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Sero-Epidemiology and Risk Factors of *Brucella abortus* in Dogs in North Central Nigeria

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ABSTRACT

Background and Objective: In veterinary hospitals in Nigeria's North Central Region, serological testing for brucellosis is Not a Common Practice (NCN). As a result, this study was carried out to find out how common brucellosis is in dogs in NCN. **Materials and Methods:** A total of 550 sera samples were collected from dogs presented to the veterinary clinics, dog kennels and dog breeder homes. The samples were analyzed using the Rose Bengal Plate Test (RBPT) which gave a prevalence of 239 (43.46%). **Results:** The result indicated the highest seroprevalence of 125 (56.82%) by RBPT for dogs 12 months old and above. This was followed by dogs aged 6-11 months with a rate of 79 (41.58%). The lowest seroprevalence 35 (25.00%) was recorded amongst dogs aged 0-6 months. Sex distribution of *Brucella abortus* among dogs in NCN indicated that female dogs had the highest seroprevalence of 185 (44.58%), while male dogs had the least percentage rate of 54 (40.00%). The seasonal distribution of *Brucella abortus* among dogs in NCN indicated that the highest seroprevalence of 165 (68.75%) was recorded in the wet season. The least percentage prevalence rates of 74 (23.87%) were recorded in the dry season. The result indicated the highest seroprevalence of 142 (59.66%) for local breeds of dogs. Mix-bred dogs had the least rate of 60 (26%). Exotic breeds of dogs however had a seroprevalence of 37 (43.53%). Based on management systems, the highest seroprevalence of 190 (53.37%) was recorded in dogs reared extensively and the least rates of 49 (25.26%) were recorded in dogs reared intensively. **Conclusion:** A cost-effective federal government policy on the control and eradication of this important zoonotic disease is indicated.

KEYWORDS

Sero-epidemiology, dogs, brucellosis, risk factors, zoonosis, veterinary clinics, questionnaire

INTRODUCTION

Brucellosis has been defined as a disease condition caused by members of the genus *Brucella*. Brucella is the etiological pathogen of brucellosis[1]. According to host preferences, Brucella is divided into six classical species, namely: *Brucella melitensis* affecting goats and sheep, *Brucella suis* in pigs,

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Brucella abortus in cattle, *Brucella ovis* in sheep, *Brucella canis* in dogs and *Brucella neotomae* in wild rats. Dogs can be infected by four of the six species of Brucella namely: *Brucella melitensis*, *B. abortus*, *B. suis* and *B. canis* excluding *Brucella ovis* and *Brucella neotomae*.

Brucellosis first came to the attention of British medical officers in 1805 during the Crimean War[2] and a causal relationship between the organism and disease was first established in 1887 by Dr. David Bruce. The first case of brucellosis in dogs caused by *Brucella suis* was published in 1931[2] and since then, it has been reported in many other countries including Nigeria[3].

Dogs are the most successful canids adapted to humans' habitation worldwide and they have contributed to the physical, social and emotional well-being of their owners[4,5]. Dogs are important household pets kept for security, hunting and leading of the blind and as a source of meat; therefore there is increased interest in keeping dogs[6,7]. The population of dogs in Nigeria has been variously estimated to be between three and five million. In Nigeria, roaming and scavenging as well as uncontrolled importation of dogs are some of the factors that favour the occurrence of diseases such as canine brucellosis among dog populations[8].

Due to a lack of pre-movement screening and an increase in the density of possibly infected foreign breeds of dogs, the introduction of dog breeding in Nigeria has contributed to the re-emergence of brucellosis as an international problem for both indigenous and foreign breeds of dogs[9]. The disease has significant financial, veterinary and public health implications[10,11]. It is one of the most economically significant diseases in pets, causing miscarriage, sterility and reduced output. In North Central Nigeria, information on canine brucellosis is scarce and in most cases unavailable. Hence, the need to carry out the study is aimed at investigating the seroepidemiology and risk factors of brucellosis in dogs in North Central Nigeria. It is hoped that the findings of this study will provide useful information on the disease in North Central Nigeria.

MATERIALS AND METHODS

Study area: This study was done between February, 2017 and November, 2019. The study area was North Central Nigeria. The study was carried out in three states, Plateau, Nassarawa, Kogi States and the FCT. Plateau State has an area of 30, 913 km^2 and a population of 3, 206, 531 people. It is located between latitude 08°24'N and longitude 008°32' and 10°38'E. Nasarawa State is located between latitude 7°45'N and longitude 9°25'E. It has a population of about 1,869,377 people according to the 2006 population estimate. Kogi state is also in the central region of Nigeria. It has a population of 3,314,043 people at the 2006 census (HASC, Population: npc.population.gov.ng 2017). Abuja is located in the centre of the country. It is located at latitudes 8°50'W and 7°10'E. The 2006 census estimated its population as 776,298.

