SURVEY FOR *BRUCELLA* ANTIBODIES IN DOGS IN BILLIRI LOCAL GOVERNMENT AREA OF GOMBE STATE, NIGERIA.

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Abstract: **Brucellosis is an infectious disease that remains endemic in many parts of the world including Nigeria. To determine the sero-prevalence of Brucella antibodies in dogs in Billiri Local Government Area (LGA) of Gombe State, Nigeria, 347 dog sera were screened using Rose Bengal Plate Test (RBPT) and competitive Enzyme Linked Immuno Sorbent Assay (c-ELISA). The sero-prevalence of Brucella antibodies was higher with RBPT (21.90%) than c-ELISA (14.70%). Location-based sero-prevalence of Brucella antibodies revealed highest prevalence rate of 26% in Kalmai Ward, while Bare Ward had the lowest prevalence rate of 4.26%. The seroprevalence of Brucella antibodies increased with age of the dogs with 18.14% in adults and 7.27% in puppies. Sex-based sero-prevalence revealed 18.35% in males and 8.53% in females. Exotic breed of dogs had the highest sero-prevalence of 19.05% compared to 15.26% and 0.00% in the Local and Cross Breeds respectively. Sick dogs had 18.95% prevalence compared to 13.04% in the apparently healthy ones. There was a statistically significant association between the sero-prevalence of Brucella antibodies and age, sex of the dogs ($p<0.05$) while there was no statistically significant association between the sero-prevalence of Brucella antibodies and breed, health status of the dogs ($P>0.05$).**

Keywords: Brucellosis, Dogs, Billiri LGA of Gombe State, Nigeria.

INTRODUCTION

Brucellosis is an infectious disease caused by members of the genus *Brucella*. Traditionally bovines are host to *Brucella abortus*, Caprine and Ovine to *Brucella melitensis*, *andBrucella canis* is associated with canine brucellosis. Brucellosis as a zoonosis poses serious human health hazards worldwide [12; 20]. While some countries have eliminated or substantially reduced the disease by extensive eradication programmes, it remains endemic in many areas of the world, including Nigeria [5; 28]. The economic burden associated with brucellosis is substantial, mainly the result of abortion or infertility, and the costs of attaining and maintaining a disease-free status. Various serological studies have documented the prevalence of brucellosis in livestock in Nigeria, with rates falling between 0.2% and 79.7% [5; 10; 14]. The same cannot be said of dogs, because of limited studies in the country. However, the isolation of *B. canis* from infected dogs has been reported [26] and serological reactions to *B. abortus* and *B. canis* documented [1; 27].

In dogs, *Brucella* primarily enters the body by ingestion and through the genital, oronasal and conjunctival mucosa, but transmission through broken skin may also be possible [13]. Most cases are thought to be acquired by venereal transmission or by contact with the fetus and fetal membranes after abortions and stillbirths. Puppies can be infected *in utero*, and may remain persistently infected even

if they appear normal. Nursing puppies can be infected from milk, but the importance of this route is controversial. Other potential sources of infection include blood transfusions and contaminated syringes [7].

Humans usually become infected with members of the genus *Brucella* by ingesting organisms or via the contamination of mucous membranes and abraded skin. In case reports, *Brucella* infections have been described after close contact with dogs especially animals that recently aborted or gave birth, and after exposure to large amounts of the organism in laboratories (e.g. contact with bacterial cultures). However, the source of the organism could not be determined in some cases [9].

