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Seroprevalence of Brucellosis in Nigerian Breed of Dog in North Bank Area of Makurdi, Benue State Nigeria

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ABSTRACT

The study was designed to evaluate the seroprevalence of brucellosis in Nigerian breeds of dog in North bank area of Makurdi, Benue State Nigeria. Serum samples from one hundred and two (102) dogs were used for serum agglutination and Rose Bengal test. Questioners were used to obtained information on the various management systems being practiced. The results showed that two of the dogs a male and a female were positive for brucellosis indicating a prevalence of 1.96%. In conclusion, brucellosis in dogs remains endemic in many parts of the world and without stronger intervention measures it might remain an unrecognized threat to human health and animal welfare.

Keywords: Seroprevalence, Brucellosis, Serum, Dogs

INTRODUCTION

Brucella canis is a gram-negative coccobacillary bacterium that primarily causes reproductive failure in dogs [1]. The genus Brucella comprises of 12 recognized species [2]. Of these, *B. melintensis*, *B. abortus* and *B. suis* are well known causes of undulant fever and influenza like symptoms in humans. It is worth noting that *B. canis* is not widely accepted as being zoonotic [3].

B. canis was first characterized in 1966 after several outbreaks of abortion and infertility in dogs in different countries [1]. Since the discovery of *B. canis* as a cause of abortion, outbreaks in breeding and research Kennels have been sporadically reported worldwide [4,5]. The primary hosts are domesticated dogs; however, *B. canis* in wild canids and human has also been reported [6,7]. Brucellosis in dogs occurs worldwide and is endemic to the Americas, Asia and Africa [8]. In the past two decades, serological studies involving dogs have been published from countries in Africa, Asia and South America; these have reported moderate to high seroprevalence, ranging from 6-35% (online Technical Appendix). This worldwide range of seroprevalence, could be attributed to multiple factors, but not limited to true disease prevalence in the region or country, sampling design, study samples and diagnostic test used. *B. canis* infection in dogs occurs predominantly through ingestion, inhalation and contact with aborted foetuses, placenta, vaginal secretion and semen [9,10]. Like the rest of the Brucella species, *B. canis* exhibits tropism for reproductive tissue. Thus, infected dogs intermittently shed low concentration of bacteria in seminal fluids and estrus vaginal secretion. Post abortion vaginal fluids contain a high level of bacteria and are a source of infection for other dogs and humans [9]. Even after castration, male dogs may still serve as a source of infection as the bacteria can persist in the prostate and lymphoid tissues [10,11]. In addition to reproductive secretions, dogs can shed the bacteria in saliva, nasal secretion and urine [12,13]. Studies have suggested that the concentration of *B. canis* in urine is higher in male than female dogs; this difference is attributed to urine contamination with seminal fluid [9]. Humans acquire *B. canis* infection through direct contact with infected dogs or their reproductive waste, secretions or blood products [14,15]. Clinical signs and symptoms include undulant fever, chills, malaise and splenomegaly [16].

The public health relevance of *B. canis* infection in human is unclear because most of the information available comes from case reports. The perceived infrequency of human infection with *B. canis* and lack of reliable diagnostic tools of the disease detection has led to few serologic surveys in

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humans. Our current understanding of prevalence of B. *canis* infection in humans comes from handful information from serological surveys that used diagnostic test available for dogs and thus, may not be true representation of the true state of this infection in humans [3,17-19]. The objective of this study therefore, was to determine the seroprevalence of brucellosis in Nigerian breeds of dog in the North bank area of Makurdi Benue State Nigeria.

MATERIALS AND METHODS

The study was conducted during the raining season (October, 2018), in the North bank area of Makurdi Benue State Nigeria. Makurdi lies approximately on Latitude 7° 44' N and longitude 8° 4' E, in the southern Guinea Savannah zone of Nigeria, has a temperature range of 22.5-40°C and annual rain fall of 1,290 mm [20].

Research animals

A free anti-rabies vaccination campaign was organized in three strategic locations at an earlier date to cover the North bank area. The dogs brought were one hundred and two dogs comprising of 51 females and 51 males all between ages 1 year to 10 years; these were the dogs used for this research.

Samples collection

3 ml of blood was collected through the jugular vein using a 5 ml syringe with a 21 gauge needle into plain sample bottle and spun at 300 rpm to harvest serum on the field. The serum samples were then kept on ice packs and transported to the laboratory to be stored at -20°C until used. Also, information on the type of feed and system of management used for each dog was obtained by use of questioners.

Serological test

Serum samples were tested for Brucella antibodies using the Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) as described by Time and Tor [21]. The antigens for the two tests were procured from Veterinary Laboratory Agency, United Kingdom.

Rose Bengal plate test (RBPT)

30 µL of antigen was placed on a white ceramic tile and same volume (30 µL) of test serum sample was placed beside the antigen. The sera and the antigen were mixed with an applicator stick and rocked gently for 4 min after which it was observed for agglutination. The formation of distinct pink granules (agglutination) was recorded as positive while the absence of agglutination was reported as negative. Known positive and negative controls were set up along with the test sera.