Sampling: A cross-sectional epidemiological study was used in the study. Simple random sampling by balloting was used to select three states and the FCT from the study area namely: Plateau, Nassarawa and Kogi States. Veterinary clinics/Kennels/breeder houses were listed and eight (4 from clinics, 2 from kennels and 2 from breeder houses) were selected using purposive sampling techniques. Dogs presented to the veterinary clinic were sampled using a systematic method chronologically until the sample size of 550 per season was attained. Scheduled visits were made to the purposively selected veterinary clinics and kennels at two weeks intervals (bi-weekly) throughout the study.

Using a sterile hypodermic needle and a 10 mL syringe, 5 mL of venous blood was aseptically collected from the cephalic vein into a clean and well-labelled sample vial devoid of the anticoagulant. The blood samples were allowed to coagulate for an hour by slanting the sample bottles and the sera were collected

by decantation and centrifugation (where decantation did not provide serum) into new, well-labelled sample bottles with sample numbers corresponding to serial numbers on the questionnaire. Sera samples were then transported to the Bacterial Zoonoses Laboratory of the National Veterinary Research Institute, Vom, in a Coleman box with ice packs for laboratory analysis.

Serology: The *Brucella abortus* antigen for the Rose Bengal Plate Test (RBPT) was obtained from Animal Health Veterinary Laboratory Agency, United Kingdom. The RBPT procedure was performed as described by Mac Millan.

Questionnaire survey: A questionnaire was used to determine the risk factors for dogs. Information on the dog's age, breed, sex, location and obstetrical history was obtained using a structured questionnaire. Information on the owner's occupation, literacy level and level of awareness of the disease was also obtained using a close-ended, structured questionnaire pre-tested for validity. The study was clearly explained to the client/dog handler/breeder/butcher/veterinary personnel and informed consent was obtained before administering the questionnaire.

Statistical analysis: Data obtained from the study was statistically analysed using Statistical Package for the Social Sciences (SPSS) Version 21, Chi-square and Fischers' Exact Tests were used to determine the seroprevalence and test for association between the presence of Brucella antibody and the various variables such as age, breed, sex, season, management and location, respectively.

RESULTS

The 550 serum samples were subjected to RBPT which gave a prevalence of 239 (43.46%) for canine brucellosis in the North Central part of Nigeria (NCN).

Seroprevalence increased with age as dogs above 12 months had the highest rates (56.82%).

Dogs aged 0-6 months had the lowest prevalence rates of 35 (25.00%). This was presented in Table 1.

The sex distribution of canine brucellosis among dogs in NCN showed that female dogs had the highest seroprevalence of 185 (44.58%) while male dogs had the least percentage prevalence rates of 54 (40.00%) as shown in Table 2. The seasonal distribution of canine *Brucella* spp., among dogs in NCN indicated that the highest seroprevalence of 165 (60.00%) was recorded in the wet season and the least percentage prevalence rate of 74 (26.91%) was recorded in the dry season as shown by Table 3.

The prevalence of canine brucellosis in NCN based on breeds was shown in Table 4. Local breeds of dogs had the highest prevalence of 142 (59.66%) while cross-bred dogs had the least prevalence of 60 (26.67%). Exotic breeds of dogs however had prevalence rates of 37 (43.53%). The prevalence of canine brucellosis in NCN based on management systems was shown in Table 5. The highest seroprevalence of 190 (53.37%) was recorded in dogs reared extensively and the least percentage prevalence rate of 49 (25.26%) was recorded in dogs reared intensively.

The prevalence of canine brucellosis in NCN based on location was mentioned in Table 6. The highest percentage seroprevalence rate of 100 (47.62%) was recorded in Plateau State, followed by Nassarawa 71 (41.77%), FCT 46 (38.33%) and the least 22 (22.00%) seroprevalence rate was recorded in Kogi State.

