Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2022, Vol 2, Issue 2: 98-104
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Michael Okpara University of Agriculture, Umudike, Nigeria
Original Research Article

Seroprevalence of *Brucella abortus* in dogs and associated risk factors in Gombe State, Nigeria

[1*]Audu, Y., [1]Mustapha M., [2]Ezema K. U., [3]Dauda, J., [2]Bamanga U. M. & [4]Okolocha E. C

[1]Department Veterinary Medicine, [2]Veterinary Teaching Hospital, [3]Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri, [4]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria*

*Corresponding author: yusufaudu99@gmail.com, +2348051421409

ABSTRACT

To determine the seroprevalence of *Brucella abortus* in dogs in Gombe state Nigeria and its associated risk factor, a total of 350 dog-sera were tested using Rose Bengal Plate Test (RBPT) and competitive Enzyme Linked Immuno-sorbent Assay (c-ELISA). Six out of the eleven Local Government Areas (LGAs) of the State (two from each senatorial zone) were selected randomly. Household, State/private Veterinary facilities were surveyed while individual dog selection was based on systematic random sampling. Questionnaires were administered to dog owners and handlers to assess their knowledge of the infection, attitudes and practices between May, 2018 and July, 2018. The prevalence of *Brucella abortus* in the study area was 26.00% with RBPT and 14.86% with c-ELISA. The sero-prevalence of *B. abortus* in dogs revealed 14.00% to 16.67% range across the LGAs of Gombe State, Nigeria. The prevalence of 18.98% was recorded among adult dogs and 8.21% in puppies. Sero-prevalence of *B. abortus* in the male was 14.68% and 15.15% in the female dogs. The local breed of dogs had a prevalence of 15.93% while the exotic and a cross breeds had 10.53% and 8.33% respectively. The sero-prevalence of *B. abortus* was higher in the sick dogs (22.43%) than apparently healthy ones (11.53%). There was a statistically significant association between the seroprevalence of *Brucella abortus* and age, health status of the dogs ($p<0.05$) while there was no statistically significant association between the seroprevalence of *Brucella abortus* and sex, breed of the dogs ($P>0.05$). From the results, 52% of dog owners were aware of zoonotic diseases from dogs compared to dog handlers (48%), but they all showed poor attitudes towards management systems and hygienic practices aimed at reducing risk of contracting zoonotic diseases. The findings of this study established the seroprevalence of *Brucella abortus* in dogs in Gombe State Nigeria and risk it poses for both dogs and humans.

Keywords: *Brucella abortus*, seroprevalence, dogs, Gombe State, Nigeria.

INTRODUCTION

Brucellosis is a worldwide zoonosis caused by *Brucella* species. The four species known to infect humans are *B. melitensis*, *B. suis*, *B. abortus*, and *B. canis* (Ramamoorthy *et al.,* 2011). Brucellosis is characterized by epididymitis and orchitis in male dogs, endometritis, placentitis, abortion and or still birth in females, and often presents as infertility in both sexes (Makloski, 2011). The disease is insidious and many infected dogs appear to be clinically healthy (Marzetti *et al.,* 2013). *Brucella* organisms can be found in the fetus, placenta, fetal fluids and vaginal discharge after an abortion or stillbirth (Chinyoka et al., 2014). The bacteria can also be shed in normal vaginal secretions, particularly during estrus, and high concentrations of *Brucella* may be found in semen for weeks or months after infection, and intermittent shedding of smaller quantities can occur for years (Hollett, 2006). *Brucella* species are also shed in urine, and low concentrations of the bacteria may be excreted in saliva, nasal and ocular secretions, and in feces (Cadmus *et al.,* 2011).

Brucellosis is one of the most devastating trans-boundary animal diseases and also a major barrier for trade (Brower *et al.,* 2007; Abubakar *et al.,* 2012). It therefore impacts negatively on the economy and public health of endemic countries (Agada *et al.,* 2017). Brucellosis constitutes a threat to dog breeding in Nigeria, along with possible zoonotic implications (Cadmus *et al.,* 2011). Despite the advances

http://doi.org/10.54328/covm.josvas.2022.064

made in surveillance and control, prevalence of brucellosis is increasing in many developing countries (Gwida *et al.*, 2010). Because brucellosis is relatively a neglected disease and its clinical signs and symptoms (in humans) are non-specific, the number of cases reported is thought to be underestimated (Pappas *et al.*, 2005; Lucero *et al.*, 2005 and Franco *et al.*, 2007). Despite the vigorous attempt to come up with a definitive diagnostic technique for brucellosis, diagnosis still relies on the combination of several tests to avoid false negative results (Poiester *et al.*, 2010).

