

SEROLOGICAL SURVEY FOR ANTIBODIES AGAINST ZONOTIC BRUCELLA ABORTUS IN DOGS IN NORTHWESTERN NIGERIA

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Abstract

A prospective study was conducted in Sokoto State, northwestern Nigeria to determine the sero-prevalence of antibodies against *Brucella abortus* in dogs. Sera samples were obtained from a total of 242 dogs in the study area and analyzed against a range of epidemiological variables. Objective was to screen dogs within the region for symptomatic and asymptomatic infection for *Brucella abortus* known to be zoonotic. Study used the Rose Bengal Plate Test (RBPT) which is validated and previously used widely as globally standard for *Brucella* screening to identify dogs infected. An overall sero-prevalence of 14.9% to *Brucella abortus* antibodies was recorded. Out of 154 males sampled, 23 (15.6%) were seropositive, while 12(13.6%) were seropositive for female. Puppies presented 25.9% prevalence against 15.3% for adult dogs. The highest breed represented was the Alsatian where 27 dogs were sampled with 11(40.7%) testing positive. The mongrel which is a term referring to the indigenous breed in Nigeria recorded 15(12.1%) isolation rate. A total of 165 household dogs were sampled, out of which 17(10.3%) tested positive as against 77 stray dogs sampled where 19(24.7) tested positive. The findings from this study indicate that brucellosis is prevalent in dogs in the study area and carrier dogs pose grave public health danger

Keywords: *Brucella*, Surveillance, Zoonosis, Prevalence, Epidemiology.

INTRODUCTION

Brucellosis is a bacterial disease caused by various *Brucella* species and is a major cause of economic losses such as abortion, infertility, low conception rate and low survival rate of neonates in the livestock industry and also a zoonosis of great public health significance (1). Brucellosis has recently been described as a re-emerging and neglected tropical disease (2) with potential for outbreaks particularly in developing countries. The four species of *Brucella* infecting dogs are: *Brucella abortus*, *Brucella canis*, *Brucella melitensis* and *Brucella suis* (3). Canine brucellosis is common in different regions of Nigeria and is an important cause of reproductive failure, particularly in kennels, accounting for abortions, stillbirths, epididymitis, orchitis and sperm abnormalities in dogs (4). The mode of transmission is similar in most domestic animals. It occurs after ingestion or contact with the organism through mucous membranes or broken skin following exposure to contaminated placenta, aborted fetuses, fetal fluid or vaginal discharges from infected females during heat, breeding (coitus), abortion or full term parturition (5). The disease is of zoonotic importance, keeping of dogs as household pets, guard dogs, for breeding purposes and contact of humans with stray dogs serves as major risk factors to humans (6). Given a dearth of information on *Brucella* infection in dogs and the specter of potential zoonosis, this study was conceived to screen canine population for the first time in study region for symptomatic and asymptomatic infections.

Data will likely serve as updated evidenced based report on the status of the disease and the need for public health measures to guarantee human and animal health. The objectives of the study therefore was to investigate the presence of *Brucella* infection in dogs sampled in the study area by screening with a globally acceptable and previously validated Rose Bengal Plate Test (RBPT) otherwise called serum agglutination test (SAT).

MATERIALS AND METHODS

A prospective serological survey for the antibodies against *Brucella abortus* and *B. canis* in dogs was done. Study designed as a comprehensive epidemiological survey for three major endemic diseases: Canine leptospirosis, salmonellosis and brucellosis in the region. A common ethical clearance was obtained for the infectious disease study from the Faculty Animal Research and Ethics Committee, later was renamed "IACUC" with approval number UDUS/IACUC/2014/AUP/R0-11

Study Area: The study was conducted in Sokoto metropolis. The state is located between longitudes 4°8E and 6°54E and latitudes 12°N and 13°58N and is bordered by Niger Republic to the north, Kebbi state to the west and by Zamfara to the east (7).

