Seroprevalence of horse (Equus caballus) brucellosis on the Mambilla plateau of Taraba State, Nigeria

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A cross-sectional study was conducted on the seroprevalence of horse brucellosis using the Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) on the Mambilla plateau of Taraba state, Nigeria where horses are reared under a free range management system on cattle farms. 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 and age. A total of 100 horses were sampled, 25 each from four locations where horses were concentrated on the plateau: Gembu, Nguroje, Dorofi, and Mayo Ndaga. Sixty-two of the horses were males, and 38 were females. Eighty of the horses were adults, while 20 were young. All horses were reared under a free range management system together with cattle. The overall seroprevalence rate was 16 (16%) according to the RBPT and 6 (6%) according to the SAT. The seroprevalence rates were 19.40% (12/62) according to the RBPT and 6.50% (4/62) according to the SAT in the males and 10.50% (4/38) according to the RBPT and 5.30% (2/38) according to the SAT in the females. The prevalence was highest in Nguroje (8/25, 32%) followed by Gembu (5/25, 20%), Dorofi (3/25, 12%) and Mayo Ndaga (0%). Adult horses showed a seroprevalence of 18.8% (n=15) according to the RBPT and 7.5% (n=1) according to the SAT. Young horses had a seroprevalence rate of only 5% (n=1) according to the RBPT and 0% (n=0) according to the SAT. There was no statistically significance association with location, sex, and age (P>0.05). From this result, it can be concluded that brucellosis in horses on the Mambilla plateau of Taraba state, Nigeria was essentially a disease of adult horses and more prevalent in male horses than female horses. Further studies need to be conducted to determine the disease status in lowland areas of the state where horses are reared on zero grazing or are tethered and also to determine the involvement of other species and humans.

Key words: Brucellosis, RBPT, SAT, Seroprevalence, Taraba state

Brucellosis is a highly contagious zoonotic disease of cattle, sheep, goats, bison, elk, deer, buffalos, pigs, dogs, and occasionally horses and donkeys caused by Brucella species and are characterized by recurrent abortion and infertility in a variety of animal species and undulating fever in humans [18]. The disease is endemic in Nigeria, resulting in huge economic losses due to decreased calving rates, delayed calving intervals, infertility, treatment cost, and decreased milk production, abortion, still births, birth of weak offspring, and loss of man-hours in infected people [3]. Serological surveys of Brucella antibodies in horses in Northern Nigeria indicated prevalence rates of 4.8% [9] and 14.7% [15]. Ocholi et al. [24] reported isolation of Brucella abortus biotype 1 from a foal. In another report, Brucella abortus was isolated from hygroma fluid in a horse with carpal bursitis [25]. It has been reported in various parts of Nigeria to have affected domestic livestock [24] and humans [7]. The few reports of equine brucellosis have focused on clinical cases [25, 28].

The prevalence of brucellosis in Nigeria varies tremendously among animal species: 0.4 to 48.8% in cattle [9, 10, 16, 32], 1.4 to 20.0% in sheep and goats [8, 11, 17, 27,

Received: October 28, 2015
Accepted: January 8, 2016
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Brucella infection in horses. It also provides information on the status of the disease in horses in relation to the association and interaction with management practices in North East Nigeria have not yet been documented, even though the horse is the closest companion to man after the dog.

The use of horses as pleasure and companion animals for polo, racing, ceremonial durbars, and security purposes, as in police mountain troops, is well documented in Nigeria. The high rate of association between cattle, horses and humans is inevitable, and brucellosis has continued to persist in the population unabated. The main source of infection for horses is cattle [2]. The magnitude and role of transmission of infection from cattle to horses and the status of the disease in horses in relation to the association and interaction with management practices in North East Nigeria have not yet been documented, even though the horse is the closest companion to man after the dog.

The aim of this research was to establish the status of Brucella infection in horses in this unique highland vegetation and low temperature region, with horses living under a free range management system in association with cattle, unlike the tethered and zero grazing management systems used for horses in other parts of Nigeria, with a view to proferring a solution for prevention and control of the disease. This report also provides information on the status of Brucella infection in horses. It also provides information on the role of rearing cattle and horses together in transmission.

Materials and Methods

Study area

The Mambilla plateau is located in the SouthEastern part of Taraba state, Nigeria. It has an average elevation of 1,524 m (5,000 ft.) above sea level and is the northern fringes of the Bamenda Highlands of Southern Cameroon. It is located at latitude 7°20’N and longitude 11°43’E. It harbors the Chappal Waddi Mountains, which are considered the highest point in Nigeria, with an average height of about 2,419 m (7,936 ft.) above sea level. The area enjoys low temperatures ranging between 12 to 25°C in most parts of the year, and it receives over 1,850 mm of rainfall annually. The four areas selected for the study were Gembu, Nguroje, Dorofi, and Mayo Ndaga (Fig. 1).