In Gombe State, dog ownership and trade are common, and humans live in close contact with dogs and other animals. In addition, there is no vaccination and control program for brucellosis, therefore *Brucella species* circulate between dogs, other animals, and human beings. The diseases reduce the productivity of dogs and induce medical problems in humans. Information that supports the prevention and control of brucellosis is not well accessible. Knowledge about the prevalence of brucellosis in dogs might help to design prevention and control strategies that will limit the spread of *Brucella* species to other animals and humans in the State. Therefore, this research aims to determine the seroprevalence of *Brucella abortus* infections in dogs and associated risk factors in Gombe State Nigeria.

MATERIALS AND METHODS

STUDY AREA

Gombe State (*Jewel in the Savannah*), located in the North East geopolitical zone of Nigeria, was created out of the then Bauchi State in 1996, with Gombe town as its administrative and commercial capital. The State covers an area of 20,265km² and is located between latitudes 9°301N and 12°301E and longitudes 8°451 and 11°451E. The State has Eleven (11) Local Government Areas (LGAs) and 114 Wards. The Local Government Areas are: Akko, Balanga, Billiri, Dukku, Funakaye, Gombe, Kaltungo, Kwami, Nafada, Shongom and Yamaltu/Deba (Misbahu, 2015).

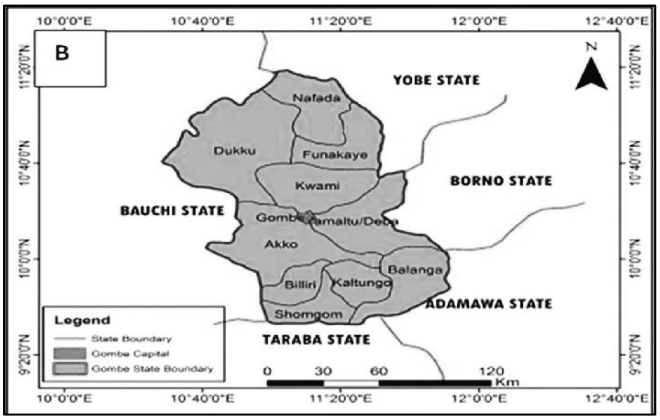

Figure I: Map of Gombe State showing the study area

STUDY DESIGN

A cross sectional study was conducted. Six LGAs (Kwami, Gombe, Yamaltu Deba, Akko, Billiri and Kaltungo) out of the eleven LGAs were selected randomly (two from each senatorial zone). Facilities suveyed were the State and Private Veterinary clinics, households and dog slaughter points. Individual dog selection was based on systematic random sampling with the selection of one out of every three dogs seen. Clinical examination was carried on each selected dog and dogs were categorized as sick if they had fever and manifested at least any 3 of the following signs; myalgia, arthritis, weakness, loss of appetite, emaciation, lymphadenitis, mucoid or mucopurulent ocular discharge, painful and swollen testicles, vaginal discharge, while those that did not meet the criteria were categorized as apparently healthy. Dogs of less than 7 months were considered as puppies while those that were 7 months and above were considered as adult based on the average age at first estrus and the variation of age in canine dentition (Salvin *et al.*, 2011). Sex of the study subjects was determined by the appearance of external reproductive structures. Breeds of dog were categorized as exotic, cross and local. Sample size was determined using the formula described by Thrusfield (2005) at 95% confidence interval and prevalence rate of 29.2% as reported by Momoh *et al.* (2014). The calculated sample size was 316 but was increased to 350 for precision.

SAMPLE COLLECTION

The sample collection was performed between May, 2018 and July, 2018. Each dog was properly restrained and 5 ml of venous blood was aseptically collected from the cephalic vein into a well labeled plain vacutainer tube using sterile hypodermic needle and 5 ml syringe. The blood samples were allowed to clot and then centrifuged at 3000 rpm for at least 5 minutes and the sera harvested by decantation into a 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.