Sample Frame: A sample frame consisting all veterinary clinics in Local Governments Areas (LGAs) making up Sokoto metropolis and client-owned dogs addressed in clinic files were used as sampling sites for the study. Local governments

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include: Sokoto North, Sokoto South, Wamakko, Dange Shuni and Kware. Both sick dogs presenting as patients in veterinary clinics in these LGAs and apparently healthy dogs owned to clients were sampled for the study. Client-owned dogs were sampled with consent from the owners. Detailed demographic data regarding ages, sex, medical history, purpose of keeping dogs and locations were recorded accordingly.

Sample Size Determination: A previously established prevalence of 32.3% reported by (8) in north-central Nigeria was used as the value of P to substitute into a formula reported by (9) for sample size calculations. Level of confidence was taken to be 5% and total samples "N" obtained was 242.

Blood Sampling: Blood samples were taken from various veterinary clinics in the state and a simple random sampling technique was used on available dogs (both domesticated and stray dogs) in different locations, irrespective of the age, sex and breed. Ages of the dogs were determined using available records obtained from owners and where this is not available; it was estimated using dentition as described by (10) using eruption and wear of the teeth. 5 millilitres (5mls) of blood samples were obtained using a syringe by venipuncture of the cephalic vein in the forelimb or hind limb as described by (11). The sample was discharged into plain test tubes, which were placed in an ice pack and then transported to the laboratory for processing. Serum was obtained using the procedure described by (12). Samples were refrigerated at 4°C for storage of about a week and afterwards, were transported in styrofoam boxes packed with dry ice and made airtight by paraffin foil to the Brucellosis Laboratory, National Veterinary Research Institute (NVRI), Vom, Nigeria. Appropriate hazard labels pasted on boxes and all sample processing and laboratory processing done using the Biosafety Cabinets (BSC) in a Biosafety Level 2 laboratory.

Sero-screening: Rose Bengal Plate Test (RBPT) method was used to screen the sera for agglutinins, using commercially prepared standardized *Brucella abortus* antigen acquired from the Veterinary Laboratory Agency (VLA) Weybridge, New Haw, Addlestone, Surrey (KT15 #NB), United Kingdom and the serological test was performed following the protocol of the manufacturer. Screening done in the Brucellosis Laboratory of the National Veterinary Research Institute (NVRI), Vom, Plateau State Nigeria as previously reported by (8).

Statistical Analysis: Data generated were imported into Graph Pad Prism 3 for Windows and Chi-square used to test association between detecting *Brucella* antibodies with various epidemiological variables recorded: sex, age and purpose of keeping dogs with level of significance at $P < 0.05$. Processed data organized into tables for clarity afterwards.

RESULTS

An overall sero-prevalence of 14.9% to *Brucella abortus* antibodies was recorded. Out of a total of 242 sera tested, 36 samples were found to be positive (Table 1). Out of 154 males sampled, 23(15.6%) were seropositive, while 12(13.6%) were seropositive out of 88 females tested (Table 1). Of the 242 sera sampled, 27 were puppies from which 7(25.9%) were found to be positive. Relatively, 91 samples were collected for the young adults category out of which 10(10.9%) was positive. For the adult (dogs above one year) category, 124 dogs were

sampled out of which 19(15.3%) were positive for *Brucella abortus* antibodies (Table 2). Based on care provided for dogs (management), entire samples were divided into stray or household. A total of 165 household dogs were sampled, out of which 17(10.3%) tested positive as against 77 stray dogs sampled where 19(24.7) tested positive (Table 3). The various breeds of dogs sampled had varying prevalence. The highest represented was the Alsatian where 27 dogs were sampled with 11(40.7) testing positive. The mongrel which is a term referring to the indigenous breed in Nigeria (based on genetics) was the second in number of isolates with 124 dogs sampled and 15(12.1%) turned out positive. Other breeds encountered were Rottweiler with 91 samples collected out of which 8(8.8%) were positive and, Caucasian with 23 samples collected and 2(8.7%) turned out positive (Table 4).