Sample collection

A total of 100 blood samples were randomly collected, 25 each from four locations in the Gembu, Nguroje, Dorofi, and Mayo Ndaga areas. All horses were reared under a free range management system on sedentary cattle farms. Horses <3 years old were considered young, while those >3 years old were considered adults. Sixty-two male and 38 female horses were sampled (Gembu, 16 males, and 9 females; Nguroje, 20 males and 5 females; Dorofi 11 males and 14 females; and Mayo Ndaga 15 males and 10 females).

Serological tests

Two serological tests were used for the detection of antibodies against Brucella: the Rose Bengal Plate Test (RBPT) and Serum (tube) Agglutination Test (SAT). Both tests are approved serological tests for screening against brucellosis [26]. RBPT antigen was obtained from the Veterinary Laboratories Agency (VLA, U.K.) and used as described previously [5] to perform a rapid agglutination plate test for the detection of Brucella-specific agglutinins. The plate test was used as a screening procedure to establish the presence or absence of homologous bacteria. Both the serum sample and reagent were allowed to thaw to room temperature at (22 ± 4°C) for testing. The antigen bottle was agitated thoroughly but gently to produce a uniform suspension. Twenty-five to 30 microliters of the serum sample was placed on the white tile plate slide. One drop of rose bengal antigen was dropped onto the test serum sample. Rose Bengal antigen and the serum sample were mixed and agitated thoroughly using a new stirring rod for each sample. The process was compared using positive and negative controls. The slide was rocked gently for 2 to 3 min. The results regarding agglutination were checked and read by observation after 4 min of gentle shaking/agitation. The presence of agglutination (presence of antibodies) was considered a seropositive (+) result, whereas no agglutination (no antibodies) was considered a seronegative (−) result.

The SAT was carried out in tubes using the Tube Agglutination Test as described [5] and standardized previously [31]. One milliliter of SAT antigen was diluted with 9 ml of prepared phenol saline buffer, which is referred to as the working solution. Then 0.8 ml of phenol saline solution was dispensed into the first tube, and 0.5 ml was dispensed into each of the remaining four tubes. Subsequently 0.2 ml of the test serum was dispensed into each of the first to fifth tubes and mixed properly. After this, 0.5 ml of the mixture was transferred serially from the first to last tubes, and 0.5 ml was discarded from the last tube. This process of doubling 0.5 ml dilutions resulted in the production of dilutions of 1:5 1:10, 1:20, and so on. Each tube then received 0.5 ml of the diluted SAT antigen (1:9) and was mixed properly giving final dilutions 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 hr, and the results were then read. Positive and negative controls were also run in the same manner.

Simple percentages and the \( \chi^2 \) test were used to analyze the data. The SPSS 17 statistical software was used for the analysis. Differences in means were considered significant at \( P<0.05 \).

**Results**

The overall seroprevalence of the disease was 16% according to the RBPT and 6% according to the SAT. Nguroje had the highest prevalence rates (32%, 8/25 according to the RBPT and 16%, 4/25 according to SAT) followed by Gembu (20%, 5/25 according to the RBPT and 4%, 1/25 according to the SAT) and Dorofi (18%, 3/25 according to the RBPT and 4%, 1/25 according to the SAT). All 25 serum samples from Mayo Ndaga were negative by both tests (Table 1). There was no statistically significant association between prevalence of the disease and location of horses in the study area. Sixty-two male horses and 38 female horses were sampled. The prevalence of the disease was higher in male horses (19.40%, 12/62, according to the RBPT and 6.5%, 4/62, according to the SAT) than in the female horses (10.50%, 4/38, according to the RBPT and 5.3%, 2/38 according to the SAT; Table 2). There was no statistically significant association between prevalence of the disease and sex of horses. Out of the 80 adult and 20 young horses sampled 18.8% (15/80) and 5%, 1/20) were positive according to the RBPT and 7.5% (6/80) and 0% were positive according to the SAT in adult and young horses respectively (Table 3). Statistically, there was no significant association between prevalence of the disease and age.

**Discussion**

The current study provides the first information on the prevalence of *Brucella* antibodies among horses on the Mambilla plateau of Taraba state, Nigeria. The overall prevalence of brucellosis detected was 16% RBPT and 6% SAT in horses on the Mambilla plateau of Taraba state Nigeria. The RBPT is highly sensitive and therefore agglutinates with antibodies of other pathogens that are similar.
to *brucella* antibodies, whereas, SAT is highly specific and can discretely segregate between mimicking antibodies of other pathogens similar to *brucella* and therefore agglutinates only with *brucella*-specific antibodies, as reflected in the results of previous study [26]. The sensitivity of the RBPT compared with the SAT may have accounted for the difference in prevalence recorded in this research compared with that described previously [9]. Only one male horse in the sampling exercise presented with lesions of fistulous withers [13]. The result obtained here are higher than those from other studies carried out in Nigeria (14.7% for a study that used the RBPT [15] and 4.8% for a study reported 31 years ago that used the SAT [9]). This could be the result of differences in the management systems for horses in the areas where these studies were carried out. Horses on the Mambilla plateau are kept under a free range management system and are always in close proximity with cattle unlike other areas of Nigeria, where horses are kept under zero grazing or tethered management systems. Although the source of infection could not be ascertained in the study, there is evidence that cattle are the main source of *Brucella abortus* infection in horses [25]. Reports on the seroprevalence of brucellosis in horses in Nigeria are rare. However, there are reports on the prevalence of the disease in other equine species including one indicating a prevalence of 5.5% in donkeys in Borno and Yobe states according to both RBPT and Microtiter Serum Agglutination Test [30]. Taraba state is located in the same zone of North East Nigeria as Adamawa, Bauchi, Borno, Gombe, and Yobe states.