Serum agglutination test (SAT)

The British method in which five test tubes were required per sample was used. For the first test tube, 0.8 ml of phenol saline was dispensed while 0.5 ml was applied to the second, third, fourth and fifth test tube using micro titer pipette fitted with corresponding tips. Similarly, 0.2 ml of the test sera was added to the first tube and mixed properly. Serial dilution was then carried out by pipetting 0.5 ml of mixture into the first; second, third, fourth and fifth test tubes, respectively. The final 0.5 ml of antigen (diluted 1:10 with phenol saline) was added to all the tubes. The tubes were covered, shaken and incubated at 37°C for 20 h. The result was then read and agglutination titer determined. Titer of 1:40 (50 IU/ml) and above was taken as diagnostic for brucellosis [22,23]. Known positive and negative control sera were set up along with the test sera.

RESULTS

The result of this research showed that out of the 102 dogs screened for brucellosis two dogs were positive **(Table 1)** male and female. The prevalence of brucellosis in both sexes was 1.96% each with total prevalence of 1.96% **(Table 2)**. Among the positive dogs, one of them feeds on abattoir waste and left over home food while the other feeds on only left over home food **(Table 3)**.

Table 1. Serological results.

S/N	Sex	Results
1	Female	Negative
2	Female	Negative
3	Female	Negative
4	Female	Negative
5	Female	Negative
6	Female	Negative
7	Female	Negative
8	Female	Negative

9	Female	Negative
10	Female	Negative
11	Female	Negative
12	Female	Negative
13	Female	Negative
14	Female	Negative
15	Female	Negative
16	Female	Negative
17	Female	Negative
18	Female	Negative
19	Female	Negative
20	Female	Negative
21	Female	Negative
22	Female	Negative
23	Female	Negative
24	Female	Negative
25	Female	Negative
26	Female	Negative
27	Female	Negative
28	Female	Negative
29	Female	Negative
30	Female	Negative
31	Female	Negative
32	Female	Negative
33	Female	Negative
34	Female	Negative
35	Female	Negative
36	Female	Negative
37	Female	Negative
38	Female	Negative
39	Female	Negative
40	Female	Negative
41	Female	Negative
42	Female	Positive
43	Female	Negative

44	Female	Negative
45	Female	Negative
46	Female	Negative
47	Female	Negative
48	Female	Negative
49	Female	Negative
50	Female	Negative
51	Female	Negative
52	Male	Negative
53	Male	Negative
54	Male	Negative
55	Male	Negative
56	Male	Negative
57	Male	Negative
58	Male	Negative
59	Male	Negative
60	Male	Negative
61	Male	Negative
62	Male	Negative
63	Male	Negative
64	Male	Negative
65	Male	Negative
66	Male	Negative
67	Male	Positive
68	Male	Negative
69	Male	Negative
70	Male	Negative
71	Male	Negative
72	Male	Negative
73	Male	Negative
74	Male	Negative
75	Male	Negative
76	Male	Negative
77	Male	Negative
78	Male	Negative

79	Male	Negative
80	Male	Negative
81	Male	Negative
82	Male	Negative
83	Male	Negative
84	Male	Negative
85	Male	Negative
86	Male	Negative
87	Male	Negative
88	Male	Negative
89	Male	Negative
90	Male	Negative
91	Male	Negative
92	Male	Negative
93	Male	Negative
94	Male	Negative
95	Male	Negative
96	Male	Negative
97	Male	Negative
98	Male	Negative
99	Male	Negative
100	Male	Negative
101	Male	Negative
102	Male	Negative
	Positive Control	Positive
	Negative Control	Negative

Summary: Out of 102 samples tested two (No. 42 and 67) were positive for Brucella antibodies by Rose Bengal Plates Test and Serum Agglutination Test

Table 2. Prevalence of Brucellosis according to sex.

Sex	**Male**	**Female**	**Total**
Prevalence (%)	0.98	0.98	1.96

Table 3. Prevalence of Brucellosis according to source of feed.

Source of feed	**Abattoir waste**	**Left over home feed**
Prevalence (%)	0.98	0.98

DISCUSSION

The prevalence of brucellosis in dogs from this study was 1.96%, which is below the findings of the serologic studies of brucellosis in dogs from Africa, Asia and South America which have reported moderate to high prevalence of 6-25% (online Technical report Appendix). This wide range of seroprevalence has been reported to be due to multiple factors such as sampling design, study samples and diagnostic test used but not the true disease prevalence in the region or country.

The positive samples were from the dogs that were fed on abattoir waste and home left over food; both dogs were also allowed to roam about. Flores-Castro and Segura [24] and Brown et al. [25] reported that when compared with owned dogs stray dogs are more likely to be intact and have a higher documented level of *B. canis* seropositivity. A higher burden of canine brucellosis in stray/roaming dog populations could lead to a spill over into human population in areas with a large number of stray dogs since these dogs are usually taken into shelters or placed in foster home pending adoption [26].

Considering the nature of the disease, the potential source of *B. canis* dissemination is breeding kennels where animals are housed in close contact and constantly moved from one breeding point to the other or point of sale [25]. Unrestricted movement of reproductively intact dogs or puppies is also known to be a risk factor for the spread of infectious diseases and has led to incidences of human infection with *B. canis* [25,26]. Quarantine periods and pre-movement health test of dogs vary from region, but no region is known to test dogs for brucellosis before there are moved. Testing of breeding animals or their offspring before interstate or international movement would decrease the risk of *B. canis* transmission between dogs and humans [27].

CONCLUSION

In conclusion, brucellosis in dogs remains endemic to many part of the world and without stronger intervention measures, it will probably remain an unrecognized threat to human health and animal welfare. Implementation of mandatory testing before interstate or international movement of dogs will be a step in the right direction in containing the disease.

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