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Seroprevalence of Leptospirosis in Vaccinated and Unvaccinated Dogs in Abuja-FCT, Nigeria

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ABSTRACT: *Leptospirosis has been a major concern and threat to animals and humans globally despite the effort in vaccination. This study investigated seroprevalence of leptospirosis in vaccinated and unvaccinated dogs in FCT, Nigeria. Two hundred (200) dogs across three Area councils in Abuja, the Federal Capital territory (FCT) of Nigeria, were selected through simple random sampling between the year 2019 and 2020.Blood samples were collected during the wet and dry season from the dogs. Vaccination status, sex, age, seasonal variation and breed variation were used to establish the prevalence of Leptospirosis. Dogs with high antibody titre to Leptospira ELISA kit was tagged positive to Leptospirosis. The study conducted in Abuja, Federal Capital Territory (FCT), Nigeria, revealed an overall prevalence of leptospirosis in dogs at 98%. Among the dogs tested, the prevalence was slightly higher in vaccinated dogs (98.31%) compared to unvaccinated dogs (97.56%). Demographic and Seasonal Distribution revealed that the prevalence varied by sex, with male dogs showing a rate of 97.73% and female dogs at 98.53%. In terms of age, adult dogs had a prevalence of 98.08%, while puppies showed a slightly lower rate of 97.37%. Seasonal analysis indicated that the prevalence remained consistent at 98% during both wet and dry seasons. Breed-Specific Prevalence shows that breed-specific prevalence are as follows: Alsatian: 98.33%, Caucasian: 96.67%, Mongrel (indigenous breed),96.67%, Boerboel 96.67%, Rottweiler 93.75%, Lhassa Apso: 91.67%, Labrador 90%, Belgian Malinois 87.50%, Springer Spaniel 75%, This study found no significant associations between leptospirosis prevalence and the variables of vaccination status, sex, age, season, or breed (p > 0.05). This study concluded that there is high and uniform prevalence of leptospirosis among dogs in the FCT, regardless of vaccination status, underscores the need for improved public awareness and disease control measures. Additionally, further investigation into the reliability and efficacy of leptospirosis vaccines used in Nigeria is warranted to optimize prevention strategies and safeguard animal and human health.*

Keywords*: Seroprevalence, leptospirosis, vaccinated, unvaccinated, dogs, Nigeria.*
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INTRODUCTION

Leptospirosis is a globally significant bacterial zoonotic disease caused by pathogenic species of the genus *Leptospira*, which are helical-shaped motile spirochaetes belonging to the family Leptospiraceae

(Eze, 2017). Recent studies using DNA homology have identified 13 major pathogenic species responsible for significant human and animal diseases (Evangelista *et al.,* 2010). Misdiagnosis often occurs due to a low index of suspicion amongst clinicians (de-Vries *et al*., 2014). The true burden, spread, and increases of leptospirosis remains largely unknown, as the quality and availability of diagnostic tests, testing facilities, and surveillance systems are highly variable and frequently absent in pertinent regions of the world (Hartskeerl *et al*., 2011). Although globally important, leptospirosis remains under-diagnosed and under-reported in Africa and, consequentially is overlooked as a public health priority (Eshetu *et al*., 2004; World Health Organization, 2006). It is not considered as a priority reportable disease in most African countries, Nigeria included. In developing countries, where the majority of people are poor and live in crowded conditions with poor sanitation, human transmission occurs through exposures to urine of Leptospira-infected livestock and or companion animals (dogs) (Ganoza *et al*., 2006).

While humans are considered to be incidental hosts, animals can serve as both reservoir or incidental hosts Ganoza *et al*. (2010). The Infection can occur in various mammals, including dogs and cats, leading to clinical manifestations that range from mild symptoms to severe multi-systemic diseases that may be fatal (Sanches-Montez *et al.,* 2015).

Diagnosis of leptospirosis is critical for effective treatment. While culture and isolation of the bacteria remain the gold standard for diagnosis, they are time-consuming and have low sensitivity (Richard *et al.,* 2020). The disease is prevalent in both temperate and tropical regions, with incidence rates in tropical areas being approximately ten times higher than in temperate zones (Hartskeel *et al.,* 2011).

The World Health Organization estimates around 873,000 cases of leptospirosis occur annually worldwide, resulting in approximately 48,600 deaths (WHO, 2019). In Africa specifically, zoonotic infections contribute significantly to human morbidity and mortality while also affecting livestock productivity and food security (Schelling *et al.,* 2007).

Despite evidence of widespread exposure to *Leptospira* in humans and animals across Africa (de Vries *et al.,* 2014), leptospirosis remains underdiagnosed and underreported as a public health concern (Maudlin *et al.,* 2009; WHO, 2006).

