 00.0% by SAT in this study is comparable to the findings of other workers carried out in horses in Nigeria; 14.7% (Ehizibolo et al., 2011) and 4.8% (Bale and Kwanashie, 1984). It is however higher than 5.5% (Sadiq et al., 2013) in other equines (donkey) in Borno and Yobe States by both RBPT and SAT and 4.2% in lowlands of central Oromiya, Ethiopia (Jegerfa et al., 2009).

The result in this study shows that male horses, 07 (07.8%) by RBPT have higher prevalence than female horses 00 (00.0%). Ehizibolo et al. (2011) also reported that all the mares in their study were negative when screened with RBPT. The higher prevalence of the *Brucella* antibodies in the male in this study could be as a result of the low population of female horses sampled There are controversial reports regarding the prevalence of brucellosis in relation to the sex of animals, as some of the research workers reported significantly higher prevalence in males than females (MacMillan and Cockrem, 1986). Whereas, others report that females have higher prevalence than males (Hussein et al., 2005).

The prevalence of the disease observed in the Rose Bengal plate test (RBPT) is higher than in serum agglutination test (SAT). This is in agreement with the works of Mbuk et al. (2011), who recorded a higher seroprevalence in RBPT than SAT. Results of RBPT, which could not be confirmed by SAT, indicate the non-specific reaction in these samples and that all animals tested are considered negative for brucellosis.

CONCLUSION AND RECOMMENDATIONS

The presence of brucellosis in horses that are on zero grazing in Jalingo metropolis is a enigmatic. It is a possibility that the horses in this study may have been exposed to *B. abortus* through the ingestion of infected pasture that was fed to them. They may also be infected at an early age or through a means that was not identified in this study.

Identification of horses with brucellosis makes it imperative that strategic planning for the control of brucellosis in Nigeria should include horses. It is recommended that horse riders be screened for the disease as a preventive measure as well as to possibly establish association.

Conflict of Interests

The authors have not declared any conflict of interests.

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