Canine brucellosis is sometimes difficult to diagnose, and the best chance of success is if multiple techniques are used in combination. The rapid slide agglutination test (RSAT), Rose Bengal Plate Test (RBPT) and the Tube Agglutination Test (TAT) are often used to detect antibodies to *Brucella* in dogs. The RSAT and RBPT are rapid commercial test that can be used for screening of rough and smooth surface *Brucella* antigens respectively. Other serological tests that have been used either clinically or in research include AGID, ELISA, an indirect fluorescent antibody (IFA) test, complement fixation test, a lateral flow immune-chromatographic assay (LFIA) and counter-immunoelectrophoresis [8]. While culture and isolation is regarded as the gold-standard test for laboratory diagnosis of brucellosis, its sensitivity is low because the *Brucellae* are fastidious micro-organisms that can easily be overgrown by contaminating bacteria. Thus, serological examinations are often used to detect evidence of exposure to *Brucella* organismssince they are relatively easy to perform and may provide a practical advantage of estimating prevalence in populations [3].

MATERIALS AND METHODS

Study Area

The study was conducted at Billiri Local Government Area of Gombe State, Nigeria. Located between latitude $9^0$51'N and longitude $11^0$13'E. It has an area of 737 km^2 and a population of 202,144 at 2006 census (Diary, 2012). People of Billiri mostly consist of crop farmers, animal rearers, fishermen, traders and civil servants. Agriculture is the main stay of their economy.

Study Design

A cross sectional study was conducted. Targeted areas were the State and Private Veterinary clinics, households and dog slaughter points. Five wards (namely; Billiri south, Kalmai, Billiri north, Bare, Banganje) out of the eleven wards were selected based on convenience, whereby individual dog selection was based on systematic random sampling with the selection of one out of every three dogs seen. Clinical examination of each dog sampled was carried.

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Sample size was determined using the formula of Mugo [24] at 95% confidence interval and prevalence rate of 29.2% as reported by Momoh *et al.* [23].

Thus $N= Z^2pq/L^2$

$N= 1.96^2 \times 0.29(1-0.29)/0.05^2 = 316$

The sample size was increased by 10% contingency to 347.

Health status

Dogs were categorized as sick if they had fever and at least 2 of the following signs; diarrhea, vomiting, weakness, loss of appetite, rapid weight loss, mucoid or mucopurulent ocular and/or nasal discharge while those that did not meet up the criteria were categorized as apparently healthy.

Sample Collection

A total of 347 dogs were sampled, comprising of 120, 100, 50, 47 and 30 from Billiri south, Kalmai, Billiri north, Bare and Banganje based on availability. The sample collection was performed around May, 2017 to July, 2017. The dogs were properly restrained and 5 ml of venous blood was aseptically collected from the cephalic vein into a clean and well labeled sample bottle devoid of anticoagulant using sterile hypodermic needle and 10 ml syringe. The blood samples were allowed to clot by laying the sample bottles in a slanting position for an hour and the sera obtained by decantation into new well labeled sample bottles. Sera samples were stored at -20°C in a freezer and finally transported to the Bacterial Zoonoses Laboratory, Ahmadu Bello University Zaria in a flask with ice packs for laboratory analysis.

Laboratory Procecedures

Rose Bengal Plate Test (RBPT)

Rose Bengal Plate Test (RBPT) was performed as described by Macmillan [19]. Thus, 30 µl of antigen (*B. abortus* antigen) was placed on the test plate using a clean Pasteur pipette and the same volume of test serum was placed beside the antigen using another clean Pasteur pipette. The two were mixed thoroughly using a sterile applicator stick and rocked gently for 4 minutes and observed for agglutination. The formation of distinct pink granules (agglutination) was recorded as positive while the absence of agglutination was recorded as negative.

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Competitive Enzyme Linked Immune Sorbent Assay (c-ELISA)

Competitive Enzyme linked Immune Sorbent Assay (c-ELISA) kits for *Brucella* species antibody was used according to Manufacturer's information. The kit utilizes ELISA based on antibody-capture technique. The test (c-ELISA) is for the detection of serum antibody to *Brucella* species and is a multispecies assay which is capable of differentiating cross-reacting antibodies from antibodies elicited by field infections of *Brucella* species in animals [18].

The antigen (*B. abortus* antigen) and c-ELISA kits was supplied by Veterinary Laboratory Agency® (VLA), New Addlestone Surrey, United Kingdom.