In Nigeria alone, only about six human cases are estimated annually (Awosanya *et al.,* 2013), highlighting the need for increased awareness and research on this disease in the region.

The research was conducted to carry out a seroprevalence of leptospirosis in vaccinated and unvaccinated dogs of different breeds in Abuja-FCT, Nigeria.

MATERIAL AND METHODS

Study area

The study was conducted in Abuja, the Federal Capital Territory (FCT) of Nigeria, which is situated north of the confluence of the River Niger and River Benue (Figure 1). It is bordered by Niger to the west and north, Kaduna State to the northeast, Nasarawa State to the east and south, and Kogi State to the southwest. Geographically, Abuja lies between latitudes 8.25°N and 9.20°N and longitudes 6.45°E and 7.39°E, covering an area of approximately 7,315 km² within the savannah region characterized by moderate climatic conditions (NPC, 2006).

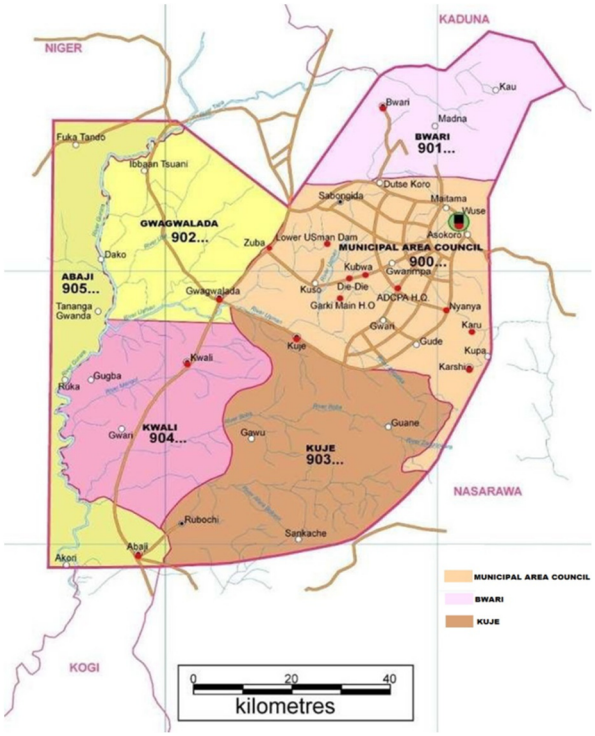

Figure 1: Map of FCT Abuja Showing the Six Area Councils (Study Area).

The FCT experiences a rainy season from April to October and a dry season from November to March. Mineral resources in the area include marble, tin, clay, mica, and tantalite. Abuja is comprised of six area councils: Abaji, Abuja, Bwari, Gwagwalada, Kuje, and Kwali. The indigenous population primarily consists of the Gbagyi people (formerly known as Gwari), who engage in farming and hunting while also producing traditional crafts such as pottery and woodwork (NPC, 2006). Other languages spoken in the region include Bassa, Gwandara, Gade, Dibo, and Koro.

Study design

The cross-sectional epidemiological method with a multi-staged sampling technique was used in this study. The use of the simple random sampling was used to select three (3) Area Councils, namely; Abuja Municipal Area Council (AMAC), Kuje Area Council and Bwari Area Council, respectively. Standard clinics with considerable number of clients were listed and purposively selected in the selected area councils. Systematic sampling method was used to select one out of every three (3) dogs that attended the clinic. Dogs belonging to the Nigerian Police Force, dog breeders and hunters were also sampled.

Sample size

The sample size was determined using Thrusfield sampling formula, N ¼ Z2P (1-P)/d2 (Thrusfield, 2007) for calculating large population using a prevalence of 16.4% (Pilau *et al*., 2022). The sample size was determined to be 200, as such those samples from dogs at Abuja FCT were targeted.

Sample collection

Blood samples collection was from dogs belonging to the Nigeria Police, dog breeders, private Veterinary Clinics, and private dog owners. Three milliliters (3ml) of venous blood were randomly collected from 200 dogs in FCT, Nigeria during the wet and dry season (duration of three months in each season). Plain sample bottle was used to collect the blood sample and centrifuged to obtain serum. The preservation and transportation of the obtained serum to the laboratory section of the Veterinary Public Health Department of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria, for prompt analysis was on ice-parks. History and careful observation were used to determine the vaccination status and age of the dogs, while breed was from World Atlas of Dog Breeds (Heather *et al.,* 2022).

Principle of the assay

The kit assay Lep-IgG/IgM level in the sample fuse purified antigen to coat microtiter plate wells, make solid-phase antibody, then add Lep-IgG or IgM to wells, combined with antigen, after washing and removing non-combinative antigen and other components then combined which with HRP labeled become antigen antibody-enzyme-antigen complex, after washing completely, add TMB substrate solution. TMB substrate becomes blue colour at HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450nm. Compared with the CUTOFF value, according to this to judge Lep-IgG or IgM exist in the sample or not.