USE OF QUESTIONNAIRE

A structured questionnaire comprising of close ended questions was used. The questionnaire was divided into two parts as follows:

i. For dog owners: Information on dog's age, breed, sex, location, history of abortion and/or stillbirth, consumption of fetuses/abattoir waste, number of dogs in the household and cohabitation with other animals, owner's occupation, and educational level were obtained.

ii. Dog handlers are individuals whose occupation were identified as high risk toward contracting zoonotic diseases. These include; all those that handle dogs in one way or the

other like the veterinarians, dog meat processors and dog retailers. For dog handlers: General information on their knowledge, attitudes and practices towards zoonotic diseases in dogs and the risk of human infection were also obtained. Informed consent was obtained from dog owners and handlers before questionnaire administration and sample collection

LABORATORY PROCEDURES

Rose Bengal Plate Test (RBPT) was performed as described by Macmillan 1990 using *B. abortus* antigen.

The competitive Enzyme Linked Immune Sorbent Assay (c-ELISA) kit that utilize *B. abortus* antigen for *Brucella* species antibody was used according to Manufacturer's information, the kit utilize ELISA based on antibody-capture technique. The antigen (*B. abortus* antigen) and c-ELISA kits was supplied by Veterinary Laboratory Agency (VLA), New Addlestone Surrey, United Kingdom in 2018.

DATA ANALYSIS

The data generated were analyzed using statistical Package for Social Sciences (SPSS) version 20. Independent samples t-test was used to test for the mean difference between the RBPT and c-ELISA values across the LGAs. Chi-square and fishers exact test were used to test for association between the presence of *Brucella* antibodies and the variables (age, sex, breed and health status). Values of $P<0.05$ were considered significant. Odds ratio and 95% confidence interval on odds ratio were used to measure the strength of association between dichotomous variables. The sero-prevalence of *Brucella abortus* in dogs was determined using the formula;

Prevalence = Positive sample/ Total sample x 100

Data generated from the questionnaire survey were presented as percentages and charts (descriptive statistics).

RESULTS

SEROPREVALENCE OF *BRUCELLA ABORTUS* IN DOGS IN LOCAL GOVERNMENT AREAS (LGAS) OF GOMBE STATE NIGERIA

Out of the 350 serum samples evaluated for *Brucella abortus*, 91 (26.00%) and 52 (14.88%) tested positive using RBPT and c-ELISA respectively. Out of the 97, 85, 50, 47, 41 and 30 serum samples collected from dogs in Billiri, Kaltungo, Akko, Gombe, Yamaltu Deba and Kwami LGAs respectively, 25 (25.77%), 22 (25.88%), 13 (26.00%), 12 (25.53%), 11 (26.83%) and 8 (26.67%) respectively were positive by RBPT while by c-ELISA 14 (14.43%), 13 (15.29%), 7 (14.00%), 7 (14.89%) 6 (14.63%) and 5 (16.67%) were positive respectively. There was no statistically significant difference between the mean values of RBPT and c-ELISA across the LGAs ($t = 2.055$; $df = 10$; $P = 0.067$) (Table I).

Table I: Seroprevalence of *Brucella abortus* in dogs in Local Government Areas (LGAs) of Gombe State Nigeria

LGA	Number of sera samples tested	Number of positive samples by RBPT (%)	Number of positive samples by c-ELISA (%)
Billiri	97	25 (25.77)	14 (14.43)
Kaltungo	85	22 (25.88)	13 (15.29)
Akko	50	13 (26.00)	7 (14.00)
Gombe	47	12 (25.53)	7 (14.89)
Yamaltu Deba	41	11 (26.83)	6 (14.63)
Kwami	30	8 (26.67)	5 (16.67)
Total	350	91 (26.00)	52 (14.86)