The microtitre has 96 wells coated with *B. abortus* antigen; twelves wells arranged horizontally (1-12 wells), while eight wells are arranged vertically (A-H).

The plate was prepared by adding 100 µl of the diluting buffer (conjugate solution) to each well, then followed by 20 µl of the test sera leaving columns 11 and 12 for controls, 20 µl of the positive control sera were added into wells A11, A12, B11, B12, C11 and C12 while 20 µl of the negative control were added into wells F11, F12, G11, G12, H11 and H12, wells D11, D12, E11 and E12 served as conjugate controls. This gives a final serum dilution of 1/6. The plate was then vigorously shaken (on the microtitre plate shaker) for 2 minutes in order to mix the serum and conjugate solution. The plate was shaken for 30 seconds and 10 seconds at 10 minutes intervals for 1 hour while the liquid was within their respective wells. The content of the plate was discarded and rinsed 5 times with washing solution and then dried by tapping on an absorbent tissue paper. The substrate chromogen was prepared immediately and mixed thoroughly, 100 µl of the solution was added to each well. The plate was left at room temperature for 10 minutes, the reaction was slowed by adding 100 µl of the stopping solution to each well. Lack of colour development indicates positive samples. The plate was read at 450 nm.

Data Analysis

Statistical Package for Social Sciences (SPSS) version 20.0 was used to analyze the data generated. Chi-square and fishers exact test were used to test for association between the presence of *Brucella* antibodies and the various variables such as age, sex, breed and health status. Values of $p \leq 0.05$ were considered significant. Odds ratio and 95% confidence interval on odds ratio was used to measure the strength of association between dichotomous variables such as *Brucella* infection in males and females. The sero-prevalence of *Brucella* in dogs was determined using the formula;

Prevalence = Positive sample/ Total sample x 100

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RESULTS

Sero-prevalence of *Brucella* antibodies in dogs in Billiri LGA using RBPT and c-ELISA based on wards

Out of the 347 serum samples evaluated for *Brucella* antibodies using RBPT and C-ELISA, a total of 76(21.71%) and 51(14.57%) tested positive respectively. Out of the 120, 100, 47, 50 and 30 serum samples collected from dogs in Billiri south, Kalmai, Bare, Billiri north and Banganje wards respectively, 23(19.17%), 37(3.00%), 3(6.38%), 6(12.00%), and 7((23.33%) were positive by RBPT respectively while 15(2.30%), 26(26.00%), 2(4.17%), 6(12.00%) and 2(6.67%) were ELISA positive respectively. There was statistically significant association (χ^2 =16.573; df=4; p= 0.002) between *Brucella* antibodies and the wards sampled. (Table 1)

Sero-prevalence of *Brucella* antibodies in dogs in Billiri LGA using RBPT and c-ELISAbased on age, sex, breed and health status of the animals.

Two hundred and thirty seven (237) adult dogs and 110 puppies were sampled, the number positive based on RBPT and c-ELISA were 59(24.89%), 43(18.14%) and 17(15.45), 8(7.27%) respectively. Out of the 218 male dogs and 129 female dogs sampled, 55(25.23%), 40(18.35%) and 21(16.28%), 11(8.53%) were positive for RBPT and c-ELISA respectively. Out of the 308 local breed of dogs sampled, 69(22.40%) and 47(15.26%) tested positives for RBPT and c-ELISA respectively. Two (11.11%) and 5(23.81%) were positive based on RBPT from the 18 and 21 samples from cross bred and exotic dogs respectively, while based on c-ELISA, none (0%) of the cross bred samples and only 4 (19.05%) of the exotic breeds were positive. Out of the 252 apparently healthy dogs sampled, 48(18.97%) and 33(13.04%) tested positive for RBPT and c-ELISA respectively, while the 95 samples from sick dogs had 28(29.47%) and 18(18.95%) positive for RBPT and c-ELISA respectively. There was statistically significant associations between the presence of *Brucella* antibodies and age (χ^2 = 6.241, P = 0.012), sex (χ^2 =5.477, P = 0.019) of the dogs. There was no statistically significant association between the presence of *Brucella* antibodies and breed (χ^2 = 3.496, P =0.174), health status (χ^2 = 1.447, P = 0.229) of the dogs (Table 2).