Assay procedure for serum examination of *leptospira* antibody

The Rat Lep-IgG and IgM ELISA for Leptospirosis was carried out as follows (Sharma *et al*., 2019): 50µL of positive and negative control was added to the microtiter control wells, along with 50µL of sample diluent into the blank sample comparison well. 40µL of sample diluent and 10µL of test sample were added to the bottom of the ELISA plate coated wells. The microtitre plates were covered with a closure plate membrane and incubated for 30 minutes at 37°C. The closure plate membrane was removed, and the plates were washed five times with washing buffer and blotted dry. 50µL of HRP-Rat conjugate reagent was added to all wells except the blank comparison well. The microtitre plates were covered with a closure plate membrane and incubated for 30 minutes at 37°C with continuous shaking. After five washes and blotting dry, 50µL of chromogen solution was added to all wells. After 15 minutes of incubation at room temperature, the color development was stopped by adding 50µL of stop solution to all wells. The optical density values were read at 450nm using an ELISA Microplate reader. The reagents used in the test were prepared according to the manufacturer's instructions. The assay procedure involves the addition of controls, samples, conjugate, chromogen, and stop solution, followed by incubation, washing, and optical density measurement to detect the presence of Leptospira-specific antibodies in the serum samples.

Data analysis

Data generated from the study was analyzed statistically for significance ($p< 0.05$) using Chi square and Fisher's exact test via the SPSS programme (SPSS, version 21).

RESULTS

Prevalence of leptospirosis in dogs in FCT, Nigeria

A total of 200 samples were collected from dogs out of which 52 samples were collected from AMAC, 54 from Kuje and 94 from Bwari. The number of positive samples in AMAC, Kuje, and Bwari were 51, 52, and 92 respectively. There was no significant relationship *(p>0.05)* in the prevalence of the disease among the Area councils (Table 1).

Prevalence of Leptospirosis in Vaccinated and Unvaccinated Dogs in FCT, Nigeria

A total of 200 samples were collected from dogs out of which 118 were from vaccinated dogs while 82 from

Table 1: Prevalence of Leptospirosis in Dogs in FCT, Nigeria

Area Council	No. of Samples Collected	No. Positive	Percentage Positive
AMAC	52	51	98.10
Kuje	54	52	96.30
Bwari	94	92	97.90
Total	200	195	97.43

x^2 = 5.1549; *p*= 0.0759 *(p>0.05)*

Table 2: Prevalence of Leptospirosis in Vaccinated and Unvaccinated Dogs in FCT, Nigeria.

Vaccination Status	No. of Samples Collected	No. Positive	Percentage Positive
Vaccinated	118	116	98.31
Unvaccinated	82	80	97.56
Total	200	196	97.94

x^2 = 0.1366'; p= 0.7116 (p> 0.05)

Table 3: Sex Distribution of Leptospirosis in Dogs in FCT, Nigeria

Sex	No. of Samples Collected	No. Positive	Percentage Positive
Male	132	129	97.73
Female	68	67	98.53
Total	200	196	98.13

x^2= 0.1473 p= 0.7011 (p > 0.05)

unvaccinated dogs. The prevalence for the vaccinated dogs was 116 (98.31%) while that of unvaccinated was 80 (97.56%). There was no significant relationship *(p>0.05)* between the vaccinated and the unvaccinated dogs (Table 2).

Sex distribution of leptospirosis in dogs in FCT, Nigeria

A total of 200 samples were collected from dogs out of which 132 were male dogs and 68 were from females. The disease prevalence was 129 (97.73 %) for male dogs and 67 (98.53%) for female dogs. There was no significant relationship (*p*> 0.05) between the infection and sex (Table 3).

Age distribution of leptospirosis in dogs in FCT, Nigeria

A total of 200 samples was collected from dogs, out of which 156 were from adult dogs and 44 were from puppies. The prevalence of Leptospirosis for the adult dogs was 153 (98.08%) and 43 (97.73%) for the puppies. There was no significant relationship *(p>0.05)* between the adult dogs and the puppies (Table 4).

Seasonal distribution of leptospirosis in dogs in FCT, Nigeria

A total of 200 samples were collected from dogs, out of which 100 samples were collected during the wet season and 100 samples were collected during the dry season. The disease prevalence for the wet and dry season was 98 (98%) for each season respectively. There was no significant relationship *(p>0.05)* between the wet and the dry season (Table 5).