Table 1: Sero-epidemiology of *Brucella abortus* in dogs based on age distribution in North Central Nigeria by RBPT

Age groups (months)	Total number of dogs examined	Total number of dogs positive	Prevalence (%)	95% CI	χ^2	p-value
0<6	140	35	25.00	18.1-33.0		
6<12	190	79	41.58	34.5-49.0	35.666	p<0.001
≥12	220	125	56.82	49.0-63.5		
Total	550	239	43.46			

Table 2: Sero-epidemiology of *Brucella abortus* in dogs based on sex distribution in North Central Nigeria using RBPT

Sex	Total number of dogs examined	Total number of dogs positive	Prevalence (%)	95% CI	OR	χ^2	p-value
Male	135	54	40.00	31.7–48.8	1.2	0.869	0.203
Female	415	185	44.58	39.7–49.5			
Total	550	239	43.46				

Table 3: Sero-epidemiology of *Brucella abortus* in dogs based on the seasonal distribution in North Central Nigeria using RBPT

Seasonal distribution	Total number of dogs examined	Total number of dogs positive	Prevalence (%)	95% CI	OR	χ^2	p-value
Dry season	310	74	23.87	31.7-48.8	0.82	0.869	p<0.001
Wet season	240	165	68.75	39.7-79.5			
Total	550	239	43.46				

Table 4: Sero-epidemiology of *Brucella abortus* in dogs based on breed distribution in North Central Nigeria using RBPT

Breed	Total number of dogs examined	Total number of dogs positive	Prevalence (%)	95% CI	χ^2	p-value
Local	238	142	59.66	53.1-66.0	136.171	
Mix	225	60	26.67	21.0-33.0		p<0.001
Exotic	87	37	42.53	32.8-54.7		
Total	550	239	43.46			

Table 5: Sero-epidemiology of *Brucella abortus* in dogs based on management system in North Central Nigeria using RBPT

Management system	Total number of dogs examined	Total number of dogs positive	Prevalence (%)	95% CI	OR	χ^2	p-value
Housed	194	49	25.26	19.3–32.0	3.4	40.390	p<0.001
Stray	356	190	53.37	48.0–58.7			
Total	550	239	43.46				

Table 6: Sero-epidemiology of *Brucella abortus* in dogs based on location in North Central Nigeria using RBPT

Location	Total number of dogs examined	Total number of dogs positive	Prevalence (%)	95% CI	χ^2	p-value
Plateau State	210	100	47.62	75.4–89.5		
Nasarawa State	160	71	41.77	34.3–49.6	18.958	p<0.001
Kogi State	100	39	39.00	24.3–41.4		
FCT Abuja	80	29	38.33	29.6–47.6		
Total	550	239	43.46			

DISCUSSION

Brucellosis is a widespread and economically important infectious disease of animals and humans caused by various species of the Gram-negative bacteria Brucella. Although the Brucella bacterium was discovered over a century ago, control of the disease remains a major challenge in many areas worldwide. Prevalence in dogs was relatively high, with *B. abortus* causing the majority of human infections for which there are limited data on disease incidence.

The study showed that the overall seroprevalence of *Brucella* spp., in North Central Nigeria is 43.46% using the RBPT. The percentage seroprevalence of *Brucella abortus* was much higher than that recorded by Momoh *et al.*[12] in Plateau State which showed a seroprevalence of 29.2% for Brucella spp using RBPT and C-ELISA. This finding is also higher than that recorded by Osinubi *et al.*[13] in VTH Zaria, Nigeria, where a seroprevalence of 21.5% was obtained. Comfort *et al.*[14] reported a seroprevalence of 12.72% using RBPT in Ibadan, Nigeria.

This report however, is in contrast to that reported by Anyaoha *et al.*[3], where zero prevalence was recorded in seroprevalence studies carried out in Enugu and Anambra States.

From the study, the seroprevalence among the dogs shows that seropositivity increased with age. This result is statistically significant ($p \leq 0.001$). The highest seroprevalence of 56.82% and a prevalence of 3.2% are recorded in dogs 12 months and above using RBPT while the lowest 25.00 and 0.00% are recorded in dogs 0-6 months old. This report is in agreement with that carried out by Comfort *et al.*[14], where the highest prevalence of 9.8% was recorded in adult dogs. It is also in agreement with the works of Momoh *et al.*[12], where a prevalence of 30.3% was recorded in 12 months old dogs and above using RBPT. Susceptibility of the disease may also be more commonly associated with sexual maturity as different studies reported that older animals are more susceptible than younger animals[15]. Sex-based prevalence of canine brucellosis showed a higher prevalence rate in female dogs (44.58%) when compared to male dogs (40.00%) using RBPT. There was however no significant association between *B. abortus* infection and sex ($p > 0.01$). Cadmus *et al.*[16] also recorded a higher prevalence rate in female dogs (6.17%) compared to male dogs (5.1%). This is also in agreement with a survey carried out by Comfort *et al.*[14], where a statistically significant higher prevalence of 5.6% was recorded in female dogs compared to male dogs (2.3%). A contributing factor to higher prevalence rates in females could be that a single male dog if infected and used to mate with several females can transmit the infection via infected semen[16].