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Table 1: Sero-prevalence of *Brucella* antibodies in dogs in Billiri LGA by RBPT and c-ELISAbased on wards

Wards	Number of sera samples tested	Number of positive RBPT samples (%)	Number of positive c-ELISA samples (%)
Billiri south	120	23(19.17)	15(12.50)
Kalmai	100	37(37.00)	26(26.00)
Billiri north	50	6(12.00)	6(12.00)
Bare	47	3(6.38)	2(4.26)
Banganje	30	7(23.33)	2(6.67)
Total	347	76(21.90)	51(14.70)

Chi-square= 16.573; P-value= 0.002

Table 2: Sero-Prevalence of *Brucella* antibodies in dogs in Billiri LGA by RBPT and c-ELISAbased on age, sex, breed and health condition of the animals

Variables	Number of sera samples tested	Number of positive samples (%)		χ^2	P-value	Odds ratio	95% confidence interval	
		RBPT	C-ELISA				Lower	Upper
AGE								
Adult	237	59(24.89)	43(18.14)	6.241	0.012	0.354	0.160	0.781
Puppy	110	17(15.45)	8(7.27)					
SEX								
Female	129	21(16.28)	11(8.53)	5.477	0.019	0.415	0.205	0.841
Male	218	55(25.23)	40(18.35)					
BREED								
Local	308	69(22.40)	47(15.26)	3.496	0.174	0	0	0
Cross	18	2(11.11)	0(0.00)					
Exotic	21	5(23.81)	4(19.05)					
HEALTH STATUS								

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Healthy	252	48(19.05)	33(13.10)	1.447	0.229	1.551	0.826	2.914
Sick	95	28(29.47)	18(18.95)					

DISCUSSIONS

The study showed that the overall sero-prevalence of *Brucella* antibodies in Billiri LGA of Gombe State using RBPT was 21.90% and c-ELISA was 14.70%. These findings could be attributed to the fact that dogs are housed together and allowed to scavenge freely thereby being exposed to infected domestic livestock. Rose Bengal Plate Test (RBPT) detect mainly IgM and IgG1, thereby used as a screening test to ascertain exposure of animals to infection due to *Brucella* species, it has a good sensitivity but its lack of specificity and the occurrence of false positive make a specific test necessary [17]. The specificity of competitive Enzyme Immuno-Sorbent Assay (c-ELISA) is very high and is able to detect all antibody isotypes (IgM, IgG1, IgG2 and IgA) making it an outstanding confirmatory assay for the diagnosis of brucellosis in most mammalian species [16]. *Brucella abortus* antigen was used, but other smooth surface antigen *Brucella* species such as *B. melitensis* and *B. suis* that have common epitope can also be detected [25]. This study is similar with the reports of other workers who also demonstrated the presence of *B. abortus* agglutinins in dog sera using RBPT [1; 4; 6; 22; 23; 27; 31] and antibody response to *B. abortus* antigens using c-ELISA [22; 23]. The findings of this study is lower than that recorded by Momoh *et al.* [23] who reported a prevalence of 29.2% using c-ELISA in Jos North and Jos South LGAs of Plateau State while a prevalence of 38.2% was reported by Adesiyun *et al.* [1] in Zaria using RBPT.

The findings of the study is also higher than 21.5% reported by Osinubi *et al.*[27] in Zaria, 5.46% by Cadmus *et al.* [6] in South western Nigeria, zero percent prevalence reported by Anyaoha [2] in Enugu and Anambra States using RBPT, as well as 1.06% reported by Modupe *et al.* [22] in South western Nigeria, using c-ELISA. The higher prevalence recorded in the study area may be as a result of high contact rate between dogs and livestock species such as cattle, sheep, goats and pigs as well as their carcasses [11]. It may also indicate lack of brucellosis control programme in animals in the study area.