Table 1. Overall sero-prevalence and relative prevalence based on sex

| Sex | No of samples | Number positive | Prevalence | χ^2 | p-value |
|--------|---------------|-----------------|------------|----------|---------|
| Male | 154 | 24 | 15.6 | 0.8161 | 0.1832 |
| Female | 88 | 12 | 13.6 | | |
| Total | 242 | 36 | 14.9 | | |

Table 2. Relative Sero-prevalence based on age groups

| Age | No of samples | Number positive | Prevalence (%) | χ^2 | p-value |
|--------------|---------------|-----------------|----------------|----------|---------|
| Puppies | 27 | 7 | 25.9 | 1.781 | 0.4104 |
| Young adults | 91 | 10 | 10.9 | | |
| Adult | 124 | 19 | 15.3 | | |
| Total | 242 | 36 | 14.9 | | |

Key: Puppy= day old to less than 6 months; young adults: 6 months to less than 1 year; adults: 1 year and above.

Table 3. Relative sero-prevalence based on dog keeping

| Dog type | Number of samples | Number of positive | Prevalence | χ^2 | p-value |
|-----------|-------------------|--------------------|------------|----------|---------|
| Household | 165 | 17 | 10.3 | 2.1640 | 0.0706 |
| Stray | 77 | 19 | 24.7 | | |
| Total | 242 | 36 | 14.9 | | |

Table 4. Relative sero-prevalence based on different breeds sampled

| Age | No of samples | Number of positive | Prevalence (%) | χ^2 | p-value |
|------------|---------------|--------------------|----------------|----------|---------|
| Alsatian | 27 | 11 | 40.7 | 1.253 | 0.2731 |
| Rottweiler | 91 | 8 | 8.8 | | |
| Mongrel | 124 | 15 | 12.1 | | |
| Caucasian | 23 | 2 | 8.7 | | |
| Total | 242 | 36 | 14.9 | | |

DISCUSSION

An overall sero-prevalence of 14.9% was obtained. A total of 36 dog sera tested positive out of 242 samples used for the study. The overall prevalence obtained is lower than a prevalence of 21.5% reported by (13) in a study conducted in Zaria and 32.3% reported (8) in a sero-survey using RBPT in north-central Nigeria. The finding from this study is higher than the prevalence of 5.46% reported by (14) from a study carried out in Ibadan, southwestern Nigeria. An area with different geographical as well as socioeconomic and cultural factors which determine outcome and progress and perpetuation of disease. Relatively, studies on canine brucellosis is scarce in northwestern Nigeria where this

research was undertaken, this underscores the significance of this sero-screening amongst dogs often used for breeding as well. To the best of knowledge, this study is the first comprehensive spatial epidemiological survey for canine brucellosis in Sokoto State Nigeria, a region strategically important in the control of diseases in Nigeria because of its borders with Niger-Republic with multiple animal trades and smuggle routes between the borderline communities. Screening is a veritable tool to raise the stakes on adequate surveillance for brucellosis amongst pets, minimize zoonotic transmissions to humans and reduce mortalities in litters in especially breeder kennels. From years of observation, priority has not been given to the screening of brucellosis in the study region probably for paucity of research to warrant data-driven health policies to prioritize the disease. Routine vaccination is not practiced at the moment in Nigeria. There is no central Federal Government of Nigeria (FGN) enforceable policy on brucellosis vaccination or screening of imported pets. Breeder bitch (es) are often not screened by a veterinarian and cases are only presented to the veterinary clinic only at terminal stage with poor prognosis.

Brucella abortus and *Brucella canis* are two most common isolates often reported in dogs, the former causes septicemia and consequential abortions in litters and source of socio-economic disturbances. While this study reported *B. abortus* antibodies because of the antigens used, numerous other studies have reported *B. canis* infections and fulminant diseases in canine out-patients and field cases in cross sectional studies respectively. (15) reported none positive for *B. abortus* while 34 (27.7%) were positive for *B. canis* out of 123 dogs sampled in southeastern Nigeria. Patterns seem to associate *B. abortus* in cases with abortions in litters, which is often a clinical case rather than asymptomatic screening in field or preceding breeding.