The result indicated that the highest percentage of the seasonal distribution prevalences were recorded in the wet season (44.58%) using RBPT when compared to the dry season (40.00%) using RBPT. This novel finding, though statistically insignificant ($p \geq 0.01$), could be because mating occurs at significantly higher rates at high precipitation (rainfall) levels only[17]. This is so because rains increase the efficiency of dogs mating by increasing odour intensity. This odour intensity is higher during the rainy season when there is high bacterial growth and decay to produce the odour. This odour rises upwards in cooler air[18] to trigger olfactory sensitivity threshold levels[19]. Also reduced temperature and increased humidity conditions ensure a higher concentration of sex pheromones in the air which also triggers high mating behaviours.

The distribution of *B. abortus* among the breeds showed a higher prevalence rate of 142 (59.66%) recorded in local breeds of the dog when compared to mixed breeds 60 (26.67%) and exotic breeds of dog 37 (42.53%) using the RBPT. Local dogs also recorded a higher prevalence rate of 4 (2.82%) when compared to mix breeds 1 (1.67%) and exotic breeds of dog 1 (2.70%). This result is statistically significant in RBPT ($p \leq 0.01$). This result is in agreement with that recorded by Comfort *et al.*[14], where it was stated that breed-specific prevalence was thirty times more likely to infect mongrels than Alsatians. This assertion was also buttressed by that recorded by Cadmus *et al.*[16], who also recorded higher prevalence rates in local breeds of dogs. These results were however in contrast to that of Osinubi *et al.*[13], where the highest seroprevalence was recorded in exotic breeds of dogs with prevalence rates of 7.9 and 55%, respectively. It is noteworthy however that all canine breeds are equally susceptible to brucellosis[20].

The prevalence rate is also higher in extensively managed dogs (53.37%) when compared to intensively managed dogs (25.26%). This result is statistically significant in RBPT ($p \leq 0.01$). The higher prevalence rate of dogs managed extensively could be because they roam about freely and are at risk of picking up food materials contaminated with the organism like aborted fetal materials and also being at risk of being mated by any dog (male) or mating any dog (female) that may be infected with brucellosis. This finding is comparable to the report of Godfroid *et al.*[10], where they stated that ingestion of tissues, foodstuff or fluid containing the organism (brucellae) is a major route of the disease transmission.

As observed, dogs in Plateau State had higher seroprevalences and prevalence rates (47.62%) when compared to dogs in Nassarawa (44.38%), Abuja (38.33%) and Kogi State (39.00%). This infers that the majority of the dogs screened in Plateau might be infected with *B. abortus*. Plateau State has a large population of dogs and dog breeders due to its cultural acceptance as food animals as well as good weather condition for keeping exotic breeds of dogs[21]. A lot of individuals in Jos breed dogs as it is a lucrative business to improve their income. The higher prevalence of brucellosis in dogs from Plateau could be due to the more common practice of feeding dogs with fetal waste or raw meat as reported by Momoh *et al*.[12] Seropositivity to Brucella infection among dogs sampled was associated with sample location, a finding similar to a previous report by Cadmus *et al*.[16]. A cost-effective federal government policy on the control and eradication of this important zoonotic disease is indicated.

CONCLUSION

This study has shown that canine brucellosis is prevalent in NCN and this is of economic and public health significance. This report provides current information on the prevalence and risk factors of brucellosis amongst dogs in NCN. The present study represents the first application of seasonal variation in Brucella-infected dogs. In this dissertation a holistic approach to the study of brucellosis in dogs and risk factors in humans in NCN, an endemic disease area, the range of disease presentations occurring in the field were appreciated, public health dilemmas understood and control realized. The present study is the first to report the prevalence of canine brucellosis in NCN and is therefore novel.

SIGNIFICANCE STATEMENT

This study represents the first application of seasonal variation in Brucella-infected dogs. In this dissertation a holistic approach to the study of brucellosis in dogs and risk factors in humans in NCN, an endemic disease area, the range of disease presentations occurring in the field were appreciated, public health dilemmas understood and control realized.

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