SEROPREVALENCE OF *BRUCELLA ABORTUS* IN DOGS IN GOMBE STATE BASED ON AGE, SEX, BREED AND HEALTH STATUS OF THE ANIMALS

Of the two hundred and sixteen (216) adult dogs and 134 puppies sampled, 59 (24.89%) adult dogs and 43 (18.14%) puppies were positive by RBPT while 17 (15.45) adult dogs and 8 (7.27%) puppies were positive by c-ELISA. There was statistically significant association between the presence of*Brucella abortus* antibodies and age ($\chi^2 = 7.587$, $P = 0.006$) of the sampled dogs. Out of the 218 male dogs and 132 female dogs sampled, 55 (25.23%) of the male dogs and 40 (18.35%) of the female dogs were positives by RBPT while 21 (16.28%) of the male dogs and 11 (8.53%) of the female dogs were positive by c-ELISA. There was no statistically significant associations between the presence of *Brucella abortus* antibodies and sex of the dogs ($\chi^2 = 0.015$, $P = 0.904$). Out of the 295 local, 36 crossed and 19 exotic breeds of dogs sampled, 66 (22.37%), 19 (52.77%) and 9 (47.36%) tested positives by RBPT respectively, while 47 (15.93%), 3 (8.33%), and 2 (10.53%) tested positives by c-ELISA respectively. There was no statistically significant association between the presence of *Brucella abortus* antibodies and breed of the dogs ($\chi^2 = 1.762$, $P = 0.414$). Out of the 243 apparently healthy dogs sampled, 51 (20.99%) and 28 (11.52%) tested positive by RBPT and by c-ELISA respectively, while the 107 samples from sick dogs had 40 (37.38%) and 24 (22.43%) positive by RBPT and by c-ELISA respectively. There was statistically significant association between the presence of *Brucella abortus* antibodies and health status of the dogs ($\chi^2 = 6.987$, $P = 0.008$) (Table II).

DEMOGRAPHIC FEATURES OF DOG OWNERS IN GOMBE STATE

Out of the 50 dog owners in Gombe State Nigeria who responded to the questionnaire, 23 (46.0%) were between the ages of 26 and 35 years, 19 (38.0%) were between the ages of 15 and 25 years, 5 (10.0%) were between the ages of 36 and

Table II: Seroprevalence of *Brucella abortus* in dogs in Gombe State Nigeria according to age, sex, breeds and health status. Key; *= Significant

Variables	Categories	Number of sera samples tested	Number of positive samples (%)		χ^2	P-value	Odds ratio	95% confidence interval	
			RBPT	c-ELISA				Upper limit	Lower limit
AGE	Adult	216	63 (29.17)	41 (18.98)	7.587*	0.006	2.620	1.295	5.299
	Puppy	134	28 (20.90)	11 (8.21)					
SEX	Female	132	42 (31.82)	20 (15.15)	0.015	0.904	0.963	0.526	1.766
	Male	218	49 (22.48)	32 (14.68)					
BREED	Local	295	66 (22.37)	47 (15.93)	1.762	0.414	0	0	0
	Cross	36	19 (52.77)	3 (8.33)					
	Exotic	19	9 (47.36)	2 (10.53)					
HEALTH STATUS	Healthy	243	51 (20.99)	28 (11.52)	6.987*	0.008	0.450	0.2469	0.823
	Sick	107	40 (37.38)	24 (22.43)					

45 years, while only 3 (6.0%) were above 45 years of age. Forty three (86.0%) of the respondents were males, while only seven (14.0%) were females. All the respondents had formal education at various levels with the highest percentage (56.0%) being the secondary school certificate holders. In terms of occupation, 22 (44.0%) were students, 12 (24.0%) were civil servants, 9 (18.0%) were business men and women while only 7 (14.0%) were farmers.

KNOWLEDGE, ATTITUDES AND PRACTICES OF DOG OWNERS TOWARD ZOONOTIC DISEASES OF DOGS IN GOMBE STATE

Out of the total of 50 respondents, 26 (52%) were aware of the occurrence of zoonotic diseases in dogs, about 31 (62%) were aware of the risk of transmission of diseases from dog to dog through mating, while 27 (54%) of the respondents were not aware of the possibility of transmission of diseases through milk from the bitch to the puppy. A total of 27 (54%) of the respondents allowed their dogs to mingle with other animals, 48 (96%) respondents play with their dogs, 26 (52%) respondents allow their dogs to eat from their hands and 46(92%) respondents did not wash their hands after handling dogs or products from a dogs. Only 4(8%) of the respondents bred their dogs under a controlled system. Twenty nine (58%) respondents allow their dogs to feed on abattoir wastes or dead fetuses while none of the respondents had ever noticed swollen and painful testicles as well as abortion and/or still births in their dogs.