The finding of higher prevalence of the disease in the male horses (19.4%, 12/62 according to the RBPT and 6.5%, 4/62 according to the SAT) than female horses (10.5%, 4/38 according to the RBPT and 5.3%, 2/38 according to the SAT) contradict the findings of some research conducted in cattle [11, 12]. The higher prevalence of the disease in the male horses in this study could be result of the relatively low population of female horses sampled. This findings, however, is in agreement with studies on the distribution of seroprevalence of brucellosis by sex in camels in which males were reported to have a higher prevalence than females [1, 14, 20, 21]. There are also controversial reports regarding the prevalence of human brucellosis in relation to sex. In Egypt researchers reported a significantly higher prevalence of human brucellosis in females than in males.

### Table 1. Seroprevalence of horse brucellosis on the Mambilla plateau

| Location      | No. examined | RBPT Positive (%) | RBPT Negative (%) | SAT Positive (%) | SAT Negative (%) | $\chi^2$ |
|---------------|--------------|-------------------|-------------------|-----------------|-----------------|---------|
| Gembu         | 25           | 5 (20)            | 20 (80)           | 1 (4)           | 24 (96)         | 1.596   |
| Nguroje       | 25           | 8 (32)            | 17 (68)           | 4 (16)          | 21 (84)         |         |
| Dorofu        | 25           | 3 (12)            | 22 (88)           | 1 (4)           | 24 (96)         |         |
| Mayo Ndaga    | 25           | 0 (0)             | 25 (100)          | 0 (0)           | 25 (100)        |         |
| Total         | 100          | 16 (16)           | 84 (84)           | 6 (6)           | 94 (100)        |         |

$P$-value=0.207, $\chi^2=1.596$, df=3, $\alpha=0.05$.

### Table 2. Sex and specific seroprevalence of horse brucellosis

| Sex        | No. examined | RBPT Positive (%) | RBPT Negative (%) | SAT Positive (%) | SAT Negative (%) | $\chi^2$ |
|------------|--------------|-------------------|-------------------|-----------------|-----------------|---------|
| Male       | 62           | 12 (19.4)         | 50 (80.6)         | 4 (6.5)         | 58 (93.5)       | 1.366   |
| Female     | 38           | 4 (10.5)          | 34 (89.5)         | 2 (5.30)        | 36 (94.7)       |         |
| Total      | 100          | 16 (16)           | 84 (84)           | 6 (6)           | 94 (100)        |         |

$P$-value=0.242, $\chi^2=1.366$, df=1, $\alpha=0.05$.

### Table 3. Age and specific seroprevalence of horse brucellosis

| Age        | No. examined | RBPT Positive (%) | RBPT Negative (%) | SAT Positive (%) | SAT Negative (%) | $\chi^2$ |
|------------|--------------|-------------------|-------------------|-----------------|-----------------|---------|
| Adult >3   | 80           | 15 (18.8)         | 65 (81.2)         | 6 (7.5)         | 74 (92.5)       | 0.059   |
| Young <3   | 20           | 1 (5)             | 19 (95)           | 0 (0)           | 20 (100)        |         |
| Total      | 100          | 16 (16)           | 84 (84)           | 6 (6)           | 94 (100)        |         |

$P$-value=0.808, $\chi^2=0.059$, df=1, $\alpha=0.05$. 
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[19], whereas other researchers [22] reported that Brucella abortus causes intermittent bacteremia in mares but not in stallions. The relatively higher incidence reported among human females than males might be due to more involvement of females in handling of livestock products. Because of the importance of horses to humans as companions and draught animals infected horses could be a source of Brucella infection for humans as a result of close contact, with infection occurring via contaminated dust or droplets entering through the respiratory system, contact with aborted fetuses, and contact with discharges from genitalia. The higher prevalence of the disease in adult horses than young horses in this study is in agreement with a study by Mbuk et al. [23] who reported a higher prevalence of the disease in adult cattle of all ages.

In conclusion, the results of this study show that brucellosis is endemic on the Mambilla plateau of Taraba state, Nigeria, and that it affects not only cattle but also horses. The rearing of horses together with cattle should be avoided to prevent infection of horses. Since cattle farmers on the plateau are sedentary, routine vaccination of cattle should be initiated upon to reduce spread of the disease to other animals and humans. There should be officially coordinated systems of brucellosis surveillance and reporting in Nigeria to enhance epidemiological track-back, enforcement, and monitoring of control measures. Public awareness on the presence of brucellosis among horses with emphasis on its economic impact and public health implications are hereby strongly recommended. There is need for improved sanitary measures with proper handling of retained placenta and aborted fetuses by burning and deep burying in the ground.

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