A higher prevalence was reported from this study in males (15.6%) than females (13.6%) (Table 1). This could be due to an increased activity in male dogs than females as hinted by the findings of (16) where he obtained a higher sero-prevalence of 13.4% in males than females. This however, is at variance with the works of (14) and (6) that reported higher sero-prevalence in female dogs. However, no statistical significant association was found between the sexes. Male preponderance in number of isolates also contrasts the reports of (15) where there was statistically significant association of infection of *Brucella* with females relative to the males. A plausible reason may be the frequent use of females for breeding in the south-eastern part of Nigeria as compared to northern Nigeria coupled with higher population of female dogs kept in the east relative to males for breeding purposes. Female dogs are highly predisposed as vulnerable groups because they are often mated with multiple males to increase chances of conception, unscreened breeder males are sources of *Brucella* for pregnant bitches. A high population of both males and females dogs are asymptomatic carriers of *Brucella* in both north and southern Nigeria. The disease is therefore endemic with cases becoming fulminant and detectable when challenged with immune-suppressive and concurrent conditions. A higher prevalence was reported in adult dogs (15.3%) than in young adults (10.9%) and puppies recorded the highest prevalence of (25.9%) (Table 2). The findings obtained from this study are similar in part to that obtained by (14) where a high prevalence was obtained in adult dogs. However, the highest prevalence reported in the puppy

category in the present study contrast the report of (13) that stated dogs of the sexually active group (1-5 years) are more predisposed to *Brucella* infection than other age groups. The high prevalence recorded in our study may be related to zero policy and practice of *Brucella* vaccination in Nigeria. Puppies likely contract the infection in early days of birth where they make contacts with fomites and various membranes. The lack of passive immunity from ingested colostrum in non-vaccinated bitches may account for natural exposure to *Brucella* evinced by detectable antibodies in puppies. It is important the Nigeria government enact an enforceable policy of compulsory vaccination of dogs against *Brucella abortus* and *B. canis* especially, similar to Rabies and Leptospirosis. The stakes are high given the zoonotic, re-emerging and economically debilitating nature of *Brucella* pathogen. A higher prevalence was also reported in stray dogs (24.7%) than household dogs (10.3%). This could be attributed to the fact that stray dogs come in contact with cattle, lead scavenging habits and maintain regular presence in abattoir which puts stray dogs at greater risk as they are likely to come in contact with contaminated membranes. Straying in dogs is very common in developing countries, where legislation that protects pets and ensures their welfare may be absent or rarely enforced (17). Stray dogs that roam free in urban and suburban areas are especially important in the transmission of diseases (18). In Nigerian cities, in both urban and rural areas, there are abundant, and largely unquantified populations of owned and stray dogs (19). The high prevalence in stray dogs is unsurprising compared to domesticated dogs for plausible reasons of the absence of care. The stray dog category represents a public health threat to pets and humans because they will continue shedding *Brucella* as asymptomatic carriers. Some of the stray dogs sampled were found in abattoirs where *Brucella* could be carried up the food chain to humans. Breed association or correlation to *Brucella* infection in canine does not present a consistent pattern for making conclusions. Studies on canine brucellosis, albeit few in Nigeria report varying prevalence with no specific breed being predisposed relative to others. Infection will depend on which breed is predominant in number in the region of studies. In a comprehensive *Brucella* survey (15) reported 36.8% in Rottweiler, 17.1% in Alsatian and 17.1% in Caucasian breeds in Southeastern Nigeria compared to our study with 40.7% positive in Alsatian, 8.8% positive in Rottweiler and 12.1% in Mongrel (Nigerian indigenous dog). It is plausible in our study, the high infection rate amongst the Alsatian group is connected to their preference for use in breeding in Sokoto State northwestern Nigeria. Different regions differ on which breed is predominantly used amongst local breeders. The high exposure of the Alsatian group makes then likely to harbor *Brucella* either as symptomatic or asymptomatic carriers. The findings from this research underscore the need to pay attention to canine breeders and preferred breeds. Our study is significant as the first comprehensive canine survey for brucellosis in the State. It is likely the data generated will compel health administrators to once again open the chapters of the risks posed by brucellosis to both humans and animals.

Conclusion

The findings from this study provide evidence of *Brucella* infection in dogs in Sokoto, northwestern Nigeria. An overall sero-prevalence of 14.9% was recorded with varying relative prevalence in analyzed variables as potential risk factors for the infection. The finding of this research is significant as it

potentially affects public health with the close contacts of dogs to humans in the study region.

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