DEMOGRAPHIC FEATURES OF DOG HANDLERS IN GOMBE STATE NIGERIA

A structured questionnaire was also administered to individuals whose occupation had been previously identified as high risk groups towards zoonotic diseases. Out of the total of 50 respondents, 17 (35%) were dog retailers, 16 (33%) were dog meat processors, 9 (18%) were dog breeders and 7(14%) were Veterinarians. All the respondents were males (100). Twenty four (48%) of them were between the ages of 26 and 35 years, 11 (22%) were between 36 and 45 years, 9 (18%) were between 15 and 25 years while only 6 (12) were above
45 years. All the respondents had formal education at various levels, 9(18%) had first school leaving certificate, 32(64%) had secondary school certificates while 9(18%) had tertiary education qualifications.

KNOWLEDGE AND PRACTICE OF DOG HANDLERS TOWARDS ZOONOTIC DISEASES OF DOG IN GOMBE STATE NIGERIA

Fifty two percent (52%) of the respondents were not aware of zoonotic diseases in dogs (Figure II). About 54% and 52% of the respondents were not aware of some of the possible mode of transmission of zoonotic diseases from dogs such as; through dog body fluid and aerosol droplet (Figure II). More than half (54%) of the respondents were not aware that apparently healthy dogs can transmit diseases to humans (Figure II) and 56% of the respondents said they do not wash their hands after handling dogs or products from dogs (Figure 3). Most of the respondents (62%) said they do not care to obtain the medical history of any dog or a product from any dog they are handling (Figure IV).

DISCUSSIONS

Brucella abortus infection in dogs has been reported under experimental and field conditions (Baek *et al.,* 2003). The study showed that the sero-prevalence of *Brucella abortus* in dogs in Gombe State Nigeria were 26.00% by RBPT and 14.86% by c-ELISA. Rose Bengal Plate Test (RBPT) detect mainly IgM and IgG_1, thereby used as a screening test to detect exposure of animals to *Brucella* species, it has a good sensitivity but its low specificity and the possible occurrence of false positive make a specific test necessary (Lobna *et al.,* 2014). Competitive Enzyme linked Immunsorbent Assay (c-ELISA) was used as a confirmatory test. The test (c-ELISA) is for the detection of serum antibody to *Brucella* species which is capable of differentiating vaccine antibodies from antibodies elicited by field infections of *Brucella* species in animals (Nielsen and Yu, 2010). The specificity of c-ELISA

is very high and is able to detect all antibody isotypes (IgM, IgG_1, IgG_2 and IgA) which made it an outstanding confirmatory assay for the laboratory diagnosis of brucellosis in most mammalian species (Kaltungo *et al.*, 2014). *Brucella abortus* antigen was used, but other smooth surface antigen *Brucella* species such as *B. melitensis* and *B. suis* with common epitope can also be detected by c-ELISA (Portanti *et al.*, 2006). This finding is higher than 1.06% reported by Ayoola *et al.* (2016) in south western Nigeria; the difference could be attributed to the fact that, in Gombe State Nigeria dogs were housed together and allowed to scavenge freely thereby being exposed to infected pets and livestock animals. Upadhyay *et al.* (2019) reported that, the spread of brucellosis between the vulnerable host animal species occurs mainly through direct and indirect contacts.

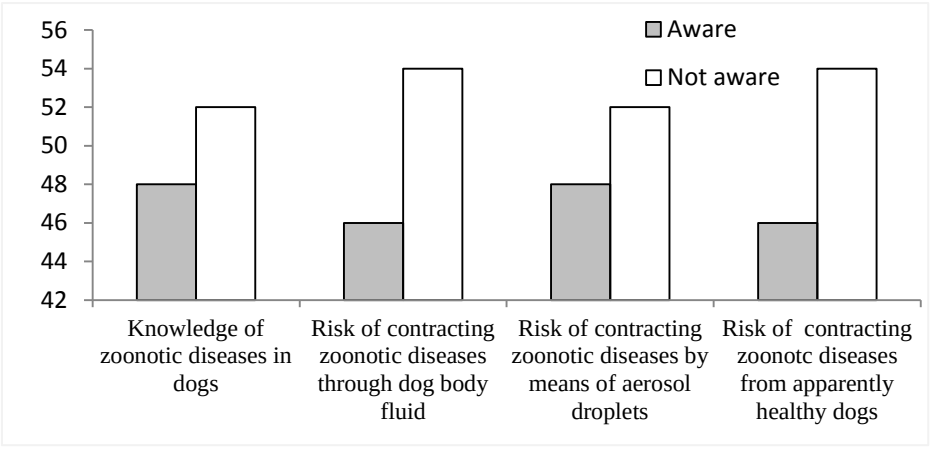

Figure II: Knowledge of dog handlers regarding zoonotic diseases in dogs and risk of acquiring the disease in Gombe State Nigeria