Breed distribution of Leptospirosis among dogs in FCT, Nigeria

A total of 200 dogs were sampled out of which 60 were Alsatian dogs, 30 Caucasian, 30 Mongrel, 30 Boerboel, 16 Rottweiler, 12 Lhasa Apso, 10 Labrador, 8 Belgian Melinios, and 4 Springer spaniel. The prevalence for Alsatian was 59 (98.33%), Caucasian 29 (96.67%), Mongrel 29 (96.67%), Boerboel 29 (96.67%), Rottweiler 15 (93.75%), Lhasa Apso 11 (91.67%), Labrador 9 (90%), Belgian Melinios 7 (87.5%), and 3 (75%) for Springer Spaniel respectively. There was no significant relationship (*p*> 0.05) between the different breed of dogs (Table 6).

DISCUSSION

The findings of this study agree with that of Awosanya *et al.* (2013), who reported cases of leptospirosis in vaccinated dogs in the Federal Capital Territory (FCT) of Nigeria. The high prevalence of *Leptospira species* antibodies observed in Abuja may be attributed to increased contact between dogs and rats, which serve as reservoir hosts for leptospirosis (Senthil *et al.,* 2013).

Table 4: Age Distribution of Leptospirosis in Dogs in FCT, Nigeria

Age	No. of Samples Collected	No. Positive	Percentage Positive
Adult	156	153	98.08
Puppy	44	43	97.73
Total	200	196	97.91

x^2= 0.9019 p= 0.3422 (P > 0.05)

Table 5: Seasonal Distribution of Leptospirosis in Dogs in FCT, Nigeria.

Breeds	No. of Samples Collected	No. Positive	Percentage Positive
Wet Season	100	98	98
Dry season	100	98	98
Total	200	196	98

x^2= 0; p= 1 (p> 0.05)

Table 6: Breed Distribution of Leptospirosis among Dogs in FCT, Nigeria.

Breeds	No. of Samples Collected	No. Positive	Percentage Positive
Alsatian	60	59	98.33
Caucasian	30	29	96.67
Mongrel	30	29	96.67
Boerboel	30	29	96.67
Rottweiler	16	15	93.75
Lhasa Apso	12	11	91.67
Labrador	10	9	90
Belgian Melinios	8	7	87.5
Springer Spaniel	4	3	75
Total	200	191	98.80

x^2 = 7.7371 p = 0.4595 (p >0.05)

These rats often scavenge for food and water in kennels, leading to contamination of the environment with their urine and feces. Additionally, factors such as vaccine failure and a shorter duration of immunity than expected could contribute to this prevalence, supporting Grassmann *et al.* (2017), who questioned the efficacy of existing leptospirosis vaccines due to reported failures. The study revealed a seroprevalence of Leptospirosis in vaccinated dogs and unvaccinated dogs, with no significant difference between the two groups. This finding agrees with the findings of Goarant (2016), who noted that infections can still occur in vaccinated animals, although they often do not progress to severe disease and are associated with reduced urinary shedding. Notably, a high seropositivity was also found in unvaccinated dogs, likely due to extensive exposure to *Leptospira* and the presence of asymptomatic carriers, as indicated by Klaasen *et al.* (2015). The distribution of leptospirosis showed no significant differences between male and female dogs, this contradicts the findings of Ward *et al.* (2002), who reported higher seropositivity in males. This can be due to variations in infective doses or geographical differences, as Ward's study was conducted in Ibadan, Oyo State. The prevalence among adult dogs and puppies was similar; corroborating the SPCA (2019) report that leptospirosis affects dogs across all ages, which agrees with Awosanya *et al.* (2013). Seasonal analysis indicated a high prevalence during both wet and dry seasons without significant differences in distribution, consistent with Lee *et al.* (2014), who noted that leptospirosis risk persists year-round due to the availability of predisposing factors and vectors across seasons. Additionally, no relationship was found between leptospirosis prevalence and different dog breeds in Abuja, supporting SPCA (2019) and Awosanya *et al.* (2013), which state that all breeds are susceptible to the disease. Overall, these findings suggest a uniform distribution of leptospirosis across the six area councils of FCT, likely due to similar immunological responses among dogs exposed to *Leptospira spp*.

Conclusion

The findings of this study conclude that, there is high and uniform prevalence of leptospirosis among dogs in the FCT, regardless of vaccination status, underscores the need for improved public awareness and disease control measures. Additionally, further investigation into the reliability and efficacy of leptospirosis vaccines used in Nigeria is warranted to optimize prevention strategies and safeguard animal and human health. Lastly, there is a need for the sequencing of a field strain of *Leptospira* in Abuja and to compare it to the component of the *Leptospira* vaccine in use.

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Conflict of Interest

The authors declare no competing interests regarding publication of this paper.

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