Location-based sero-prevalence of *Brucella* antibodies in dogs revealed a highest prevalence rate of 26% in Kalmai ward as compared to other wards, this may be as a result of the location of the dog market and dog slaughter slab as well as the activities of dog meat processors and dog retailers in the ward. The lowest prevalence rate (4.17%) was recorded in Bare ward, this may be as a result of less contact rate between dogs and other livestock species in the study area as observed by Anyaoha *et al.* [2] in Enugu and Anambra States.

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From the study, the sero-prevalence of *Brucella* antibodies increased with age of the dogs, this could be attributed to the fact that young animals are conferred with maternal immunity and do not normally scavenge for food like sexually matured animals. Free scavenging of abattoir wastes and the practice of feeding the dogs with dead fetuses of livestock animals may contribute to the high prevalence in adult dogs, this concur with the findings of Cadmus *et al.* [6] and Modupe *et al.*[22] in Southwestern Nigeria. Uncontrolled breeding and reckless mating of sexually matured dogs is a means of rapid transmission of *Brucella* infections among adult dogs in the study area, as also observed by Osinubi *et al.* [27] and Hollett [13] who reported that dogs of sexually active age (1-5 years) are more predisposed to *Brucella* infections than other age groups, and that most diseased animals carry the infection throughout their lives.

Sex-based sero-prevalence of *Brucella* antibodies in dogs showed a higher prevalence rate in male dogs (18.35%) than in the female dogs (8.53%). Male dogs are mostly used for hunting activities than the female dogs in the study area, which may also increase their chances of being exposed to infected wildlife species. Hunters and hunting dogs are at high risk of contracting brucellosis from wildlife [29]. In male dogs, *Brucella* organisms have been implicated in epididymytis, orchitis, poor sperm quality and loss of libido [13], thereby limiting transmission of *Brucella* species from infected male dogs to female dogs through mating. The findings are similar with those of Osinubi *et al.* [27] who reported higher sero-positivity to *B. abortus* antigen in the male dogs (9.1%) than in the female dogs (5.9%) in Zaria, and Modupe *et al.* [22] who reported 13.4% sero-prevalence in the male as compared to 12.2% in the female in South-western Nigeria.

The sero-prevalence of *Brucella* antibodies in dogs according to breed showed a higher prevalence rate of 19.05% in exotic breeds when compared to 15.26% in the local breeds and 0.00% in crossed breeds, this is similar with the findings of Momoh *et al.* [23]. The high prevalence in exotic breed may be as a result of lack of screening of dogs, especially exotic breed which may lead to an increase in the density of possibly infected foreign breed of dogs, which is in accordance with the findings of Ryan *et al.* [11]. The zero percent prevalence in crossed breed may indicate less breeding activity between the exotic and local breed of dogs in the study area, while the 15.26% prevalence in the local breed of dogs may be as a result of inadequate care and attention given to the breed by the owners, possibly because of their less commercial value which may make them wander in the environment, exposing them to *Brucella* infection, as observed by Modupe *et al.*, [22].

The findings of this study showed high prevalence in sick dogs (18.95%) as compared to the apparently healthy dogs (13.04%), this may be as a result of lack of recognition of brucellosis as a diagnostic possibility in sick dogs, thereby neglecting the diagnosis and management of the disease resulting in high incidence of the disease. McDermott *et al.* [21] stated that brucellosis remained a major neglected zoonosis of developing nations. It has been recognized that dogs can act as mechanical and biological vector of *B. abortus*,

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B. suis and B. melitensis. In nearly all cases, the source of the infection could be traced to the consumption of materials from infected domestic and wild animals [15].

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