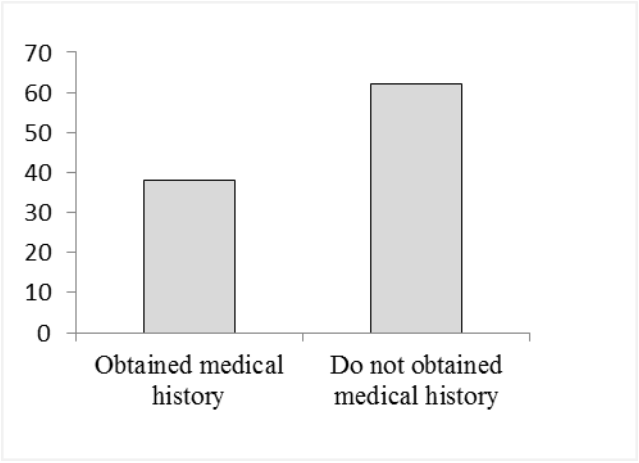

Figure III: Attitude of dog handlers regarding the medical history of dogs they handled in Gombe State Nigeria

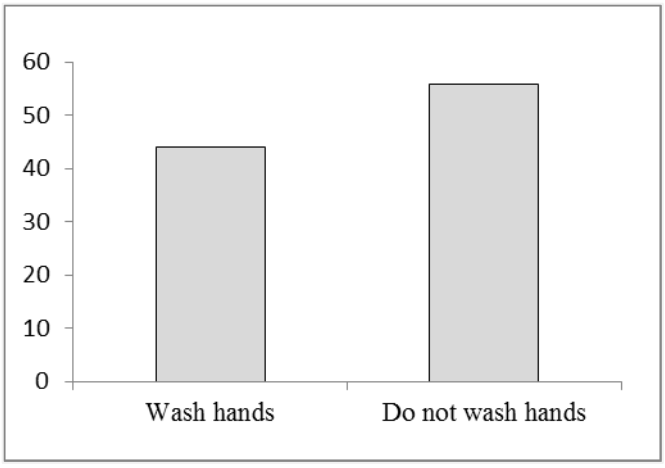

Figure IV: Hygienic practice of dog handlers after handling dogs or their products in Gombe State Nigeria

The seroprevalence across the different locations sampled within the state showed similarities in rates ranging from 14.00% to 16.67%. This could be attributed to the abundance and extensive breeding of livestock as well as uncontrolled movement and trading of dogs within the study area. Despite *B. canis* being the major causative organism for brucellosis in dogs, infection with other *Brucella* species (*B. abortus*, *B. melitensis* and *B. suis*) have been extensively reported to be closely associated with mingling of dogs with infected domestic livestock and wildlife (Hinić *et al.*, 2010; Ramamoorthy *et al.*, 2011). Naturally acquired *B. abortus* infection in dogs associated with infected cattle has been reported and horizontal dog-to-dog, livestock-to-dog, dog-to-livestock, and dog-to-human transfer of the infection have been demonstrated (Eduardo *et al.*, 2019).

The seroprevalence of *B. abortus* was higher in adult dogs (18.98%) than in puppies (8.21%), this could be attributed to the fact that young animals do not normally scavenge for food like sexually matured dogs. Scavenging of food and feeding of dogs with abattoir wastes may have contributed to the high prevalence in adult dogs. In agreement with the findings is the report of Cadmus *et al.* 2011 in Southwestern Nigeria which suggest that the unhygienic practice of feeding the dogs with dead fetuses of livestock and other abattoir waste is a means of exposure of dogs to *Brucella* species. Also Hollett (2006) reported that, sexually matured dogs were more prone to *Brucella* infections than other age groups and that most of the infected dogs remained carriers even without obvious sign of the disease.

The sero-prevalence of *B abortus* in dogs is higher in female dogs (15.15%) than in the male dogs (14.68%). This observation is similar to the findings of Momoh *et al.* (2014)

and Chinyoka *et al*. (2014) who also reported higher serpositivity in the females than in the male dogs in Plateau State Nigeria and in Zimbabwe respectively. The higher seroposivity may be due to an indiscriminate mating of an intact stud with multiple bitches thereby putting the bitches at high risk of getting infected by discharging cells into females. If the males are infected the infection would be discharged along will the cell (sperm) whereas infection of males by infected females can only occur by chance.

Breed based seroprevalence of *B. abortus* showed a higher prevalence rate of 15.93% in the local breed when compared with the 10.53% in the exotic and 8.33% in the cross breed dogs. This is similar with findings of Ayoola *et al.* (2016) in south western Nigeria who also reported higher seropositivity in the local breed compared to the exotic breed of dogs. This may be as a result of insufficient care and attention given to the breed by the owners, probably because of their less economic value which may render them roam freely in the environment, thereby increasing their vulnerability to *Brucella* infections (Audu *et al.,* 2018).

The findings in this study showed a higher prevalence of *B. abortus* in sick dogs (18.95%) when compared to the apparently healthy dogs (13.04%), this may be because brucellosis is not considered as a differential in the disease diagnosis in sick dogs, thereby ignoring the treatment and management of brucellosis which may further compromise the immune system resulting in high incidences of clinical cases. Osinubi *et al*. (2004) states that *Brucella abortus* infection in the Nigerian dog population appears to be an emerging health problem and the incidence may be higher if *Brucellae* are not considered possible etiological agent in febrile illnesses.

Brucellosis is a thoughtful, globally distributed zoonotic disease that needs diverse approach in order to control the incidence of the disease (Eduardo *et al.,* 2019). The findings of the knowledge, attitude and practices of dog owners showed that, most of the respondents (52%) were aware of zoonotic diseases occurring in dogs and some of the possible modes of transmission to humans, but their attitude towards reducing the risk of contracting such diseases was poor. These include; allowing their dogs to mingle with other animals and scavenge freely for abattoir wastes and other environmental debris, playing with the dogs and allowing them to eat from their hands without proper personal hygiene. This is risky as *Brucellae* can enter through intact skin and abrasions (Manthur & Amarnath, 2008). Baek *et al.* (2003) observed that close contact when feeding or handling *Brucella* infected dog has resulted in human infection. Most of the respondents had never bred their dogs under controlled system and none of the respondents ever noticed any of the classical signs of brucellosis in dogs such as swollen and painful testicles as well as abortion and/or still birth, this may be as a result of non-specificity of clinical signs of brucellosis in dogs or negligence of dog owners regarding the health status of their animals. Brucellosis can be clinically inapparent in dogs and be transmitted to human being (Kim *et al.,* 2012)

From the questionnaire result, most of the dog handlers were not aware of zoonotic diseases and some of their possible mode of transmission such as through contact with infected body fluid (blood, serum, uterine fluid, semen) and aerosol droplets. Most of the respondents were not aware that apparently healthy dogs can harbor zoonotic diseases thereby handling any dog or product from any dog recklessly without enquiring about the medical history of the dog. Hollett. (2006) stated that brucellosis in dogs continues to be a problem, simply because of poor knowledge about the disease. Krueger *et al.* (2014) observed that, the most common routes of transmission of brucellosis from dogs to humans are through contact with infected dogs and or their secretions, or occupationally through exposure to clinical cases during assisted parturition or abortion, handling stillbirths, or contact with canine biological samples including blood, urine, placenta or aborted fetuses.

CONCLUSION

The present study showed relatively high seroprevalence of *Brucella abortus* infection in dogs and their risk factors associated with the infection in humans in Gombe State and possibly in the whole country. Therefore, the findings are important toward prevention and control of brucellosis in Nigeria.

ACKWNOLEDGEMENT

The authors acknowledged the contribution of the staffs of the Department of Veterinary Public health and Preventive Medicine, Ahmadu Bello University Zaria for their contribution toward the success of this work. The authors specifically appreciated Malam Yahuza Sulaiman of the bacterial zoonosis laboratory of the same institution for his technical support.

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